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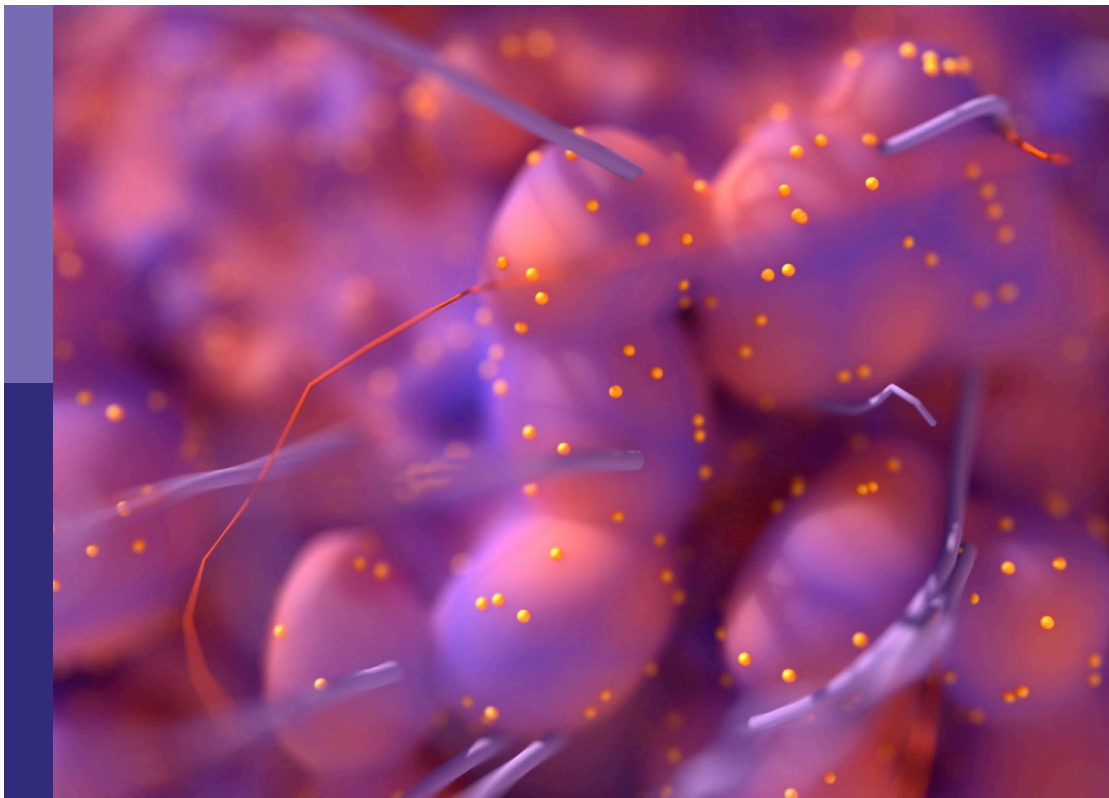
Reviews in hematologic malignancies 2023

Edited by
Billy Jebaraj, Felix Seyfried and Michael Liew

Published in
Frontiers in Oncology



ISSN 1664-8714
ISBN 978-2-8325-4898-1
DOI 10.3389/978-2-8325-4898-1

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Reviews in hematologic malignancies: 2023

Topic editors

Billy Jebaraj — University of Ulm, Germany

Felix Seyfried — Ulm University Medical Center, Germany

Michael Liew — ARUP Laboratories, United States

Citation

Jebaraj, B., Seyfried, F., Liew, M., eds. (2024). *Reviews in hematologic malignancies: 2023*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4898-1

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frontiers | Frontiers in Oncology

TYPE Editorial
PUBLISHED 02 May 2024
DOI 10.3389/fonc.2024.1412843

OPEN ACCESS

EDITED AND REVIEWED BY
Alessandro Isidori,
AORMN Hospital, Italy

*CORRESPONDENCE
Felix Seyfried
felix.seyfried@uni-ulm.de

RECEIVED 05 April 2024
ACCEPTED 24 April 2024
PUBLISHED 02 May 2024

CITATION
Jebaraj BMC, Liew M and Seyfried F (2024) Editorial: Reviews in hematologic malignancies: 2023.
Front. Oncol. 14:1412843.
doi: 10.3389/fonc.2024.1412843

Editorial: Reviews in hematologic malignancies: 2023

Billy Michael Chelliah Jebaraj[1], Michael Liew[2] and Felix Seyfried[3]*

[1]Division of Chronic Lymphocytic Leukemia, Department of Internal Medicine III, Ulm University Medical Center, Ulm, Germany, [2]ARUP Institute for Clinical and Experimental Pathology®, Salt Lake City, UT, United States, [3]Department of Pediatrics and Adolescent Medicine, Ulm University Medical Center, Ulm, Germany

KEYWORDS

hematologic malignancies, reviews, molecular mechanisms, disease pathology, lymphoma, multiple myeloma, clinical trends

Editorial on the Research Topic
Reviews in hematologic malignancies: 2023

Editorial

The research being applied to hematological malignancies is as diverse and heterogeneous as the disease itself, all moving towards the ultimate goal of better therapeutic outcomes. In this article Research Topic, molecular pathway targets for therapy and the tumor microenvironment are highlighted for further research. Along with current clinical trends, a focus on hematological composite tumors and treatment guidance for developing countries like India are provided. Lastly, this Research Topic would not be complete without discussing next generation sequencing (NGS) and machine learning, which revolutionize molecular characterization, diagnostics and treatment optimization.

Molecular pathways

Exploring molecular pathways to prevent tumor progression is an important area of research. For instance, Schmid and Hobeika in their review on B-cell receptor (BCR) signaling in chronic lymphocytic leukemia (CLL) present a detailed account on the biology of various components of the signaling pathway. The importance of BCR signaling for CLL is exemplified by the clinical success of inhibitors targeting Bruton's tyrosine kinase (BTK), a key component of the pathway. The authors also present an update on pre-clinical and clinical efficacy of next-generation inhibitors of BCR, mechanisms that mediate resistance to these inhibitors and strategies to overcome resistance. Uncontrolled cell proliferation is a hallmark of cancers and William et al. explore the role of SKP2 (S-Phase kinase-related protein 2), in promoting cell cycle progression through ubiquitin-mediated degradation of cell cycle regulator proteins. Overexpression of *SKP2* has been associated with poor prognosis in solid tumors. This also appears to be the case in hematological

malignancies. In addition, *SKP2* overexpression is associated with drug resistance in hematological malignancies. Research is needed to develop novel inhibitors of SKP2 to overcome the drug resistance.

A small nucleocytoplasmic shuttling protein myeloid leukemia factor 1 (MLF1) is known to act as a 'double-edged sword' in a context dependent manner. Li et al. review the role of MLF1 in myeloid neoplasms. The precise role of MLF1 is poorly understood, but it has been implicated in the development of acute myeloid leukemia (AML) as well as myelodysplastic syndrome (MDS). For example, high expression of MLF1 is associated with poor prognosis in AML, but in a drosophila model of leukemia MLF1 reduced RUX1-ETO-dependent leukemia cell proliferation. More research into the role of MLF1 in the normal functioning of a cell will help determine if it is a candidate for cancer therapeutics. On the other hand, long non-coding RNAs (lncRNAs) are described as being involved in a plethora of processes including tumorigenesis. However, an in-depth understanding of each lncRNA is lacking. Nylund et al. comprehensively summarize recent studies supporting the clinical relevance of lncRNAs in multiple myeloma (MM). Sequencing analyses have provided evidence that lncRNAs contribute to disease development, treatment resistance, and patient prognosis in MM. lncRNAs have been shown to modulate chromatin remodeling and to impact gene expression. Targeting lncRNAs is emerging as a possible therapeutic approach for cancer, including MM.

Studying cell death mechanisms is important for refining cancer therapy. Ferroptosis is a form of cell death which is different from autophagy, apoptosis and necrosis and Chen et al. review its role in leukemia. Ferroptosis is characterized by iron-dependent lipid peroxidation and reactive oxygen species (ROS) accumulation, which eventually becomes fatal to a cell. If ferroptosis could be enhanced specifically in leukemic cells, this could serve as a possible therapeutic. Several studies have demonstrated that current therapies do induce ferroptosis, but some cancers are able to evade cell death. Research is needed to investigate the specific mechanisms that prevent ferroptosis.

Tumor microenvironment

The tumor microenvironment plays an important role in the pathogenesis and progression of various hematological malignancies. In their review, Ding et al. summarize the recent findings on a crucial component of the tumor microenvironment called cancer-associated fibroblasts (CAFs). Various cells of mesenchymal stem cell origin can be reprogrammed into activated CAFs by tumors. The CAFs in-turn support tumor growth, drug resistance and metastasis. The authors highlight that a comprehensive understanding of CAFs in hematologic cancer would be important for innovative and next-generation cancer drug design. Extracellular vesicles (EVs) are capable of mediating complex crosstalk between tumor cells, as well as their microenvironment. EVs shuttle various proteins, lipids and nucleic acids between cells. Bernardi et al. in their review compiled key findings on the role of EVs in chronic myeloid leukemia (CML). Of importance, the authors emphasize on translational aspects such as the potential value of EVs for monitoring minimal residual disease (MRD), as biomarkers for optimizing treatments, and to analyze therapy efficacy. They also present from the literature, interesting prospects to reprogram EVs as targeted drug delivery vehicles for CML treatment.

Clinical trends

In the clinical setting, use of tyrosine kinase inhibitors (TKIs) substantially changed the treatment perspective of CML, but chronic use is associated with adverse events. Cheng et al. discuss dose optimization strategies for TKIs in chronic myeloid leukemia (CML). The authors summarize recent clinical trials and real-life practices in which an increasing number of CML patients have undergone a dose optimization strategy involving dose reduction and discontinuation of TKI therapy. They discuss how treatment discontinuation has now emerged as a therapeutic goal for CML patients with a deep molecular response and has proven to be feasible in about half of patients.

Survival of patients with acute lymphoblastic leukemia (ALL) has greatly improved in the recent decade. However, for developing countries like India, it remains a challenge due to direct costs such as the financial cost of treatment and indirect costs such as the loss of productive years of the patient and caregiver and the rise of more resistant forms of the disease due to difficulties in timely treatment delivery. Mathews et al. describe how a panel of 15 actively practicing clinicians developed a consensus document for B-ALL management to offer assistance to Indian hematologists/oncologists. Strategies like this are very important to ensure that effective treatments are available to everyone.

The complexity of hematologic malignancies being managed clinically is illustrated by a case discussed by Gu et al. Composite lymphomas (CL) are an unusual type of hematologic malignancy, accounting for 1-4.7% of all lymphomas. Even more uncommon are CLs that comprise of both a B-cell and a T-cell tumor. The authors present their case of a mixture of diffuse large B cell lymphoma (DLBCL) and peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS). The mechanism by which CL arises has not been elucidated, but hypotheses include virological and a specific mutation in a progenitor cell. These are a challenging class of lymphoma and will require further study to improve their poor prognosis. Hepatitis B virus (HBV) is the most common cause of liver disease worldwide and it is associated with lymphoma in endemic regions. Rosenberg et al. provide an overview of hepatitis B virus (HBV) infection in B-cell lymphoma. The authors emphasize the importance of systematic screening and preventive antiviral therapy for non-Hodgkin's lymphoma (NHL) patients. The review summarizes studies showing a connection between HBV and lymphoma, particularly DLBCL. In addition, recent studies have revealed that HBV-positive DLBCL has distinct mutational signatures with differential outcomes.

In another setting of hematological disorders, Nassani et al. present an important clinical summary on the benefits and adverse effects of androgen therapy in different BMF syndromes. Androgens are an important class of molecules that are in clinical use for treating various bone marrow failure (BMF) syndromes since decades. Though their mechanism of action in stimulating hematopoiesis is unresolved, they continue to prove to be an important class of treatment for specific clinical scenarios. The authors give practical recommendations for use of androgens for BMF patients.

NGS and deep learning

Over the past decade, substantial advances have been made in NGS technologies. Tomacinschii et al. provide a comprehensive review of recent developments in NGS for the diagnosis and clinical management of NHL patients. The data generated by NGS allows the identification of genetic markers specific to different subtypes, leading to a more accurate diagnosis and classification. The integration of genomic and transcriptomic data can improve the understanding of the mechanisms of tumor development and can help select the optimal therapy. DL and artificial intelligence (AI) are revolutionizing every field. Elsayed et al. review recent studies that examine the use of DL in the diagnosis of ALL, focusing on the analysis of bone marrow images. DL approaches, especially those using Convolutional Neural Networks (CNN) techniques, have achieved excellent results in classifying cancer cells. The authors propose that DL methods have high potential for reliable classification of ALL in a clinical context. Further models aim to combine both image analysis and genomic data, which could lead to improvements in ALL classification.

In conclusion, there are still avenues open for exploration to improve the treatment of hematological malignancies. As current therapies, clinical guidelines, and the use of AI continue to get refined and improved, they contribute to steadily improving outcomes for patients with hematological malignancies.

Author contributions

BMCJ: Writing – original draft, Writing – review & editing. ML: Writing – original draft, Writing – review & editing. FS: Writing – original draft, Writing – review & editing.

Acknowledgments

FS would like to thank for the support provided by the Advanced Clinician Scientist Programme from the Medical Faculty of Ulm University.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 06 February 2023
DOI 10.3389/fonc.2023.1124978

OPEN ACCESS

EDITED BY
Michele Redell,
Baylor College of Medicine, United States

REVIEWED BY
Ju Huang,
Wenzhou Medical University, China

*CORRESPONDENCE
Mingxia Shi
shmxia2002@sina.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Hematologic Malignancies, a section of the journal Frontiers in Oncology

RECEIVED 15 December 2022
ACCEPTED 17 January 2023
PUBLISHED 06 February 2023

CITATION
Li Z, Yang Y, Wu K, Li Y and Shi M (2023) Myeloid leukemia factor 1: A "double-edged sword" in health and disease. *Front. Oncol.* 13:1124978. doi: 10.3389/fonc.2023.1124978

Myeloid leukemia factor 1: A "double-edged sword" in health and disease

Zixuan Li[1,2†], Yuanyuan Yang[1,2†], Kun Wu[3], Yuntao Li[1,2] and Mingxia Shi[1,2*]

[1]Department of Hematology, the First Affiliated Hospital of Kunming Medical University, Kunming, China, [2]Hematology Research Center of Yunnan Province, Kunming, China, [3]Department of Clinical Laboratory, First Affiliated Hospital of Kunming Medical University, Kunming, China

The occurrence and development of malignancies are closely related to abnormal cell cycle regulation. Myeloid leukemia factor 1 (MLF1) is a small nucleocytoplasmic shuttling protein associated with cell cycle exit, apoptosis, and certain immune functions. Therefore, it is pertinent to explore the role of MLF1 in health and diseases. Studies to date have suggested that MLF1 could act as a double-edged sword, regulating biochemical activities directly or indirectly. In hematopoietic cells, it serves as a protective factor for the development of lineages, and in malignancies, it serves as an oncogenesis factor. The diversity of its functions depends on the binding partners, including tumor inhibitors, scaffolding molecules, mitochondrial membrane proteins, and transcription factors. Emerging evidence indicates that MLF1 influences immune responses as well. This paper reviews the structure, biological function, and research progress on MLF1 in health and diseases to provide new insights for future research.

KEYWORDS

myeloid leukemia factor 1, nucleo-cytoplasmic shuttling protein, cell cycle regulation, immune function, cell development and differentiation, malignancy development

Introduction

Myeloid leukemia factor (MLF) is a poorly characterized family of conserved proteins which earliest member, myeloid leukemia factor 1 (MLF1), is associated with hemopoietic lineage commitment and malignancies. MLF1 has so far been shown to be a double-edged sword, acting as either a tumor suppressor or an oncogene, depending on the context of the cell. MLF1 has been initially described in the leukemic fusion protein NPM-MLF1, which is generated by a rare t(3;5)(q25.1;q34) chromosomal translocation in patients with acute myeloid leukemia (AML) (1), and implicated in the development of AML and myelodysplastic syndrome (MDS) (2). Although the role of NPM in the pathogenesis of leukemia has been well studied (3–6), the contribution of MLF1 to normal hematopoiesis and oncogenesis has not been adequately characterized. Several studies have demonstrated that MLF1 can regulate cell cycle exit and differentiation, promote apoptosis, inhibit proliferation in various cell types, enhance immune function, or impair the lymphocyte population.

However, its biochemical activity remains largely unclear. Up to now, no systematic overview of MLF1 studies in pathology and physiology has been published. In this review, we summarized current knowledge of MLF1 and provided a valuable reference for future research.

MLF1 structure and function

MLF1 gene is located on human chromosome 3 and encodes MLF1 protein and its isoforms (7). MLF1 protein is a small nucleocytoplasmic shuttling protein (268 amino acids), which has a functional N-terminal nuclear export signal (NES) and two C-terminal nuclear localization signals (NLS), allowing MLF1 to shuttle between the nucleus and the cytoplasm (8, 9). MLF1 has a characterized central domain preserved within the MLF family (10, 11), comprising two identifiable motifs that bind to 14-3-3 protein and the COP9 signalosome by Ser34 and subunit 3 (CNS3), and a SAM domain, which is involved in many different biological processes and has RNA binding properties (12). Above features of MLF1 are summarized in Figure 1. MLF is highly conserved across species from Drosophila, murine, and shrimp to humans (13–15). The phenotypic defects associated with MLF loss in Drosophila can be rescued by human MLF1 (16). MLF overexpression reduces Drosophila wing and eye size (17), which is demonstrated by the fact that MLF activates the bsk-JNK pathway by interacting with DREF (18). Additionally, overexpressed MLF causes abnormal DNA synthesis in Drosophila (19). Enforced expression of murine MLF1 suppresses a rise in the cell cycle inhibitor p27Kip1 to disturb the development and the differentiation of erythrocytes (20, 21). Microarray analysis performed with MLF1-expressing cells has concluded that MLF1, when expressed in the nucleus, inhibited calcium cycle proteins and CR6 (cytokine response protein) associated with differentiation and growth arrest (8). Immune function is also associated with MLF1. It has been identified in kuruma shrimp and characterized as MjMLF, which plays a critical role both in antiviral and antibacterial immunity. MjMLF could inhibit the lethal white spot syndrome virus (WSSV) replication *in vivo* and accelerate *Vibrio anguillarum*, a gram-negative bacteria, clearance in shrimp (22, 23). In contrast, a study about lymphoma has shown that the overexpression of MLF1 increases lymphocyte apoptosis *in vitro* (13). Furthermore, MLF1 absence is consistently associated with the expansion of B- and T-cell numbers in the spleen (24). These findings imply that MLF1 might function as a context-dependent factor involved in the regulation of normal physiological processes and that its absence or overexpression leads to disease.

MLF1 and its distribution

MLF1 is widely expressed in different tissues. It is highly presented in the testis, heart, lung, brain, thyroid gland, gall bladder, kidney, and digestive system and is expressed to some extent in human bone marrow, spleen, and lymph nodes (25). At the cellular level, MLF1 transcripts are dominantly expressed in CD34+ cells but only slightly in GlyA+, CD3+, CD19+, or CD14+ cells and granulocytes (2). These facts indicate that the expression of MLF1 in CD34+ progenitor cells decreases during differentiation to each lineage, especially toward the myeloid and erythroid lineage (15). Cells at an early stage seem to need MLF1. At the subcellular level, MLF1 is mostly found in the cytoplasm. The apoptosis-inducing domain contained in MLF1 is unique because it requires dimerization and nuclear transportation to induce cell death, whereas most of the

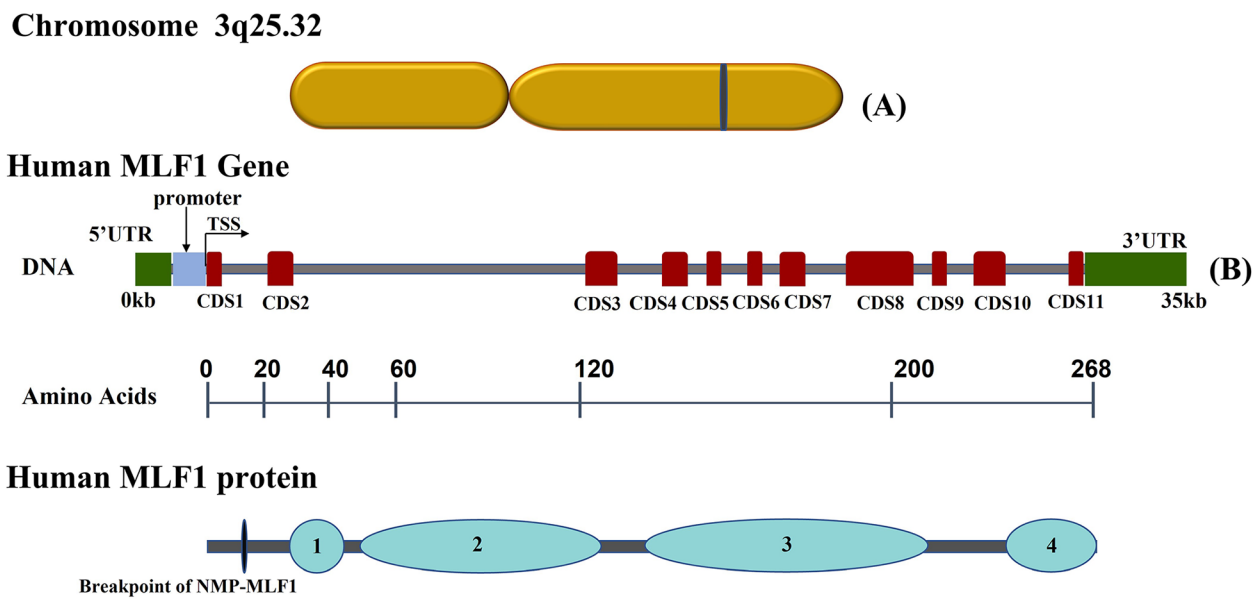

FIGURE 1
Schematic representation of human Myeloid Leukemia Factor 1 (MLF1) chromosome, gene and corresponding protein domains: **(A)** Diagram of *MLF1* gene chromosome location. **(B)** The grey horizontal line represents the DNA sequence, red boxes on the sequence represent coding sequences (CDS) in MLF1, and two green boxes at the ends indicate UTRs. Underneath, the numbers over the black lines indicate the amino acid positions, which correspond to human MLF1 protein domains ([1] 14-3-3 protein binding domain (Ser34); [2] the COP9 signalosome subunit 3(CNS3) binding domain; [3] MLF family characteristic domain; [4] a SAM domain). All sequences were obtained from the NCBI database.

well-known 'death domains' function in the cytoplasm (26). However, the relationship between the increased accumulation of MLF1 in the cytoplasm and diseases is still unclear. Notably, a functional NES sequence is important for both MLF1 protein and NMP-MLF1 fusion protein to exert proleukemic effects. Additionally, studies have demonstrated that an MLF1 mutant containing only NES sequence inhibited proliferation more strongly than WT protein (9, 27). The contribution of NPM to NPM-MLF1-induced leukemogenesis is debatable (28), whereas NPM-MLF1 fusion protein without NES sequence loses oncogenic transformation ability (9). However, the regulatory mechanisms of the abnormal localization of MLF1 in the nucleus remain unknown.

MLF1 networks

MLF1 plays an essential role in cell development by interacting with multiple factors, which are summarized in Figure 2.

1. MLF1 in cell development and apoptosis. MLF1, shuttling from the cytoplasm to the nucleus, binds COP9 subunit 3 (CSN3), which leads to the downregulation of COP1; therefore, the cell cycle of hematopoietic cells becomes arrested because the bonding accelerates the accumulation of wild-type p53 in the nucleus (29). The above-mentioned process has also been demonstrated in Drosophila (17). Tumor suppressor p53 remains a vital mechanism of inhibiting tumor escape from apoptosis, and emerging evidence suggests that mutant p53 also promotes inflammation and supports tumor immune evasion (30, 31). Yoneda-Kato et al. have demonstrated that the MLF1-induced growth arrest depended on the integrity of the p53 allele (29). This raises the question of whether MLF1 still acts as a protective mechanism when p53 is mutated or if it enhances the oncogenicity of mutated p53. Overexpression of MLF1 promotes apoptotic death of the cells but is negatively regulated by 14-3-3 protein blocking its Bcl-XL homology domain 3 (BH3), which prevents the cell from apoptosis (32, 33). 14-3-3 (RSXSXP) motifs are involved in important cell processes, such as death, differentiation, and division (34–36). Bcl-XL, a Bcl-2 family member, maintains a fully functional immune system that ensures an efficient clearance of senescent cells (37). The above-presented conclusion has been obtained in lymphocytes, suggesting that MLF1 is required for lymphocytes to respond to apoptotic stimulations. Additionally, the nuclear content of MLF1 is also regulated by 14-3-3 protein, which sequesters MLF1 in the murine cytoplasm (32), However, another study has suggested the opposite conclusion that the distribution of full-length human MLF1 is 14-3-3 protein-independent (38). Therefore, the subcellular localization of MLF1 is probably regulated by other unknown proteins. A yeast two-hybrid screen has identified that MLF1 binds with an adaptor, which contains a 220-

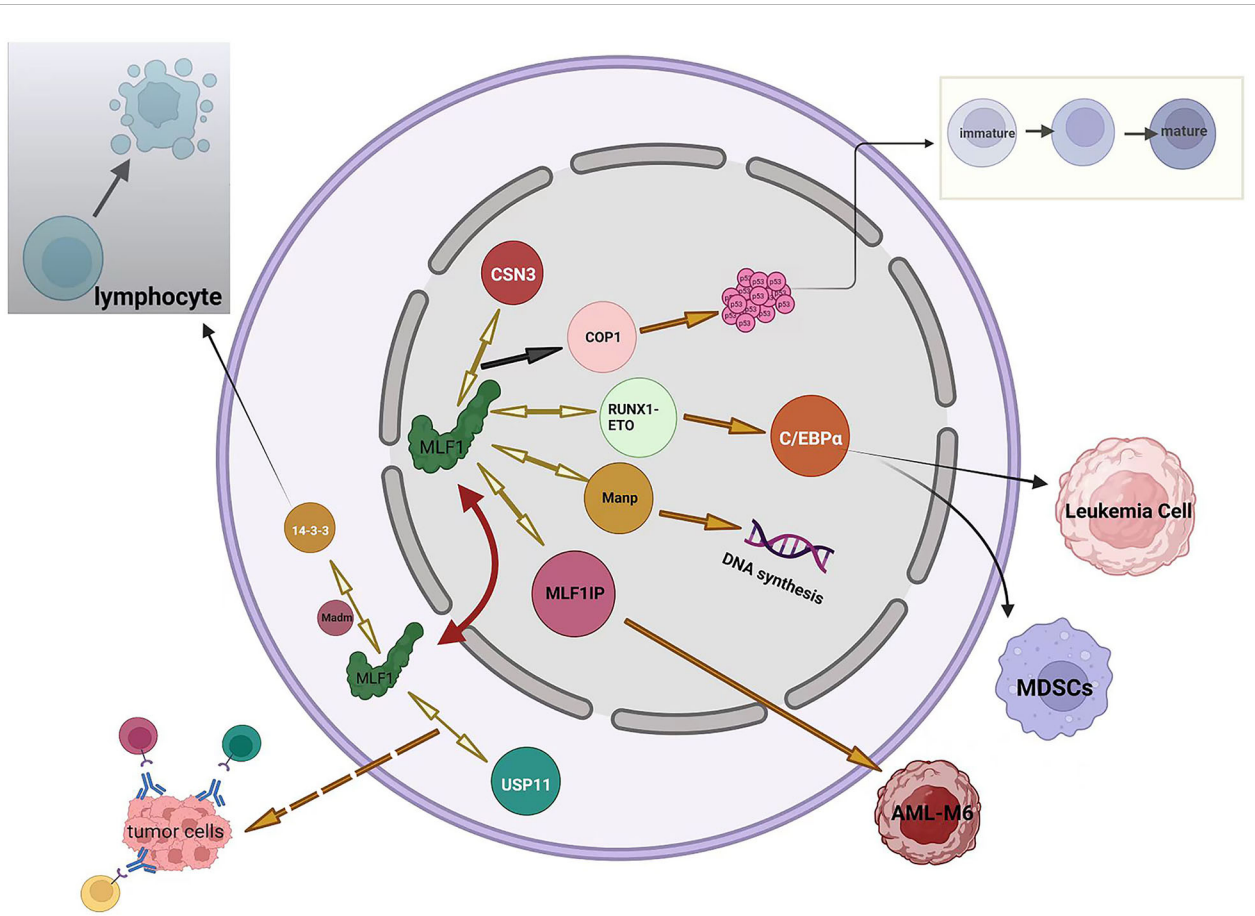

FIGURE 2
Summary of MLF1 networks. Black arrows represent negative regulation (including inhibition or downregulation), orange arrows represent positive regulation (including promotion or upregulation), orange dotted arrow represents speculation, and bidirectional yellow arrows indicate proteins interacting with MLF1 protein. The red two-way arrow illustrates the shuttling of MLF1 between the nucleus and the cytoplasm.

bp cDNA fragment and several potential phosphorylation sites in the vicinity of both the NLS and NES. At the time of its isolation, it had no homology to sequences in the database and was named Madm for MLF1-adaptor molecule. Madm mediates phosphorylation of 14-3-3 binding site of MLF1, which then immunoprecipitates and localizes to the cytoplasm. Thus, Madm might regulate the localization of MLF1 in the cytoplasm. In contrast to MLF1, which promotes the maturation, ectopic expression of Madm suppresses differentiation in myeloid cells (32). Louise N et al. have reported that MLF1 interacts with Manp, also known as scaffold attachment factor-A (SAF-A), which is a member of the heterogeneous nuclear ribonucleoprotein (hnRNP) family and homologous to hnRNP-U. Manp localizes exclusively in the nucleus and redirects MLF1 into the nucleus (8). Recent studies have suggested that hnRNP-U regulates DNA replication, organizes large-scale chromosome structures, and protects the genome from instability (39–41). The effects of MLF on DNA synthesis have been previously discussed (19). However, the relationship between MLF1 and hn-RNP in DNA synthesis remains unclear.

2. MLF1 in immune function and leukemia. In a drosophila model of leukemia, MLF has been demonstrated to control the development of hematopoietic stem cells by stabilizing the RUNX transcription factor Lozenge (LZ). MLF controls LZ activity and prevents its degradation, which is critical to control crystal cell number in the fly (42). Further study has shown that MLF and DnaJ-1 interact through conserved domains to form a chaperone complex that directly regulates LZ activity. Importantly, the interaction controls RUNX transcription factor activity and Notch signaling during blood cell development *in vivo* (43). RUNX members are key regulators of hematopoiesis; particularly, RUNX1 functions as a positive regulator for definitive hematopoietic stem cell emergence and megakaryocyte and lymphocyte differentiation (44). RUNX1-ETO, the mutant and infusion form of the RUNX1 protein, has been identified in cancer. MLF1 stabilizes the human oncogenic fusion protein RUNX1-ETO. Further study has indicated that MLF1 impairs RUNX1-ETO accumulation and reduces RUNX1-ETO-dependent leukemia cell proliferation (42). It is reasonable to conclude that MLF1 functions as a tumor suppressor gene in leukemia. However, the expression level of MLF1 in healthy adults' bone marrow is not as high as expected. Moreover, high expression of MLF1 is associated with poor prognosis for AML and MDS (2). To some extent, MLF1 is required to inhibit the development of leukemia. However, it does not always appear to be a protective factor, and when leukemia is developed, MLF1 is positively correlated with leukemia (2). Reasonably, it can be inferred that *MLF1* is a context-dependent gene, with its elevated expression being associated with leukemia promotion and suppression in different settings. CCAAT/enhancer-binding protein-α (C/EBPα) is a key transcription factor regulating myeloid differentiation in normal hematopoiesis and is frequently dysregulated in AML (45). Studies have shown that Trib1 and RUNX1-ETO downregulate C/EBPa and induce AML in mouse models (46, 47). MLF1 treatment upregulates the level of C/EBPα by suppressing Trib1 or RUNX1-ETO, which causes the inactivation of myeloid-derived suppressor cells (MDSCs) with potent antitumor responses across different tumor models and cancer patients (48). In mouse or leukemia cell models, the distribution of C/EBPa is paralleled with MLF1 (49). MLF1-interacting protein (MLF1IP), also known as PB1P1, KLIP1, KLP1, CENP-U, and CENP-50, specifically binds with MLF1, as shown by yeast two-hybrid analysis and pulldown assays (50). MLF1IP differs from MLF1 without other known protein homology. MLF1IP is a centromere-binding protein (51) that shows 25% identity to the SMC family of proteins and some homology to myosin, which is involved in actin cytoskeletal organization (52). *MLF1IP* may be an erythroid lineage-specific gene, as it is expressed exclusively in CFU-E erythroid precursor cells but not in mature erythrocytes (50, 53). MLF1 drives the occurrence of erythroleukemia as well (20, 21). A study has found that NMP-MLF1 infusion protein is more likely to occur in M6 (according to FAB classification) patients than in other leukemia types (54). Therefore, the interaction between *MLF1IP* and *MLF1* will most likely play a role in the occurrence of M6. High expression of *MLF1IP* is associated with poor prognosis in several cancers, such as breast cancer, glioma, and diffuse large B-cell lymphoma (DLBC) (55). Furthermore, *MLF1IP* also plays a role in the development of the immune system (56). However, the functional consequences of *MLF1* and *MLF1IP* interaction remain largely unknown. HAX-1, a 35-kDa inner mitochondrial membrane protein, functions as an anti-apoptosis protein (57), and its deficiency and overexpression result in the loss of lymphocytes and tumorigenesis, respectively (58, 59). This expression balance of MLF1 is also critical for its function. MLF1 has been recently revealed to directly associate with HAX-1 by co-immunoprecipitation assay. Animal experiments have confirmed that the two have interaction, and severe splenocyte and thymocyte lymphopenia in Hax1−/− mice can be reversed by MLF1 deficiency (13). However, it is unclear whether their effects on lymphocytes are synergistic or antagonistic. As of now, despite conflicting evidence, the relationship between MLF1 and immune function has not been adequately investigated. Further research is required to clarify this issue.

3. MLF1 in antitumor protection. MLF1 protein is directly associated with the deubiquitinase ubiquitin-specific peptidase 11 (USP11), which is a promising therapeutic target. Additionally, USP11 has promoted the accumulation of MLF2 in all tested cells (60), whereas MLF1 and MLF2 are approximately 40% similar (11). USP11 plays a dual role in the development of tumors (61, 62). Based on the studies conducted so far, *MLF1* may act as both a tumor suppressor and tumor oncogene, depending on the context of the cell. It is worth mentioning that MLF1 is a positive factor in various biological processes, such as progenitor cell development and tumor regression. Whether MLF1, together with upregulated USP11 protein, enhances antitumor ability still needs further research.

MLF1 and disease

MLF1 functions as a double-edged sword in various diseases. An early clinical study has found that t(3;5) is more likely to occur in M6 patients than in patients with other leukemia types (54). A preclinical study has confirmed that MLF1 expression drives the occurrence of erythroleukemia (20, 21). A significantly higher level of MLF1 expression is detected in over 25% of patients with immature AML subtypes and higher malignant MDS (2). MLF1 is also upregulated in lung squamous cell carcinoma and esophageal carcinomas (63, 64). MLF1 overexpression results in aggregate formation; however, there

is still controversy over the cause and effect of protein aggregate in neurodegenerative diseases (65). The whole-exome sequencing of small intestine neuroendocrine tumors has revealed that *MLF1* is therapeutically relevant (66). The presence of MLF1 protein inhibits apoptosis caused by neurotoxicity induced by Huntingtin (HTT) aggregates (67). An extensive genome-wide association study (GWAS) has indicated that *MLF1* expression is high in neuroblastoma and that silenced MLF1 significantly suppresses tumor proliferation (68). Recent research in the heart has shown that the increased expression of *MLF1* leads to accelerated apoptosis and reduced cardiac cell proliferation (69). However, an aberrant downregulation of *MLF1* is also related to tumorigenesis. Aberrant DNA methylation plays a significant role and is extensive (70), as indicated by the higher incidence of aberrant DNA methylation of known tumor-suppressor genes than that of mutations (71). *MLF1* is methylation-silenced in the gastric cancer cell line and is upregulated 27-fold after 5-AZA-dC treatment. There is a possibility that *MLF1* silencing is causally related to the development and progression of gastric cancer (72, 73). A comparative study has identified that *MLF1* is also a methylation marker for the detection of early gastric neoplasia and field cancerization (74). Shuang Zhao et al. have found that the expression levels of *MLF1* were downregulated in tumor tissues compared to normal tissues, which suggested that *MLF1* influences tumor initiation and progression in nasopharyngeal carcinoma (75). Defects in the centrosome and cilium are associated with a set of human diseases. Ramona A. Hoh et al. have found that MLF1 was associated with diseases, was upregulated during ciliogenesis, and localized to centrioles and cilia (76). Hypermethylated *MLF1* gene in mantle cell lymphoma (MCL) has been confirmed by genome-wide DNA methylation analysis, and aberrant methylation is associated with inverse changes in mRNA levels (77). Marcela B. Mansur et al. have identified a recurrent somatic deletion on chromosome 3. This loss results in the complete deletion of MLF1 and has not been previously described in infant T-cell acute lymphoblastic leukemia (78).

Conclusion and future perspectives

In conclusion, MLF1 is a small shuttling protein playing a critical role in biological and pathological processes. Currently, research regarding MLF1 has mainly focused on cancer development, which is still an obscure and disputed topic. In general, although there is more evidence supporting the point that MLF1 contributes to tumor suppression, a few studies have confirmed the tumorigenesis of MLF1 in solid and hematologic tumors, which cannot be neglected. Additionally, the functions of MLF1 in immune response still need further investigations despite some already reported studies. Given the complexity and variety of involved proteins, we may draw a conclusion that MLF1 might be a double-edged factor in the regulation of cell cycle, immunity, stem cell development, and cancer. However, studies about MLF1 are still inadequate; therefore, expanding the research on MLF1 is significant and may enrich the knowledge of MLF1 in the above-mentioned conditions. On the other hand, exploring regulations of MLF1 shuttling will provide a better understanding of MLF1, which helps develop novel specific MLF1-aimed drugs that might provide a promising strategy for cancer treatment, as well as other pathologies, such as neurological diseases. Therefore, analysis of the partner protein, localization, and shuttling mechanism might provide new insights for future research.

Author contributions

All authors contributed to the article and approved the submitted version.

Funding

The present study was supported by the National Natural Science Foundation of China (No. 81760035).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Yoneda-Kato N, Look AT, Kirstein MN, Valentine MB, Raimondi SC, Cohen KJ, et al. The T (3,5)(Q25.1;Q34) of myelodysplastic syndrome and acute myeloid leukemia produces a novel fusion gene, npm-Mlf1. *Oncogene* (1996) 12(2):265–75.

2. Matsumoto N, Yoneda-Kato N, Iguchi T, Kishimoto Y, Kyo T, Sawada H, et al. Elevated Mlf1 expression correlates with malignant progression from myelodysplastic syndrome. *Leukemia* (2000) 14(10):1757–65. doi: 10.1038/sj.leu.2401897

3. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *New Engl J Med* (2005) 352(3):254–66. doi: 10.1056/NEJMoa041974

4. Grisendi S, Pandolfi PP. Npm mutations in acute myelogenous leukemia. *New Engl J Med* (2005) 352(3):291–2. doi: 10.1056/NEJMe048337

5. Nakagawa M, Kameoka Y, Suzuki R. Nucleophosmin in acute myelogenous leukemia. *New Engl J Med* (2005) 352(17):1819–20. doi: 10.1056/NEJM200504283521719

6. Wang R, Xu P, Chang L-L, Zhang S-Z, Zhu H-H. Targeted therapy in Npm1-mutated aml: Knowns and unknowns. *Front In Oncol* (2022) 12:972606. doi: 10.3389/fonc.2022.972606

7. Muzny DM, Scherer SE, Kaul R, Wang J, Yu J, Sudbrak R, et al. The DNA sequence, annotation and analysis of human chromosome 3. *Nature* (2006) 440(7088):1194–8. doi: 10.1038/nature04728

8. Winteringham LN, Endersby R, Kobelke S, McCulloch RK, Williams JH, Stillitano J, et al. Myeloid leukemia factor 1 associates with a novel heterogeneous nuclear ribonucleoprotein U-like molecule. *J Biol Chem* (2006) 281(50):38791–800. doi: 10.1074/jbc.M605401200

9. Yoneda-Kato N, Kato J-Y. Shuttling imbalance of Mlf1 results in P53 instability and increases susceptibility to oncogenic transformation. *Mol Cell Biol* (2008) 28(1):422–34. doi: 10.1128/MCB.02335-06

10. Gobert V, Haenlin M, Waltzer L. Myeloid leukemia factor: A return ticket from human leukemia to fly hematopoiesis. *Transcription* (2012) 3(5):250–4. doi: 10.4161/trns.21490

11. Kuefer MU, Look AT, Williams DC, Valentine V, Naeve CW, Behm FG, et al. Cdna cloning, tissue distribution, and chromosomal localization of Myelodysplasia/Myeloid leukemia factor 2 (Mlf2). *Genomics* (1996) 35(2):392–6. doi: 10.1006/geno.1996.0376

12. Kim CA, Bowie JU. Sam Domains: Uniform structure, diversity of function. *Trends In Biochem Sci* (2003) 28(12):625–8. doi: 10.1016/j.tibs.2003.11.001

13. Sun Y, Chao J-R, Xu W, Pourpak A, Boyd K, Moshiach S, et al. Mlf1 is a proapoptotic antagonist of hop complex-mediated survival. *Biochim Et Biophys Acta Mol Cell Res* (2017) 1864(4):719–27. doi: 10.1016/j.bbamcr.2017.01.016

14. Ohno K, Takahashi Y, Hirose F, Inoue YH, Taguchi O, Nishida Y, et al. Characterization of a drosophila homologue of the human Myelodysplasia/Myeloid leukemia factor (Mlf). *Gene* (2000) 260(1-2):133–43. doi: 10.1016/S0378-1119(00)00447-9

15. Hitzler JK, Witte DP, Jenkins NA, Copeland NG, Gilbert DJ, Naeve CW, et al. Cdna cloning, expression pattern, and chromosomal localization of Mlf1, murine homologue of a gene involved in myelodysplasia and acute myeloid leukemia. *Am J Pathol* (1999) 155(1):53–9. doi: 10.1016/S0002-9440(10)65098-5

16. Martin-Lannerée S, Lasbleiz C, Sanial M, Fouix S, Besse F, Tricoire H, et al. Characterization of the drosophila myeloid leukemia factor. *Genes To Cells: Devoted To Mol Cell Mech* (2006) 11(12):1317–35. doi: 10.1111/j.1365-2443.2006.01023.x

17. Sugano W, Ohno K, Yoneda-Kato N, Kato J-Y, Yamaguchi M. The myeloid leukemia factor interacts with Cop9 signalosome subunit 3 in drosophila melanogaster. *FEBS J* (2008) 275(3):588–600. doi: 10.1111/j.1742-4658.2007.06229.x

18. Yanai H, Yoshioka Y, Yoshida H, Nakao Y, Plessis A, Yamaguchi M. Drosophila myeloid leukemia factor acts with dref to activate the jnk signaling pathway. *Oncogenesis* (2014) 3:e98. doi: 10.1038/oncsis.2014.13

19. Fouix S, Martin-Lannerée S, Sanial M, Morla L, Lamour-Isnard C, Plessis A. Over-expression of a novel nuclear interactor of suppressor of fused, the drosophila Myelodysplasia/Myeloid leukaemia factor, induces abnormal morphogenesis associated with increased apoptosis and DNA synthesis. *Genes To Cells* (2003) 8(11):897–911. doi: 10.1046/j.1365-2443.2003.00685.x

20. Williams JH, Daly LN, Ingley E, Beaumont JG, Tilbrook PA, Lalonde JP, et al. Hls7, a hemopoietic lineage switch gene homologous to the leukemia-inducing gene Mlf1. *EMBO J* (1999) 18(20):5559–66. doi: 10.1093/emboj/18.20.5559

21. Winteringham LN, Kobelke S, Williams JH, Ingley E, Klinken SP. Myeloid leukemia factor 1 inhibits erythropoietin-induced differentiation, cell cycle exit and P27kip1 accumulation. *Oncogene* (2004) 23(29):5105–9. doi: 10.1038/sj.onc.1207661

22. Feng X-W, Huo L-J, Sun J-J, Xu J-D, Niu G-J, Wang J-X, et al. Myeloid leukemia factor functions in anti-wssv immune reaction of kuruma shrimp, marsupenaeus japonicus. *Fish Shellfish Immunol* (2017) 70:416–25. doi: 10.1016/j.fsi.2017.09.028

23. Feng XW, Huo LJ, Shi XZ. Myeloid leukemia factor participates in the antibacterial immune reaction of kuruma shrimp marsupenaeus japonicus. *Invertebrate Survival J* (2018) 15:203–9. doi: 10.25431/1824-307X/isj.v15i1.203-209

24. Sun Y, Fu A, Xu W, Chao J-R, Moshiach S, Morris SW. Myeloid leukemia factor 1 interfered with bcl-xl to promote apoptosis and its function was regulated by 14-3-3. *J Physiol Biochem* (2015) 71(4):807–21. doi: 10.1007/s13105-015-0445-5

25. Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol Cell Proteomics* (2014) 13(2):397–406. doi: 10.1074/mcp.M113.035600

26. Ashkenazi A, Dixit VM. Death receptors: Signaling and modulation. *Science* (1998) 281(5381):1305–8. doi: 10.1126/science.281.5381.1305

27. Yoneda-Kato N, Fukuhara S, Kato J. Apoptosis induced by the myelodysplastic syndrome-associated npm-Mlf1 chimeric protein. *Oncogene* (1999) 18(25):3716–24. doi: 10.1038/sj.onc.1202711

28. Falini B, Nicoletti I, Bolli N, Martelli MP, Liso A, Gorello P, et al. Translocations and mutations involving the nucleophosmin (Npm1) gene in lymphomas and leukemias. *Haematologica* (2007) 92(4):519–32. doi: 10.3324/haematol.11007

29. Yoneda-Kato N, Tomoda K, Umehara M, Arata Y, Kato J-Y. Myeloid leukemia factor 1 regulates P53 by suppressing Cop1 *Via* Cop9 signalosome subunit 3. *EMBO J* (2005) 24(9):1739–49. doi: 10.1038/sj.emboj.7600656

30. Cui Y, Guo G. Immunomodulatory function of the tumor suppressor P53 in host immune response and the tumor microenvironment. *Int J Mol Sci* (2016) 17(11):1942. doi: 10.3390/ijms17111942

31. Daver NG, Maiti A, Kadia TM, Vyas P, Majeti R, Wei AH, et al. Tp53-mutated myelodysplastic syndrome and acute myeloid leukemia: Biology, current therapy, and future directions. *Cancer Discovery* (2022) 12(11):2516–29. doi: 10.1158/2159-8290.CD-22-0332

32. Lim R, Winteringham LN, Williams JH, McCulloch RK, Ingley E, Tiao JYH, et al. Madm, a novel adaptor protein that mediates phosphorylation of the 14-3-3 binding site of myeloid leukemia factor 1. *J Biol Chem* (2002) 277(43):40997–1008. doi: 10.1074/jbc.M206041200

33. Molzan M, Weyand M, Rose R, Ottmann C. Structural insights of the Mlf1/14-3-3 interaction. *FEBS J* (2012) 279(4):563–71. doi: 10.1111/j.1742-4658.2011.08445.x

34. Peng CY, Graves PR, Thoma RS, Wu Z, Shaw AS, Piwnica-Worms H. Mitotic and G2 checkpoint control: Regulation of 14-3-3 protein binding by phosphorylation of Cdc25c on serine-216. *Sci (New York NY)* (1997) 277(5331):1501–5. doi: 10.1126/science.277.5331.1501

35. Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ. Serine phosphorylation of death agonist bad in response to survival factor results in binding to 14-3-3 not bcl-X(L). *Cell* (1996) 87(4):619–28. doi: 10.1016/S0092-8674(00)81382-3

36. Sluchanko NN. Recent advances in structural studies of 14-3-3 protein complexes. *Adv In Protein Chem Struct Biol* (2022) 130:289–324. doi: 10.1016/bs.apcsb.2021.12.004

37. Mas-Bargues C, Borrás C, Viña J. Bcl-xl as a modulator of senescence and aging. *Int J Mol Sci* (2021) 22(4):1527. doi: 10.3390/ijms22041527

38. Molzan M, Ottmann C. Subcellular localization of full-length human myeloid leukemia factor 1 (Mlf1) is independent of 14-3-3 proteins. *Cell Mol Biol Lett* (2013) 18(1):137–48. doi: 10.2478/s11658-012-0044-1

39. Marenda M, Lazarova E, Gilbert N. The role of saf-a/Hnrnp U in regulating chromatin structure. *Curr Opin In Genet Dev* (2022) 72:38–44. doi: 10.1016/j.gde.2021.10.008

40. Nozawa R-S, Boteva L, Soares DC, Naughton C, Dun AR, Buckle A, et al. Saf-a regulates interphase chromosome structure through oligomerization with chromatin-associated rnas. *Cell* (2017) 169(7):1214–1227.e18. doi: 10.1016/j.cell.2017.05.029

41. Connolly C, Takahashi S, Miura H, Hiratani I, Gilbert N, Donaldson AD, et al. Saf-a promotes origin licensing and replication fork progression to ensure robust DNA replication. *J Cell Sci* (2022) 135(2):jcs258991. doi: 10.1242/jcs.258991

42. Bras S, Martin-Lannerée S, Gobert V, Augé B, Breig O, Sanial M, et al. Myeloid leukemia factor is a conserved regulator of runx transcription factor activity involved in hematopoiesis. *Proc Natl Acad Sci United States America* (2012) 109(13):4986–91. doi: 10.1073/pnas.1117317109

43. Miller M, Chen A, Gobert V, Augé B, Beau M, Burlet-Schiltz O, et al. Control of runx-induced repression of notch signaling by mlf and its partner dnaj-1 during drosophila hematopoiesis. *PloS Genet* (2017) 13(7):e1006932. doi: 10.1371/journal.pgen.1006932

44. Lin T-C. Runx1 and cancer. *Biochim Et Biophys Acta Rev On Cancer* (2022) 1877(3):188715. doi: 10.1016/j.bbcan.2022.188715

45. Nakamae I, Kato J-Y, Yokoyama T, Ito H, Yoneda-Kato N. Myeloid leukemia factor 1 stabilizes tumor suppressor C/Ebpα to prevent Trib1-driven acute myeloid leukemia. *Blood Adv* (2017) 1(20):1682–93. doi: 10.1182/bloodadvances.2017007054

46. Pabst T, Mueller BU, Harakawa N, Schoch C, Haferlach T, Behre G, et al. Aml1-eto downregulates the granulocytic differentiation factor C/Ebpalpha in T (8,21) myeloid leukemia. *Nat Med* (2001) 7(4):444–51. doi: 10.1038/86515

47. Dedhia PH, Keeshan K, Uljon S, Xu L, Vega ME, Shestova O, et al. Differential ability of tribbles family members to promote degradation of C/Ebpalpha and induce acute myelogenous leukemia. *Blood* (2010) 116(8):1321–8. doi: 10.1182/blood-2009-07-229450

48. Hashimoto A, Sarker D, Reebye V, Jarvis S, Sodergren MH, Kossenkov A, et al. Upregulation of C/Ebpα inhibits suppressive activity of myeloid cells and potentiates antitumor response in mice and patients with cancer. *Clin Cancer Res* (2021) 27(21):5961–78. doi: 10.1158/1078-0432.CCR-21-0986

49. Nakamae I, Kato JY, Yokoyama T, Ito H, Yoneda-Kato N. Myeloid leukemia factor 1 stabilizes tumor suppressor C/Ebpalpha to prevent Trib1-driven acute myeloid leukemia. *Blood Adv* (2017) 1(20):1682–93. doi: 10.1182/bloodadvances.2017007054

50. Hanissian SH, Akbar U, Teng B, Janjetovic Z, Hoffmann A, Hitzler JK, et al. Cdna cloning and characterization of a novel gene encoding the Mlf1-interacting protein Mlf1ip. *Oncogene* (2004) 23(20):3700–7. doi: 10.1038/sj.onc.1207448

51. Minoshima Y, Hori T, Okada M, Kimura H, Haraguchi T, Hiraoka Y, et al. The constitutive centromere component cenp-50 is required for recovery from spindle damage. *Mol Cell Biol* (2005) 25(23):10315–28. doi: 10.1128/MCB.25.23.10315-10328.2005

52. Cope MJ, Whisstock J, Rayment I, Kendrick-Jones J. Conservation within the myosin motor domain: Implications for structure and function. *Structure* (1996) 4(8):969–87. doi: 10.1016/S0969-2126(96)00103-7

53. Feng G, Zhang T, Liu J, Ma X, Li B, Yang L, et al. Mlf1ip promotes normal erythroid proliferation and is involved in the pathogenesis of polycythemia Vera. *FEBS Lett* (2017) 591(5):760–73. doi: 10.1002/1873-3468.12587

54. Raimondi SC, Dubé ID, Valentine MB, Mirro J, Watt HJ, Larson RA, et al. Clinicopathologic manifestations and breakpoints of the T (3,5) in patients with acute nonlymphocytic leukemia. *Leukemia* (1989) 3(1):42–7.

55. Xu Y, Zhang L, Wang Q, Zheng M. Overexpression of Mlf1ip promotes colorectal cancer cell proliferation through Brca1/Akt/P27 signaling pathway. *Cell Signal* (2022) 92:110273. doi: 10.1016/j.cellsig.2022.110273

56. Wang X, Marcinkiewicz M, Gatain Y, Bouchard M, Mao J, Tremblay M, et al. Investigation of tissue-specific expression and functions of Mlf1-ip during development and in the immune system. *PloS One* (2013) 8(5):e63783. doi: 10.1371/journal.pone.0063783

57. Suzuki Y, Demoliere C, Kitamura D, Takeshita H, Deuschle U, Watanabe T. Hax-1, a novel intracellular protein, localized on mitochondria, directly associates with Hs1, a substrate of src family tyrosine kinases. *J Immunol* (1997) 158(6):2736–44. doi: 10.4049/jimmunol.158.6.2736

58. Trebinska A, Rembiszewska A, Ciosek K, Ptaszynski K, Rowinski S, Kupryjanczyk J, et al. Hax-1 overexpression, splicing and cellular localization in tumors. *BMC Cancer* (2010) 10:76. doi: 10.1186/1471-2407-10-76

59. Dong Q, Li D, Zhao H, Zhang X, Liu Y, Hu Y, et al. Anti-apoptotic hax-1 suppresses cell apoptosis by promoting c-abl kinase-involved ros clearance. *Cell Death Dis* (2022) 13(4):298. doi: 10.1038/s41419-022-04748-2

60. Huang D, Liu C, Sun X, Sun X, Qu Y, Tang Y, et al. Crl4 and Usp11 oppositely regulate the stability of myeloid leukemia factors (Mlfs). *Biochem Biophys Res Commun* (2020) 529(2):127–32. doi: 10.1016/j.bbrc.2020.05.186

61. Liao Y, Zhou D, Wang P, Yang M, Jiang N. Ubiquitin specific peptidase 11 as a novel therapeutic target for cancer management. *Cell Death Discovery* (2022) 8(1):292. doi: 10.1038/s41420-022-01083-5

62. Guo T, Tang H, Yuan Z, Zhang E, Wang X. The dual role of Usp11 in cancer. *J Oncol* (2022) 2022:9963905. doi: 10.1155/2022/9963905

63. Sun W, Zhang K, Zhang X, Lei W, Xiao T, Ma J, et al. Identification of differentially expressed genes in human lung squamous cell carcinoma using suppression subtractive hybridization. *Cancer Lett* (2004) 212(1):83–93. doi: 10.1016/j.canlet.2004.03.023

64. Chen J, Guo L, Peiffer DA, Zhou L, Chan OTM, Bibikova M, et al. Genomic profiling of 766 cancer-related genes in archived esophageal normal and carcinoma tissues. *Int J Cancer* (2008) 122(10):2249–54. doi: 10.1002/ijc.23397

65. Li Z-F, Wu X, Jiang Y, Liu J, Wu C, Inagaki M, et al. Non-pathogenic protein aggregates in skeletal muscle in Mlf1 transgenic mice. *J Neurol Sci* (2008) 264(1-2):77–86. doi: 10.1016/j.jns.2007.07.027

66. Banck MS, Kanwar R, Kulkarni AA, Boora GK, Metge F, Kipp BR, et al. The genomic landscape of small intestine neuroendocrine tumors. *J Clin Invest* (2013) 123 (6):2502–8. doi: 10.1172/JCI67963

67. Banerjee M, Datta M, Bhattacharyya NP. Modulation of mutant huntingtin aggregates and toxicity by human myeloid leukemia factors. *Int J Biochem Cell Biol* (2017) 82:1–9. doi: 10.1016/j.biocel.2016.11.008

68. McDaniel LD, Conkrite KL, Chang X, Capasso M, Vaksman Z, Oldridge DA, et al. Common variants upstream of Mlf1 at 3q25 and within cpz at 4p16 associated with neuroblastoma. *PloS Genet* (2017) 13(5):e1006787. doi: 10.1371/journal.pgen.1006787

69. Rangrez AY, Pott J, Kluge A, Frauen R, Stiebeling K, Hoppe P, et al. Myeloid leukemia factor-1 is a novel modulator of neonatal rat cardiomyocyte proliferation. *Biochim Et Biophys Acta Mol Cell Res* (2017) 1864(4):634–44. doi: 10.1016/j.bbamcr.2017.01.004

70. Dawson MA, Kouzarides T. Cancer epigenetics: From mechanism to therapy. *Cell* (2012) 150(1):12–27. doi: 10.1016/j.cell.2012.06.013

71. Ushijima T, Sasako M. Focus on gastric cancer. *Cancer Cell* (2004) 5(2):121–5. doi: 10.1016/S1535-6108(04)00033-9

72. Yamashita S, Tsujino Y, Moriguchi K, Tatematsu M, Ushijima T. Chemical genomic screening for methylation-silenced genes in gastric cancer cell lines using 5-Aza-2'-Deoxycytidine treatment and oligonucleotide microarray. *Cancer Sci* (2006) 97 (1):64–71. doi: 10.1111/j.1349-7006.2006.00136.x

73. Shi J, Zhang G, Yao D, Liu W, Wang N, Ji M, et al. Prognostic significance of aberrant gene methylation in gastric cancer. *Am J Cancer Res* (2012) 2(1):116–29.

74. Watanabe Y, Kim HS, Castoro RJ, Chung W, Estecio MRH, Kondo K, et al. Sensitive and specific detection of early gastric cancer with DNA methylation analysis of gastric washes. *Gastroenterology* (2009) 136(7):2149–58. doi: 10.1053/j.gastro.2009.02.085

75. Zhao S, Dong X, Ni X, Li L, Lu X, Zhang K, et al. Exploration of a novel prognostic risk signature and its effect on the immune response in nasopharyngeal carcinoma. *Front In Oncol* (2021) 11:709931. doi: 10.3389/fonc.2021.709931

76. Hoh RA, Stowe TR, Turk E, Stearns T. Transcriptional program of ciliated epithelial cells reveals new cilium and centrosome components and links to human disease. *PloS One* (2012) 7(12):e52166. doi: 10.1371/journal.pone.0052166

77. Leshchenko VV, Kuo P-Y, Shaknovich R, Yang DT, Gellen T, Petrich A, et al. Genomewide DNA methylation analysis reveals novel targets for drug development in mantle cell lymphoma. *Blood* (2010) 116(7):1025–34. doi: 10.1182/blood-2009-12-257485

78. Mansur MB, van Delft FW, Colman SM, Furness CL, Gibson J, Emerenciano M, et al. Distinctive genotypes in infants with T-cell acute lymphoblastic leukaemia. *Br J Haematol* (2015) 171(4):574–84. doi: 10.1111/bjh.13613

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 05 April 2023
DOI 10.3389/fonc.2023.1146108

OPEN ACCESS

EDITED BY
Massimo Breccia,
Sapienza University of Rome, Italy

REVIEWED BY
Elisabetta Abruzzese,
University of Rome Tor Vergata, Italy
Junbin Huang,
Seventh Affiliated Hospital, Sun Yat-sen University, China

*CORRESPONDENCE
Yu Zhang
zhangwkp@163.com
Weiming Li
lee937@126.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Hematologic Malignancies, a section of the journal Frontiers in Oncology

RECEIVED 04 February 2023
ACCEPTED 23 March 2023
PUBLISHED 05 April 2023

CITATION
Cheng F, Li Q, Cui Z, Hong M, Li W and Zhang Y (2023) Dose optimization strategy of the tyrosine kinase inhibitors imatinib, dasatinib, and nilotinib for chronic myeloid leukemia: From clinical trials to real-life settings.
Front. Oncol. 13:1146108.
doi: 10.3389/fonc.2023.1146108

Dose optimization strategy of the tyrosine kinase inhibitors imatinib, dasatinib, and nilotinib for chronic myeloid leukemia: From clinical trials to real-life settings

Fang Cheng[1,2,3†], Qiang Li[1,2†], Zheng Cui[1,2], Mei Hong[3], Weiming Li[4*] and Yu Zhang[1,2*]

[1]Department of Pharmacy, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2]Hubei Province Clinical Research Center for Precision Medicine for Critical Illness, Wuhan, China, [3]Hubei Key Laboratory of Biological Targeted Therapy, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [4]Department of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

With the advent of tyrosine kinase inhibitors (TKIs), the treatment prospects of chronic myeloid leukemia (CML) have changed markedly. This innovation can lengthen the long-term survival of patients suffering from CML. However, long-term exposure to TKIs is accompanied by various adverse events (AEs). The latter affect the quality of life and compliance of patients with CML, and may lead to serious disease progression (and even death). Recently, increasing numbers of patients with CML have begun to pursue a dose optimization strategy. Dose optimization may be considered at all stages of the entire treatment, which includes dose reduction and discontinuation of TKIs therapy. In general, reduction of the TKI dose is considered to be an important measure to reduce AEs and improve quality of life on the premise of maintaining molecular responses. Furthermore, discontinuation of TKIs therapy has been demonstrated to be feasible and safe for about half of patients with a stable optimal response and a longer duration of TKI treatment. This review focuses mainly on the latest research of dose optimization of imatinib, dasatinib, and nilotinib in CML clinical trials and real-life settings. We consider dose reduction in newly diagnosed patients, or in optimal response, or for improving AEs, either as a prelude to treatment-free remission (TFR) or as maintenance therapy in those patients unable to discontinue TKIs therapy. In addition, we also focus on discontinuation of TKIs therapy and second attempts to achieve TFR.

KEYWORDS

tyrosine kinase inhibitors, dose optimization, chronic myeloid leukemia, dose reduction, treatment-free remission

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative tumor formed by clonal adult cases of leukemia (1). With the advent of tyrosine kinase inhibitors (TKIs) targeting BCR::ABL1, the therapeutic prospect of CML has changed markedly (2). The long-term survival of patients with CML in the chronic phase (CP) has become close to normal life expectancy (3). Imatinib, dasatinib and nilotinib are the most commonly used TKIs for CML patients in clinical practice in China.

Long-term treatment with TKIs is accompanied with various adverse events (AEs) that significantly affect the quality of life and compliance of patients with CML, and have the potential to cause significant disease progression and mortality. Severe AEs associated with second-generation TKIs have also been reported. These include pleural effusion (PE) and pulmonary hypertension induced by dasatinib (4, 5) as well as nilotinib-related dyslipidemia and arterial thrombosis (6, 7).

Recently, increasing numbers of patients with CML have begun to pursue a dose optimization strategy, which included dose reduction and discontinuation of TKIs therapy. Dose reduction of TKIs has been suggested to be safe and feasible, and to elicit an optimal response, in patients with CML. Also, the prevention and management of AEs must also be considered to improve patient compliance and reduce the risk of treatment interruption (8, 9). Fassoni and colleagues developed a patient data-based mathematical model which suggested that a reduction ≥50% of the full dose of a TKI did not exacerbate outcomes from long-term treatment (10). Importantly, the dose reduction of TKIs should be considered as early as possible, but the clinical benefit of this approach is controversial if chronic toxicity occurs, especially in some specific settings (11). Furthermore, some patients with a sustained deep molecular response (DMR, BCR::ABL1^{IS} ≤ 0.01%) can achieve relatively long-lasting safe discontinuation of TKIs therapy [i.e., treatment-free response (TFR)].

In recent years, several clinical trials and real-life practices have indicated that treatment discontinuation has become a new therapeutic goal for patients with CML who are stable and have a DMR (12, 13). However, about half of patients have molecular recurrence and need re-introduction of TKIs therapy. Imatinib was first applied to the treatment of CML two decades ago, and only 5%–10% of patients can maintain TFR (14). Eighty percent of patients continue to need long-term therapy with a TKI to achieve long-term survival (though 20% of them meet the conditions for treatment discontinuation) (15). Therefore, this review focuses mainly on the latest research on the dose optimization of the TKIs imatinib, dasatinib, and nilotinib in patients with CML. This information includes dose reduction (Table 1) and TFR (Table 2). In this way, we aim to provide important references for the formulation of individualized therapeutic regimen for patients with CML.

TABLE 1 Clinical trials evaluating different imatinib, dasatinib and nilotinib doses.

TKIs	Study	TKIs dose	Patients	Publication time
Imatinib	RIGHT trial (15)	800mg	115	2009
	Baccarani et.al (16)	800mg VS. 400mg	216	2009
	Cortes et.al (17)	800mg VS. 400mg	476	2010
	Michel et.al (18)	800mg VS. 400mg	422	2019
	Cervantes et.al (19)	300mg	246	2017
TDM-guide imatinib dose optimization	Lankheet et.al (20)	NR	109	2017
	Adeagbo et.al (21)	NR	126	2017
Dasatinib	DASISION (22)	100mg/day VS. <100mg/day	519	2017
	CA180-034 (23)	100mg qd *VS.* 140mg qd VS. 70mg bid *VS.* 50mg bid	670	2016
	Naqvi et.al (24)	50mg	81	2020
	DAVLEC (25)	20mg	52	2021
	Latagliata et.al (26)	100mg/day VS. <100mg/day	65	2016
	Iurlo et.al (27)	100mg/day VS. <100mg/day VS. >100mg/day	853	2018
TDM-guide imatinib dose optimization	Shin et.al (28)	NR	102	2021
	Rousselot et.al (29)	NR	287	2021
Nilotinib	ENESTnd (30)	300mg bid VS. 400mg bid	563	2016
	NILO-RED (31)	300/400mg bid VS. 300/400mg qd	67	2017
	ENESTswift (32)	300mg bid VS. 400mg bid	20	2018

TKIs, tyrosine kinase inhibitors; qd, once daily; bid, twice daily; NR, not report.

TABLE 2 Characteristics of TKIs discontinuation trials.

Study	N	TKI	Minimum TKIs duration(y)	Minimum DMR duration (y)	TFR rate	Resumed treatment
France in 2007 (33)	12	IM	1.875	UMRD≥2	50% in the first 5 months	positive BCR::ABL1 transcripts
STIM1 (34)	100	IM	3	UMRD≥2	43% at 6 months and 38% at 60 months	Significant increase of 1-log or loss of MMR
A-STIM (35)	80	IM	3	MR4≥2	64% at 24 months and 61% at 36 months	Loss of MMR, UMRD
STIM2 (36)	124	IM	3	DMR≥2	61.2% at 12 months	Loss of MMR
TWISTER (37)	40	IM	3	UMRD≥2	47.1% at 24 months	Loss of UMRD
KID (38)	90	IM	2	MR4.5>2	62.2% at 12 months and 58.5% at 24 months	Loss of MMR
ISAV (39)	108	IM	2	UMRD≥1	48% at 36 months	Loss of MMR
DOMEST (40)	99	IM	2	MR4≥2	70% at 6 months, 68% at 12 months, and 64% at 24 months	Loss of MR4
DADI (41)	63	DA	1	DMR≥2	49% at six months and 48% at 12 months	Loss of MR4
First-line DADI (42)	58	DA	3	DMR≥2	55% at 6 months	Loss of MR4
D-STOP (43)	54	DA	2	DMR≥2	62.9% at 1 year	Loss of MMR
DASFREE (44)	84	DA	2	MR4.5≥1	48% at 12 months and 46% at 24 months	Loss of MMR
ENESTfreedom (45)	190	NL	3	MR4.5≥2	51.6 at 48 weeks	Loss of MMR
STAT2 (46)	78	NL	2	MR4.5≥2	67.9% at 12 months and 62.8% at 24 months	Loss of MR4.5
ENESTop (47–50)	126	NL	3	MR4.5≥1	57.9% and 53.2% at 48 week and 96 week, 52.0% and 46% at 144 weeks and 192 weeks, 42.9% at 5 years	Loss of MMR or MR4
NILSt (51)	149	NL	NR	MR4.5≥2	The TFR rate was 60.9% at both 1 and 3 years	Loss of MR4.5
STOP-2G (52)	60	DA NL	3	MR4.5≥2	63.3% at 12 months and 53.7% at 48 months	Loss of MMR
LAST (53)	172	IM NL DA BO	3	MR4≥2	60.8% at 12 months	Loss of MMR
EURO-SKI (54)	755	IM NL DA	3	MR4≥1	61% at 6 months and 50% at 24 months	Loss of MMR
GIMEMA (55)	293	IM NL DA BO	7	DMR≥3	68% and 73% in imatinib and second-generation TKIs at 12 months and 62% at 34 months	Loss of MMR

TKI, tyrosine kinase inhibitor; IM, imatinib; DA, dasatinib; NL, nilotinib; BO, bosutinib; DMR, deep molecular response; MMR, major molecular response; TFR, treatment-free remission; UMRD, undetected minimum residual disease; MR, molecular response.

Dose reduction

Imatinib

Several clinical trials have explored the efficacy of high-dose imatinib (800 mg/day) treatment compared with standard-dose therapy (16–19). High-dose imatinib appeared to elicit a faster major molecular response (MMR, *BCR::ABL1*^{IS} ≤ 0.1%), but the prevalence of MMR was similar at 1 years or 2 years between the two groups being assessed. However, an increased prevalence of severe AEs and worse compliance by patients was reported in the high-dose-imatinib arm. As a result, patients received imatinib at 800 mg/day initially which was later reduced to 400 mg/day. In addition, dose reduction was accompanied by a reduction in the prevalence of AEs and medical costs, and could improve patient compliance. Claudiani and coworkers (8) conducted a retrospective study of 246 patients with CML receiving treatment with a lower dose of a TKI (imatinib, n = 90; dasatinib, n = 88; nilotinib, n = 81; bosutinib, n = 39) after achievement of MMR because of intolerable AEs. A "lower dose" of a TKI (mg/day) was defined as 200 or 300 for imatinib, 70, 50, 40 or ≤20 for dasatinib; 400, 300, or ≤200 for nilotinib; 300, 200, or <200 for bosutinib. Their findings suggested that dose reduction should not be recommended as routine clinical practice, but could be an acceptable and safe option for patients who cannot tolerate a standard dose of a TKI. Cervantes and collaborators (56) found that a reduction to 300 mg/day in 43

patients with CML who received imatinib (400 mg/day) initially as first-line treatment with a sustained DMR improved tolerability significantly and maintained a DMR continuously.

Therapeutic drug monitoring (TDM) is gradually becoming a practical tool to achieve individualized medicine for patients receiving targeted drugs (57). Peng and colleagues showed that fixed-dose imatinib showed high inter-patient variability to plasma exposure in patients with CML (58). An imatinib concentration in plasma >1000 ng/mL in patients with CML can lead to a beneficial clinical outcome (59, 60). Therefore, a TDM-based dose-adjustment strategy could improve the efficacy, and reduce the toxicity and medical cost, of imatinib therapy (20). In daily practice, Lankheet and colleagues (21) monitored the proportion of patients who reached the target trough concentration (C_{min}) of a TKI (imatinib, sunitinib, or pazopanib) after a TDM-based dose-adjustment strategy. The proportion of patients with the target C_{min} increased from 38% to 64%, which suggested that a TDM-based dose-adjustment strategy may be an effective strategy to enable patients who received a TKI to achieve the target C_{min}. The population pharmacokinetics of imatinib in patients with CML in Nigeria (61) showed that treatment with a standard dose of imatinib may not elicit the desired effect in most patients, and that exposure to low concentrations continuously might lead to drug resistance. They suggested the need for a TDM-guided dose-adjustment strategy of imatinib in this population. In summary, those data indicated that dose reduction could be a feasible and safe option for patients with a stable optimal response but who cannot tolerate a standard dose of imatinib (22). If possible, the imatinib concentration in plasma could be monitored to provide an important reference for the dose adjustment of imatinib.

Dasatinib

Several studies have explored the efficacy and safety of standard-dose dasatinib (100 mg/day) compared with low-dose therapy (<100 mg/day) in clinical trials and real-life settings. A retrospective analysis of the DASISION trial (23) revealed that dose reduction of dasatinib could maintain a superior prevalence of MMR while reducing the risk of dasatinib-related AEs. Of 65 patients with CML (age >65 years) receiving first-line treatment with dasatinib (100 mg/day VS. <100 mg/day),10 patients who required permanent drug withdrawal due to toxicity all received an initial dose of 100 mg/day (24). Iurlo and colleagues (25) retrospectively evaluated 853 CML patients who received dasatinib as first-line and second-line therapy (100 mg/day VS. <100 mg/day). A total of 196 episodes of PE (23.0%) were identified, and 70.4% of PE events were observed in patients who received 100 mg/day.

The CA180-034 study (26) enrolled patients with CML who were resistant and intolerant to imatinib and who were switched to dasatinib. The result of 7-year follow-up indicated that the clinical response at 100 mg/day was similar to that of 70-mg twice daily or 140 mg/day, and was more beneficial in terms of toxicity. Initial half-dose dasatinib therapy (50 mg/day) was suggested to be a safe option for newly diagnosed CP-CML patients. The clinical response and toxicity profile of initial treatment with half-dose dasatinib were more favorable compared with those documented in the DASISION trial (27). The DAVLEC study (28) suggested that low-dose dasatinib (20 mg/day) as the initial dose for older patients with newly diagnosed CP-CML was worthy of consideration.

Dasatinib exposure may be related to the clinical response and toxicity profile. The dose-limiting toxicities (DLTs) and clinical response of dasatinib were analyzed in patients with CP-CML at 17 hospitals in South Korea (62). Those results suggested that the initial dasatinib dose could be reduced to 80 mg/day according to dose adjusted for bodyweight (dose/BW) in South Korean CML patients, especially for those with lower BW. Mizuta and coworkers found that Patients experienced a higher risk of altered treatment with a higher C_{min}/D/W (dasatinib concentration adjusted by dose (g), and bodyweight (kg)) (63). Therefore, TDM-guided dose-adjustment strategy may have potential benefits for dasatinib treatment (29). Rousselot and co-workers evaluated whether TDM could reduce the prevalence of dasatinib-induced AEs at 12 months (30). All eligible patients received an initial dose of 100 mg/day of dasatinib, followed by assessment of the C_{min} of dasatinib. Patients were assigned randomly to a dose-reduction strategy (TDM) group and standard-dose strategy (control) group according to C_{min} ≥3 nmol/L. The cumulative prevalence of PE was reduced significantly in the TDM group (15% vs. 4%, 35% vs. 11%, and 39% vs.12% at 1, 2 and 3 years, respectively, p = 0.0094), whereas the prevalence of MMR was similar. A TDM-guided dose-adjustment strategy for dasatinib was feasible and resulted in a significant reduction in the incidence of PE events without impairing the MMR rate upon long-term treatment.

Nilotinib

The ENESTnd study (64) reported that nilotinib (400 mg, twice daily (bid) VS. 300 mg, bid) had equivalent efficacy, but high-dose therapy led to longer 5-year overall survival compared with imatinib. However, a higher prevalence of cardiovascular events was observed in the high-dose arm. Furthermore, the ENESTswift trial (31) suggested crossover with nilotinib (300 mg, bid) to be efficacious and well tolerated in most patients treated with nilotinib as second-line therapy. In the NILO-RED study (32), patients were recommended to receive dose adjustments to a lower-dose once-daily (qd) regimen after achieving a MMR with standard-dose nilotinib bid schedule (first-line 300 mg, bid; and second-line 400 mg, bid) solely in case of severe toxicity. Switching to a nilotinib qd regimen as maintenance therapy after achievement of MMR on standard-dose schedule is feasible and safe in CP-CML patients regardless of prior treatment history.

Discontinuation of TKIs therapy

Imatinib

In recent years, the experience of discontinuation of TKIs therapy in patients with CML has been reported worldwide. A

research team in France reported the first key study in 2007 (33). They suggested that a certain proportion of patients with molecular diseases not detected for ≥2 years could discontinue TKIs treatment and maintain molecular remission. One-hundred patients with CP-CML with undetectable molecular disease for ≥2 years were involved in the STIM1 trial (34). Molecular relapse was defined as a significant increase of 1-log reduction or loss of MMR in two consecutive samples. The prevalence of molecular recurrence-free survival was 43% at 6 months and 38% at 60 months, respectively. The cumulative prevalence of molecular recurrence was estimated to be ~60%. Furthermore, 55 patients who suffered molecular relapse achieved a faster DMR after resumption of TKIs treatment, and no patient had disease progression or mutations of the ABL1 kinase domain. Eighty patients with CML who received imatinib treatment were involved in the A-STIM study (35). Molecular relapse was defined as loss of MMR. The TFR prevalence was 64% at 24 months and 61% at 26 months, respectively. In the STIM2 study, 50% of patients continued to have TFR at 24 months (36). Forty patients with CP-CML enrolled in the TWISTER study in Australia (37) received imatinib treatment for >3 years and achieved 4.5-log reduction (MR4.5) for ≥2 years. Molecular relapse was defined as loss of MMR. At 2-year follow-up, the TFR prevalence was 47.1%, and most molecular relapses occurred in the first 4 months after treatment discontinuation. No patient had disease progression or mutations of the ABL1 kinase domain, and imatinib therapy was restarted successfully in all patients who suffered molecular relapse. The Korean Imatinib Discontinuation (KID) study (38) aimed to identify the predictors for safe and successful discontinuation of imatinib therapy, and 90 patients with CML were enrolled. The probability of achieving a sustained MMR at 12 months and 24 months was 62.2% and 58.5%, respectively. The ISAV study in Italy (39) enrolled CML patients with 112 who received imatinib treatment and who had undergone interferon-α treatment previously. If patients maintained MR4.5 for ≥2 years, then imatinib treatment was stopped. In that study, 50.9% of patients lost their MMR. The DOMEST trial (40) was a multicenter phase-II trial conducted in Japan to assess the clinical efficacy and safety of discontinuing imatinib therapy in patients with CML. Patients with sustained MR4.0 for ≥2 years were included. Molecular relapse was defined as the loss of MR4.0, and resumed dasatinib or other TKIs therapy. The prevalence of molecular recurrence-free survival was 69.6%, 68.6%, and 64.3% at 6, 12, and 24 months, respectively.

Lee and collaborators (65) aimed to identify the predictors for successful discontinuation of imatinib therapy, and 48 patients with CP-CML were enrolled. Patients were eligible for therapy cessation after receiving imatinib treatment for ≥3 years, and to maintain undetectable minimal residual disease (MRD) for ≥2 years. That study also included 20 patients who suffered a post-transplant relapse. Molecular relapse was defined as loss of UMRD or MMR. After a median follow-up of 15.8 months, nine patients lost UMRD and MMR in the non-transplant group, whereas all patients in the post-transplantation group maintained UMRD. Previous transplantation, imatinib duration, and UMRD duration were significantly associated with sustained molecular responses. Campiotti and coworkers (66) conducted a systematic review to assess the long-term safety of discontinuation of imatinib therapy in patients with CML. Approximately 50% of patients had TFR, and no death occurred 2 years after discontinuation of imatinib therapy. Those results indicated that discontinuation of imatinib therapy was feasible and safe for patients with CP-CML who had a sustained DMR.

Dasatinib

STOP-2G (52) was the first multicenter observational study to investigate the feasibility of discontinuation of second-generation TKIs therapy. The discontinuation criteria were patients with ≥3 years for first-line or subsequent lines of dasatinib or nilotinib therapy with sustained MR4.5 for >2 years. Molecular relapse was defined as loss of MMR. Sixty patients were enrolled and the follow-up was 12 months: 43.3% of patients suffered a relapse at a median of 4 months. The TFR prevalence at 1 year and 2 years was 63.3% and 53.6%, respectively. The DADI trial (41) in Japan included 63 patients with CML with a sustained DMR for >1 year. Molecular relapse was considered to be the loss of DMR at any time point. They found that 52.4% of patients experienced a relapse at a median follow-up of 20 months, and all patients regained DMR 6 months after resumption of dasatinib therapy. The first-line DADI trial (42) was a multicenter phase-II trial in 23 Japanese hospitals, and aimed to assess molecular relapse-free survival at 6 months after discontinuation therapy. Fifty-eight patients with CML received dasatinib as first-line treatment and had a sustained DMR for >1 year. Thirty-two patients maintained TFR at 6 months and TFR prevalence at 6 months was 55.2%. The D-STOP trial (43) explored the long-term outcome of 54 patients with CML who stopped dasatinib treatment after achieving a sustained DMR for ≥2 years. At a median follow-up of 16.2 months, 12 patients suffered molecular relapse. The TFR prevalence at 12 months and 36 months was 62.9% and 44.4%, respectively. The DASFREE study (44) enrolled 74 patients with CML who received dasatinib treatment for >2 years and maintained MR4.5 for ≥1 year. At 2-year follow-up, 51% of patients in the first-line-treatment arm and 42% in the second-line-treatment arm continued to have TFR. The prevalence of TFR was 44% for patients who were resistant or intolerant to first-line dasatinib treatment.

A meta-analysis was conducted in patients with CML under a stable DMR to assess the prevalence of TFR and the long-term safety of discontinuation of second-generation TKIs therapy (67). Five single-armed, prospective cohort studies were included, and 517 patients were enrolled. The overall estimated TFR prevalence at a follow-up of 12 months and 24 months was 57% and 53%, respectively. Molecular recurrence occurred mainly in first 12 months after discontinuation therapy. Investigators discovered that 96.5% of patients who resumed TKIs treatment after molecular relapse could achieve MMR rapidly. During 2-year follow-up, four patients died (including two non-CML-related deaths: one died from arterial hemorrhage during the consolidation phase, and the other death was due to heart failure).

Nilotinib

The phase-II ENESTFreedom trial (45) was the first to evaluate discontinuation of nilotinib therapy. It enrolled 215 patients who received first-line nilotinib treatment and had stable MR4.5 for ≥2 years. All patients continued to receive 1 year of consolidated nilotinib treatment after enrollment, and 190 patients underwent discontinuation of nilotinib treatment. 48.9% of patients maintained TFR at 96-week follow-up. Furthermore, TFR prevalence was closely associated with the Sokal score at the diagnosis (low risk: 61.3%; intermediate risk: 50.0%; high risk: 28.6%). At 5-year follow-up, 81 patients (42.6%) continued to have TFR, and 76 (40.0%) had MR4.5. Patients who suffered a relapse regained MMR (98.9%) and 92.3% had a DMR (68). The STAT2 trial (46) evaluated the efficacy of 2-year consolidated nilotinib (300 mg, bid) therapy for achieving TFR in CML patients with sustained DMR. Molecular relapse was defined as loss of DMR. Fifty-three patients continued to have TFR in the first 12 months among the 78 patients who were eligible to discontinue nilotinib therapy. The TFR prevalence at 3 years was estimated to be 62.8%. Of the 29 patients who suffered a relapse, 25 patients regained DMR after treatment resumption. The ENESTop study evaluated the TFR prevalence in patients with CP-CML treated with TKIs for >3 years and who achieved a sustained DMR after replacing imatinib with nilotinib. The TFR prevalence was 57.9% and 53.2% at 48 weeks and 96 weeks, respectively (47). Treatment-free survival was 52.0% and 46% at 144 weeks (48) and 192 weeks (49). At 5-year follow-up (50), 42.9% (54/126) of patients continued to have TFR. Of the 59 patients who lost the MMR or DMR and were re-introduced to nilotinib treatment, 98.3% regained the MMR, 94.9% regained MR4, and 93.2% regained MR4.5. Overall, AE rates decreased over the 5 years of TFR, and no patients suffered disease progression or CML-related death. The NILSt study (51) enrolled patients with DMR who received nilotinib consolidation therapy for ≤24 months, and who maintained MR4.5 proceeded to discontinuation of nilotinib treatment. Molecular relapse was defined as loss of MR4.5. Eighty-seven patients (58.4%) underwent discontinuation of nilotinib therapy. The TFR prevalence was 60.9% at 1 year and 3 years, respectively. The phase-II study GIMEMA CML 0307 (69) found that 24 (32.9%) patients with a stable DMR discontinued nilotinib treatment at 10-year follow-up, and the TFR prevalence at 2 years was 72.6%. The overall TFR prevalence was estimated to be 24.7%.

LAST (53) was a prospective clinical trial that included 172 patients with CML from 14 academic medical centers in the USA, which aimed to evaluate molecular relapse and patient-reported outcomes after discontinuation of TKIs treatment. Molecular relapse was defined as loss of the MMR. At a median follow-up of 41.6 months, 112 (65.5%) continued to maintained MMR, and 104 (60.8%) achieved TFR. A total of 755 patients were enrolled across Europe in the EURO-SKI trial (54): 94% of patients discontinued imatinib therapy, and 2% and 4% discontinued dasatinib therapy and nilotinib therapy, respectively. Patients received TKIs treatment for ≥3 years and had sustained MR4 for ≥1 year. Relapse-free survival was 61% at 6 months and 50% at 24 months. Disease progression was not observed. The optimal duration of sustained MR4 before treatment discontinuation was 3.1 years calculated by a prognostic model, with 61% probability of retaining MMR. The cutoff for imatinib therapy was 5.8 years, and the molecular relapse-free survival was 63%. Also, 86% of patients regained the MMR after restarting TKIs treatment. The GIMEMA trial enrolled 293 Italian patients with CP-CML who discontinued TKIs therapy (55). 72% patients received imatinib treatment, and the remainder of patients received second-generation TKIs before treatment discontinuation. At 12 months, the TFR prevalence was 68% in the imatinib arm and 73% in the second-generation-TKIs arm. At a median follow-up of 34 months, the overall estimated TFR prevalence was 62%, and disease progression did not occur.

Recently, several retrospective studies have assessed the safety of discontinuation of TKIs therapy outside of clinical trials. One research team (70) enrolled 236 patients with CML from 33 Spanish centers to evaluate the safety of discontinuation of TKIs treatment in a real-life setting. Overall, the TFR prevalence was 64% at 4 years, and no patients suffered disease progression. Most patients who experienced molecular relapse regained the DMR after resuming TKIs therapy for 3–5 months. Iino and coworkers (71) assessed the outcome of 21 patients with CML who discontinued TKIs treatment. The TFR prevalence at 2 years was 66.7%, and no patients experienced disease progression or died. A retrospective study demonstrated that discontinuation of TKIs therapy was safe (especially for patients with a stable DMR with a longer duration of TKI treatment) (72): the prevalence of molecular relapse was 25% in patients with a stable DMR and 85% in those with an unstable DMR. Overall, discontinuation of dasatinib or nilotinib therapy was feasible and safe for patients with a sustained DMR and a longer duration of TKI treatment in clinical trials and real-world settings.

Dose reduction before therapy discontinuation

The DESTINY study (73) aimed to evaluate the outcome of gradual dose reduction before TKIs discontinuation as well as the safety of TFR for patients with less deep (but stable) remission. In detail, patients from 20 UK hospitals were assigned to a MR4 group and MMR group. TKIs treatment was reduced to half of the standard dose (imatinib = 200 mg/day; dasatinib = 50 mg/day; nilotinib = 200 mg, bid) for 12 months, then discontinued for a further 24 months. Molecular relapse was defined as loss of MMR that necessitated resumption of TKIs treatment at the full dose. The primary endpoint was the proportion of patients who could first experience half-dose therapy for 1 year, and then stop treatment completely for a further 2 years, without losing the MMR. Of the 174 patients, 148, 10, and 16 were treated with imatinib, dasatinib, and nilotinib, respectively. Forty-nine patients were assigned to the MMR group and 125 to the MR4 group. Three patients in the DMR group and nine patients in the MMR group suffered molecular relapse during dose reduction. Eighty-four (67%) patients achieved the primary endpoint and recurrence-free survival was 72% in the

DMR group. Sixteen (33%) patients achieved the primary endpoint and recurrence-free survival was 36% in the MMR group. No patients suffered disease progression and two patients died due to unrelated causes. All patients who relapsed regained the MMR within 5 months of resumption of TKIs therapy.

In a retrospective analysis in 2020 (74), 26 patients with CML received a low-dose TKI before discontinuation, and the TFR prevalence at 5 years was 47.5% in the full-dose group and 58.8% in the low-dose group. That study suggested that low-dose TKI regimens before discontinuation of TKI therapy did not impair the chance of achieving TFR in patients with CML. An investigation on the attitude of hematologists practicing in Italy towards a low-dose TKI regimen and its impact on TFR was undertaken (75). Results showed that 64.4% of hematologists believed that TFR should not be affected by low-dose TKIs. Furthermore, this strategy was applied to 194 patients with CML. Except for three patients, all patients reached a DMR with a median treatment duration of 61.0 months at the time of TFR. At a median follow-up of 29.2 months, 138 (71.1%) patients continued to have TFR, and the TFR prevalence was improved significantly after dose reduction due to AEs. However, outside of clinical trials, one-third of Italian hematologists continued to harbor doubts about the safety of TFR after patients received a low dose of TKIs. Interestingly, only 28.9% of patients suffered molecular relapse, which was lower than that reported in the standard dose therapy. That survey suggested that TFR may be an effective and safe option, even in patients who receive treatment with low-dose TKIs. Those findings suggest that low-dose TKIs do not impair the opportunity to achieve TFR. However, more prospective and multicenter clinical trials must be undertaken to explore the efficacy and safety for patients receiving low-dose TKIs before discontinuation of TKI therapy.

Second attempt to achieve TFR

A second attempt to achieve TFR may be considered for some patients. The details of trials focusing on a second opportunity to achieve TFR are shown in Table 3. Ross and collaborators (76) conducted a study on a second discontinuation for 12 patients who regained MR4.5 with restarted treatment after a first molecular relapse. At a median of 8.6 years follow-up, the TFR prevalence was 50%. Patients who relapsed after the first discontinuation of TKIs therapy and who regained a DMR were enrolled in the RE-STIM trial (77). The TFR prevalence after a second attempt at therapy discontinuation was 44.3% at 24 months, 38.5% at 36 months, and 33.2% at 48 months in 70 patients. In the TRAD trial (78), patients who suffered a relapse (loss of MR4 or MMR) after discontinuation of imatinib therapy were resumed on dasatinib therapy (100 mg/day). Patients who regained MR4 that was sustained for >1 year had a second attempt at achieving TFR. The TFR prevalence after a successful attempt at therapy discontinuation was 21.5% at 6 months. In the 2020 A-STIM study (79), 32 (49.2%) patients underwent a second attempt to achieve TFR. The TFR prevalence at the second attempt at treatment discontinuation was 35.8%. Although the TFR prevalence of the second therapy discontinuation was lower than that of the first treatment discontinuation (46.8%), the failure of the first treatment discontinuation did not preclude the success of the second treatment discontinuation. However, patients who lost the MMR rapidly after the first treatment discontinuation had a negligible chance of achieving TFR on a second occasion using TKIs therapy alone.

Switching TKIs

Switching TKIs are required if there are intolerable toxicities, failure to achieve treatment milestones, or a BCR::ABL1 mutation that leads to resistance to specific TKI, (80, 81). The change is mandatory and should be accompanied by BCR::ABL1 KD-mutations tests in cases of failure/resistance. In the absence of BCR::ABL1 KD-mutations, there are no definitive recommendation for any particular TKIs. The criteria for selection of the second-line TKIs are almost entirely patient-related and dependent upon comorbidities, age, and the toxicity of the first TKI. If there is a mutation for a specific TKI, further TKI selection should be select accordingly. In case of warning response, the change is optional, and dependent upon the patients' long-term treatment goals and personal factors (e.g., age, complications, tolerance and economic situation). In the case of treatment-related complications and intolerance, the decision to switch TKIs is in part subjective, dependent upon the patient, physician, supportive care, and also upon the clinical response levels. The choice of dose of converted TKIs must take into account the clinical response and tolerance of the patient, as well as the standard- or reduced-dose regimens.

Pediatrics CML

In addition to imatinib, dasatinib and nilotinib were approved recently for pediatric CML treatment, which has expanded the therapeutic options. Moreover, allogeneic stem cell transplantation suggest to be third-line treatment for most pediatric cases (82).

TABLE 3 Characteristics of secondary TFR trials.

Study	N	TFR rate	Resumed treatment
Ross et.al (76)	40	50% at a median of 8.6 years follow-up	Loss of MMR
RE-STIM (77)	70	44.3% at 24 months, 38.5% at 36 months and 33.2% at 48 months	Loss of MMR
TRAD (78)	25	21.5% at 6 months	Loss of MR4 or MMR
2020 A-STIM (79)	65	46.8% and 35.8% at 1 year and 3 year	Loss of MMR

MMR, major molecular response; TFR, treatment-free remission; UMRD, undetected minimum residual disease; MR, molecular response.

However, children are actively growing during TKIs treatment, so they develop unique AEs, such as growth disturbance (83). Currently, there are lacking of sufficient data on efficacy and safety to pediatric patients, TKI selection mostly to be reliant on the clinical effects observed in adults.

Some research teams based in European groups recommend a lower starting dose of imatinib in children with CP-CML (260–300 mg/m^2/day) (84). However, Children's Oncology Group CML Working Group suggested a higher dose of imatinib was also well tolerated (85), and the initial recommended dose is 340 mg/m^2/day (maximum to 600 mg). The initial dose of dasatinib is 400-100 mg/m^2 qd (maximum to 100 mg) in children with CP-CML (86), and 230 mg/m^2/dose bid for nilotinib (maximum single dose of 400 mg) (87). The dose should be recalculated every 3 months based on changes in body-weight or more frequently if required, and could be adjust on the basis of clinical response and tolerability, but the maximum dose should not be exceeded.

Limited evidence regarding discontinuation of TKIs therapy is available for pediatric CML, mostly in small studies and case series. The Japanese Pediatric Leukemia/Lymphoma Study Group (88) reported the first prospective pediatric discontinuation of TKIs trial in 22 patients with CP-CML who had been taking TKIs for >3 years and had sustained MMR (MR4.0) for >2 years. TFR at 12 months was 50%. Of seven pediatric patients who discontinued imatinib, two patients achieved TFR (85). The STOP IMAPED study enrolled 14 pediatric patients who were treated with imatinib for ≥3 years and sustained DMR for ≥2 years to discontinued imatinib, the TFR rate at 6 months was 28.6% (89). Millot and colleagues (90) reported a TFR rate of 56% at 36 months after discontinuation of imatinib in 18 pediatric patients with sustained DMR for ≥23.9 months. Shima and coworkers (91) evaluated the feasibility of discontinuation of TKIs in pediatric CML patients. Twenty-two patients were eligible to discontinue TKIs if they treated with TKIs for ≥3 years, and sustained MR4.0 for ≥2 years. Their results showed the TFR rate to be 50.0% at 12 months, and that all patients regained MR4.0 after resumption of TKIs therapy. Therefore, discontinuation of TKIs therapy in pediatric CML is not recommended outside of clinical trials, and more prospective studies in pediatric CML are needed.

Conclusions

Recently, increasing numbers of patients with CML have begun to pursue a dose optimization strategy, which included dose reduction and discontinuation of TKIs therapy. In the real-life settings, we will comprehensively consider the dose optimization strategy base on the treatment goal, clinical response, tolerance, and economic situation of patients. A dose-reduction regimen could allow for broader clinical use of TKIs (even in patients with comorbidities). For example, if the elderly patient with multiple comorbidities or is previously intolerant to other TKIs, we may suggest a half-dose of dasatinib treatment (50 mg/day). If conditions permit, the dose can also be adjusted according to blood concentration monitoring. For patients with sustained optimal clinical response (MMR or DMR), reducing TKIs dose can reduce AE and improve treatment compliance. The proposal of TFR as a possible final treatment endpoint should be discussed with patients (especially younger patients) at the diagnosis to achieve a DMR rapidly and improve long-term compliance. For patients with stable DMR and long duration of TKIs treatment, it is feasible and safe to stop TKI treatment. Patients who discontinued TKIs should follow the discontinuation standards recommended in ELN or NCCN guidelines. For patients with stable DMR who want to stop TKIs treatment but are afraid of relapse, we recommend to reduce TKIs dose before discontinuation of TKIs therapy. For patients who cannot achieve TFR, the TKIs dose must be reduced without affecting the clinical response. Importantly, patients who underwent dose optimization should be advised for more intensive molecular monitoring, especially during the first 6 months. Once the patients lose the optimal response, physicians should take measures immediately, such as resuming to standard-dose therapy, reintroducing TKIs treatment, or switching to other TKIs, etc. However, evidence for dose optimization in pediatrics CML is limited. Hence, evidence from novel, prospective clinical trials and real-life clinical practice are required to explore dose-optimization strategies, which may provide more promising options for CML treatment.

Author contributions

FC: Writing (original draft) and data curation. QL: Validation, data curation, and writing (original draft). ZC: Data curation. MH: Revision of the original draft. WL: Conceptualization, visualization, and data curation. YZ: Project administration and funding acquisition. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by Open fund of Hubei Key Laboratory of Biological Targeted Therapy (No. 2022swbx001).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Cortes J, Pavlovsky C, Saußele S. Chronic myeloid leukaemia. *Lancet* (2021) 398 (10314):1914–26. doi: 10.1016/s0140-6736(21)01204-6

2. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2020 update on diagnosis, therapy and monitoring. *Am J Hematol* (2020) 95(6):691–709. doi: 10.1002/ajh.25792

3. Rosti G, Castagnetti F, Gugliotta G, Baccarani M. Tyrosine kinase inhibitors in chronic myeloid leukaemia: Which, when, for whom? *Nat Rev Clin Oncol* (2017) 14 (3):141–54. doi: 10.1038/nrclinonc.2016.139

4. Nekoukar Z, Moghimi M, Salehifar E. A narrative review on adverse effects of dasatinib with a focus on pharmacotherapy of dasatinib-induced pulmonary toxicities. *Blood Res* (2021) 56(4):229–42. doi: 10.5045/br.2021.2021117

5. Weatherald J, Bondeelle L, Chaumais MC, Guignabert C, Savale L, Jaïs X, et al. Pulmonary complications of bcr-abl tyrosine kinase inhibitors. *Eur Respir J* (2020) 56 (4):2000279. doi: 10.1183/13993003.00279-2020

6. Wang Z, Jiang L, Yan H, Xu Z, Luo P. Adverse events associated with nilotinib in chronic myeloid leukemia: Mechanisms and management strategies. *Expert Rev Clin Pharmacol* (2021) 14(4):445–56. doi: 10.1080/17512433.2021.1894129

7. Cirmi S, El Abd A, Letinier L, Navarra M, Salvo F. Cardiovascular toxicity of tyrosine kinase inhibitors used in chronic myeloid leukemia: An analysis of the FDA adverse event reporting system database (FAERS). *Cancers (Basel)* (2020) 12(4):826. doi: 10.3390/cancers12040826

8. Claudiani S, Apperley JF, Szydlo R, Khan A, Nesr G, Hayden C, et al. TKI dose reduction can effectively maintain major molecular remission in patients with chronic myeloid leukaemia. *Br J Haematol* (2021) 193(2):346–55. doi: 10.1111/bjh.17286

9. Iurlo A, Cattaneo D, Bucelli C, Breccia M. Dose optimization of tyrosine kinase inhibitors in chronic myeloid leukemia: A new therapeutic challenge. *J Clin Med* (2021) 10(3):515. doi: 10.3390/jcm10030515

10. Fassoni AC, Baldow C, Roeder I, Glauche I. Reduced tyrosine kinase inhibitor dose is predicted to be as effective as standard dose in chronic myeloid leukemia: A simulation study based on phase III trial data. *Haematologica* (2018) 103(11):1825–1834. doi: 567 10.3324/haematol.2018.194522

11. Okamoto S, Ureshino H, Kawaguchi A, Miyazono M, Ikeda Y, Kimura S. Assessment of estimated glomerular filtration rate in patients with chronic myeloid leukemia following discontinuation of tyrosine kinase inhibitors. *Int J Hematol* (2020) 112(1):41–5. doi: 10.1007/s12185-020-02880-3

12. Atallah E, Sweet K. Treatment-free remission: the new goal in CML therapy. *Curr Hematol Malig Rep* (2021) 16(5):433–9. doi: 10.1007/s11899-021-00653-1

13. Breccia M, Scalzulli E, Pepe S, Colafigli G, Bisegna ML, Capriata M, et al. Emerging concepts for assessing and predicting treatment-free remission in chronic myeloid leukemia patients. *Expert Rev Hematol* (2022) 15(1):25–32. doi: 10.1080/17474086.2022.2018296

14. Cortes J, Rea D, Lipton JH. Treatment-free remission with first- and second-generation tyrosine kinase inhibitors. *Am J Hematol* (2019) 94(3):346–57. doi: 10.1002/ajh.25342

15. Saglio G, Gale RP. Prospects for achieving treatment-free remission in chronic myeloid leukaemia. *Br J Haematol* (2020) 190(3):318–27. doi: 10.1111/bjh.16506

16. Cortes JE, Kantarjian HM, Goldberg SL, Powell BL, Giles FJ, Wetzler M, et al. High-dose imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: high rates of rapid cytogenetic and molecular responses. *J Clin Oncol* (2009) 27(28):4754–9. doi: 10.1200/jco.2008.20.3869

17. Baccarani M, Rosti G, Castagnetti F, Haznedaroglu I, Porkka K, Abruzzese E, et al. Comparison of imatinib 400 mg and 800 mg daily in the front-line treatment of high-risk, Philadelphia-positive chronic myeloid leukemia: a European LeukemiaNet study. *Blood* (2009) 113(19):4497–504. doi: 10.1182/blood-2008-12-191254

18. Cortes JE, Baccarani M, Guilhot F, Druker BJ, Branford S, Kim DW, et al. Randomized, open-label study of daily imatinib mesylate 400 mg versus 800 mg in patients with newly diagnosed, previously untreated chronic myeloid leukemia in chronic phase using molecular end points: Tyrosine kinase inhibitor optimization and selectivity study. *J Clin Oncol* (2010) 28(3):424–30. doi: 10.1200/jco.2009.25.3724

19. Michel C, Burchert A, Hochhaus A, Saussele S, Neubauer A, Lauseker M, et al. Imatinib dose reduction in major molecular response of chronic myeloid leukemia: Results from the German chronic myeloid leukemia-study IV. *Haematologica* (2019) 104(5):955–62. doi: 10.3324/haematol.2018.206797

20. Clarke WA, Chatelut E, Fotoohi AK, Larson RA, Martin JH, Mathijssen RHJ, et al. Therapeutic drug monitoring in oncology: International association of therapeutic drug monitoring and clinical toxicology consensus guidelines for imatinib therapy. *Eur J Cancer* (2021) 157:428–40. doi: 10.1016/j.ejca.2021.08.033

21. Lankheet NAG, Desar IME, Mulder SF, Burger DM, Kweekel DM, van Herpen CML, et al. Optimizing the dose in cancer patients treated with imatinib, sunitinib and pazopanib. *Br J Clin Pharmacol* (2017) 83(10):2195–204. doi: 10.1111/bcp.13327

22. Westerdijk K, Desar IME, Steeghs N, van der Graaf WTA, van Erp NP. Imatinib, sunitinib and pazopanib: From flat-fixed dosing towards a pharmacokinetically guided personalized dose. *Br J Clin Pharmacol* (2020) 86(2):258–73. doi: 10.1111/bcp.14185

23. Cortes JE, Hochhaus A, Kantarjian HM, Guilhot F, Kota VK, Hughes TP, et al. Impact of dose reductions on 5-year efficacy in newly diagnosed patients with chronic myeloid leukemia in chronic phase (CML-CP) from DASISION. *J Clin Oncol* (2017) 35 (15_suppl):7051–. doi: 10.1200/JCO.2017.35.15_suppl.7051

24. Latagliata R, Stagno F, Annunziata M, Abruzzese E, Iurlo A, Guarini A, et al. Frontline dasatinib treatment in a "Real-life" cohort of patients older than 65 years with chronic myeloid leukemia. *Neoplasia* (2016) 18(9):536–40. doi: 10.1016/j.neo.2016.07.005

25. Iurlo A, Galimberti S, Abruzzese E, Annunziata M, Bonifacio M, Latagliata R, et al. Pleural effusion and molecular response in dasatinib-treated chronic myeloid leukemia patients in a real-life Italian multicenter series. *Ann Hematol* (2018) 97(1):95–100. doi: 10.1007/s00277-017-3144-1

26. Shah NP, Rousselot P, Schiffer C, Rea D, Cortes JE, Milone J, et al. Dasatinib in imatinib-resistant or -intolerant chronic-phase, chronic myeloid leukemia patients: 7-year follow-up of study CA180-034. *Am J Hematol* (2016) 91(9):869–74. doi: 10.1002/ajh.24423

27. Naqvi K, Jabbour E, Skinner J, Anderson K, Dellasala S, Yilmaz M, et al. Long-term follow-up of lower dose dasatinib (50 mg daily) as frontline therapy in newly diagnosed chronic-phase chronic myeloid leukemia. *Cancer* (2020) 126(1):67–75. doi: 10.1002/cncr.32504

28. Murai K, Ureshino H, Kumagai T, Tanaka H, Nishiwaki K, Wakita S, et al. Low-dose dasatinib in older patients with chronic myeloid leukaemia in chronic phase (DAVLEC): A single-arm, multicentre, phase 2 trial. *Lancet Haematol* (2021) 8(12): e902–e11. doi: 10.1016/s2352-3026(21)00333-1

29. He S, Bian J, Shao Q, Zhang Y, Hao X, Luo X, et al. Therapeutic drug monitoring and individualized medicine of dasatinib: Focus on clinical pharmacokinetics and pharmacodynamics. *Front Pharmacol* (2021) 12:797881. doi: 10.3389/fphar.2021.797881

30. Rousselot P, Mollica L, Guilhot J, Guerci A, Nicolini FE, Etienne G, et al. Dasatinib dose optimisation based on therapeutic drug monitoring reduces pleural effusion rates in chronic myeloid leukaemia patients. *Br J Haematol* (2021) 194(2):393–402. doi: 10.1111/bjh.17654

31. Hiwase D, Tan P, D'Rozario J, Taper J, Powell A, Irving I, et al. Efficacy and safety of nilotinib 300mg twice daily in patients with chronic myeloid leukemia in chronic phase who are intolerant to prior tyrosine kinase inhibitors: Results from the phase IIIb ENESTswift study. *Leuk Res* (2018) 67:109–15. doi: 10.1016/j.leukres.2018.02.013

32. Rea D, Cayuela J-M, Dulucq S, Etienne G. Molecular responses after switching from a standard-dose twice-daily nilotinib regimen to a reduced-dose once-daily schedule in patients with chronic myeloid leukemia: A real life observational study (NILO-RED). *Blood* (2017) 130:318. doi: 10.1182/blood.V130.Suppl_1.318.318

33. Rousselot P, Huguet F, Rea D, Legros L, Cayuela JM, Maarek O, et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. *Blood* (2007) 109(1):58–60. doi: 10.1182/blood-2006-03-011239

34. Etienne G, Guilhot J, Rea D, Rigal-Huguet F, Nicolini F, Charbonnier A, et al. Long-term follow-up of the French stop imatinib (STIM1) study in patients with chronic myeloid leukemia. *J Clin Oncol* (2017) 35(3):298–305. doi: 10.1200/jco.2016.68.2914

35. Rousselot P, Charbonnier A, Cony-Makhoul P, Agape P, Nicolini FE, Varet B, et al. Loss of major molecular response as a trigger for restarting tyrosine kinase inhibitor therapy in patients with chronic-phase chronic myelogenous leukemia who have stopped imatinib after durable undetectable disease. *J Clin Oncol* (2014) 32 (5):424–30. doi: 10.1200/jco.2012.48.5797

36. Nicolini FE, Dulucq S, Boureau L, Cony-Makhoul P, Charbonnier A, Escoffre-Barbe M, et al. Evaluation of residual disease and TKI duration are critical predictive factors for molecular recurrence after stopping imatinib first-line in chronic phase CML patients. *Clin Cancer Res* (2019) 25(22):6606–13. doi: 10.1158/1078-0432.Ccr-18-3373

37. Ross DM, Branford S, Seymour JF, Schwarer AP, Arthur C, Yeung DT, et al. Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER study. *Blood* (2013) 122(4):515–22. doi: 10.1182/blood-2013-02-483750

38. Lee SE, Choi SY, Song HY, Kim SH, Choi MY, Park JS, et al. Imatinib withdrawal syndrome and longer duration of imatinib have a close association with a lower molecular relapse after treatment discontinuation: the KID study. *Haematologica* (2016) 101(6):717–23. doi: 10.3324/haematol.2015.139899

39. Mori S, Vagge E, le Coutre P, Abruzzese E, Martino B, Pungolino E, et al. Age and dPCR can predict relapse in CML patients who discontinued imatinib: the ISAV study. *Am J Hematol* (2015) 90(10):910–4. doi: 10.1002/ajh.24120

40. Fujisawa S, Ueda Y, Usuki K, Kobayashi H, Kondo E, Doki N, et al. Feasibility of the imatinib stop study in the Japanese clinical setting: delightedly overcome CML expert stop TKI trial (DOMEST trial). *Int J Clin Oncol* (2019) 24(4):445–53. doi: 10.1007/s10147-018-1368-2

41. Imagawa J, Tanaka H, Okada M, Nakamae H, Hino M, Murai K, et al. Discontinuation of dasatinib in patients with chronic myeloid leukaemia who have maintained deep molecular response for longer than 1 year (DADI trial): A multicentre phase 2 trial. *Lancet Haematol* (2015) 2(12):e528–35. doi: 10.1016/s2352-3026(15)00196-9

42. Kimura S, Imagawa J, Murai K, Hino M, Kitawaki T, Okada M, et al. Treatment-free remission after first-line dasatinib discontinuation in patients with chronic myeloid leukaemia (first-line DADI trial): A single-arm, multicentre, phase 2 trial. *Lancet Haematol* (2020) 7(3):e218–e25. doi: 10.1016/s2352-3026(19)30235-2

43. Kumagai T, Nakaseko C, Nishiwaki K, Yoshida C, Ohashi K, Takezako N, et al. Discontinuation of dasatinib after deep molecular response for over 2 years in patients with chronic myelogenous leukemia and the unique profiles of lymphocyte subsets for successful discontinuation: A prospective, multicenter Japanese trial (D-STOP trial). *Blood* (2016) 128(22):791. doi: 10.1182/blood.V128.22.791.791

44. Shah NP, García-Gutiérrez V, Jiménez-Velasco A, Larson S, Saussele S, Rea D, et al. Dasatinib discontinuation in patients with chronic-phase chronic myeloid leukemia and stable deep molecular response: the DASFREE study. *Leuk Lymphoma* (2020) 61(3):650–9. doi: 10.1080/10428194.2019.1675879

45. Ross DM, Masszi T, Gómez Casares MT, Hellmann A, Stentoft J, Conneally E, et al. Durable treatment-free remission in patients with chronic myeloid leukemia in chronic phase following frontline nilotinib: 96-week update of the ENESTfreedom study. *J Cancer Res Clin Oncol* (2018) 144(5):945–54. doi: 10.1007/s00432-018-2604-x

46. Takahashi N, Nishiwaki K, Nakaseko C, Aotsuka N, Sano K, Ohwada C, et al. Treatment-free remission after two-year consolidation therapy with nilotinib in patients with chronic myeloid leukemia: STAT2 trial in Japan. *Haematologica* (2018) 103(11):1835–42. doi: 10.3324/haematol.2018.194894

47. Radich J, Mahon F-X, Hochhaus A, Hughes T, Ailawadhi S, Lipton J, et al. ENESTfreedom and ENESTop update: Durable treatment-free remission (TFR) at 96 weeks after nilotinib treatment cessation in patients with chronic myeloid leukemia in chronic phase (CML-CP). *Clin Lymphoma Myeloma Leukemia* (2017) 17:S314–S5. doi: 10.1016/j.clml.2017.07.115

48. Hughes TP, Boquimpani C, Takahashi N, Benyamini N, Clementino NCD, Shuvaev V, et al. ENESTop 144-week update: Long-term treatment-free remission (TFR) in patients with chronic myeloid leukemia in chronic phase (CML-CP) after stopping second-line nilotinib. *Clin Lymphoma Myeloma Leukemia* (2018) 18:S222. doi: 10.1016/j.clml.2018.07.092

49. Mahon F-X, Boquimpani C, Takahashi N, Benyamini N, Clementino NCD, Shuvaev V, et al. ENESTop 192-weeks results: Treatment-free remission (TFR) in patients with chronic myeloid leukemia in chronic phase (CML-CP) after stopping second-line nilotinib. *Clin Lymphoma Myeloma Leukemia* (2019) 19:S288–S9. doi: 10.1016/j.clml.2019.07.233

50. Hughes TP, Clementino NCD, Fominykh M, Lipton JH, Turkina AG, Moiraghi EB, et al. Long-term treatment-free remission in patients with chronic myeloid leukemia after second-line nilotinib: ENESTop 5-year update. *Leukemia* (2021) 35 (6):1631–42. doi: 10.1038/s41375-021-01260-y

51. Nagafuji K, Matsumura I, Shimose T, Kawaguchi T, Kuroda J, Nakamae H, et al. Cessation of nilotinib in patients with chronic myelogenous leukemia who have maintained deep molecular responses for 2 years: A multicenter phase 2 trial, stop nilotinib (NILSt). *Int J Hematol* (2019) 110(6):675–82. doi: 10.1007/s12185-019-02736-5

52. Rea D, Nicolini FE, Tulliez M, Guilhot F, Guilhot J, Guerci-Bresler A, et al. Discontinuation of dasatinib or nilotinib in chronic myeloid leukemia: Interim analysis of the STOP 2G-TKI study. *Blood* (2017) 129(7):846–54. doi: 10.1182/blood-2016-09-742205

53. Atallah E, Schiffer CA, Radich JP, Weinfurt KP, Zhang MJ, Pinilla-Ibarz J, et al. Assessment of outcomes after stopping tyrosine kinase inhibitors among patients with chronic myeloid leukemia: A nonrandomized clinical trial. *JAMA Oncol* (2021) 7 (1):42–50. doi: 10.1001/jamaoncol.2020.5774

54. Saussele S, Richter J, Guilhot J, Gruber FX, Hjorth-Hansen H, Almeida A, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): A prespecified interim analysis of a prospective, multicentre, non-randomised, trial. *Lancet Oncol* (2018) 19(6):747–57. doi: 10.1016/s1470-2045(18) 30192-x

55. Fava C, Rege-Cambrin G, Dogliotti I, Cerrano M, Berchialla P, Dragani M, et al. Observational study of chronic myeloid leukemia Italian patients who discontinued tyrosine kinase inhibitors in clinical practice. *Haematologica* (2019) 104(8):1589–96. doi: 10.3324/haematol.2018.205054

56. Cervantes F, Correa JG, Pérez I, García-Gutiérrez V, Redondo S, Colomer D, et al. Imatinib dose reduction in patients with chronic myeloid leukemia in sustained deep molecular response. *Ann Hematol* (2017) 96(1):81–5. doi: 10.1007/s00277-016-2839-z

57. Mueller-Schoell A, Groenland SL, Scherf-Clavel O, van Dyk M, Huisinga W, Michelet R, et al. Therapeutic drug monitoring of oral targeted antineoplastic drugs. *Eur J Clin Pharmacol* (2021) 77(4):441–64. doi: 10.1007/s00228-020-03014-8

58. Peng B, Lloyd P, Schran H. Clinical pharmacokinetics of imatinib. *Clin Pharmacokinet* (2005) 44(9):879–94. doi: 10.2165/00003088-200544090-00001

59. De Francia S, D'Avolio A, Ariaudo A, Pirro E, Piccione F, Simiele M, et al. Plasma and intracellular imatinib concentrations in patients with chronic myeloid leukemia. *Ther Drug Monit* (2014) 36(3):410–2. doi: 10.1097/ftd.0000000000000013

60. Cortes JE, Egorin MJ, Guilhot F, Molimard M, Mahon FX. Pharmacokinetic/pharmacodynamic correlation and blood-level testing in imatinib therapy for chronic myeloid leukemia. *Leukemia* (2009) 23(9):1537–44. doi: 10.1038/leu.2009.88

61. Adeagbo BA, Olugbade TA, Durosinmi MA, Bolarinwa RA, Ogungbenro K, Bolaji OO. Population pharmacokinetics of imatinib in nigerians with chronic myeloid leukemia: Clinical implications for dosing and resistance. *J Clin Pharmacol* (2017) 57 (12):1554–63. doi: 10.1002/jcph.953

62. Shin H, Ha JE, Zang DY, Kim SH, Do YR, Lee WS, et al. Appropriate starting dose of dasatinib based on analyses of dose-limiting toxicities and molecular responses in Asian patients with chronic myeloid leukemia. *Clin Lymphoma Myeloma Leuk* (2021) 21(6):e521–e9. doi: 10.1016/j.clml.2021.01.020

63. Mizuta S, Sawa M, Tsurumi H, Matsumoto K, Miyao K, Hara T, et al. Plasma concentrations of dasatinib have a clinical impact on the frequency of dasatinib dose reduction and interruption in chronic myeloid leukemia: an analysis of the DARIA 01 study. *Int J Clin Oncol* (2018) 23(5):980–8. doi: 10.1007/s10147-018-1300-9

64. Hochhaus A, Saglio G, Hughes TP, Larson RA, Kim DW, Issaragrisil S, et al. Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. *Leukemia* (2016) 30(5):1044–54. doi: 10.1038/leu.2016.5

65. Lee SE, Choi SY, Bang JH, Kim SH, Jang EJ, Byeun JY, et al. Predictive factors for successful imatinib cessation in chronic myeloid leukemia patients treated with imatinib. *Am J Hematol* (2013) 88(6):449–54. doi: 10.1002/ajh.23427

66. Campiotti L, Suter MB, Guasti L, Piazza R, Gambacorti-Passerini C, Grandi AM, et al. Imatinib discontinuation in chronic myeloid leukaemia patients with undetectable BCR-ABL transcript level: A systematic review and a meta-analysis. *Eur J Cancer* (2017) 77:48–56. doi: 10.1016/j.ejca.2017.02.028

67. Di Q, Deng H, Zhao Y, Li BY, Qin L. Second-generation tyrosine kinase inhibitor discontinuation in chronic myeloid leukemia patients with stable deep molecular response: A systematic review and a meta-analysis. *Comput Math Methods Med* (2021) 2021:3110622. doi: 10.1155/2021/3110622

68. Radich JP, Hochhaus A, Masszi T, Hellmann A, Stentoft J, Casares MTG, et al. Treatment-free remission following frontline nilotinib in patients with chronic phase chronic myeloid leukemia: 5-year update of the ENESTfreedom trial. *Leukemia* (2021) 35(5):1344–55. doi: 10.1038/s41375-021-01205-5

69. Gugliotta G, Castagnetti F, Breccia M, Levato L, Intermesoli T, D'Adda M, et al. Treatment-free remission in chronic myeloid leukemia patients treated front-line with nilotinib: 10-year follow-up of the GIMEMA CML 0307 study. *Haematologica* (2022) 107(10):2356–64. doi: 10.3324/haematol.2021.280175

70. Hernández-Boluda JC, Pereira A, Pastor-Galán I, Alvarez-Larrán A, Savchuk A, Puerta JM, et al. Feasibility of treatment discontinuation in chronic myeloid leukemia in clinical practice: Results from a nationwide series of 236 patients. *Blood Cancer J* (2018) 8(10):91. doi: 10.1038/s41408-018-0125-0

71. Iino M, Yamamoto T, Sakamoto Y. Outcomes of unplanned tyrosine kinase inhibitor discontinuation in patients with chronic myeloid leukemia: Retrospective analysis of real-world experience in a single institution. *Hematology* (2019) 24(1):355–61. doi: 10.1080/16078454.2019.1590964

72. Cerveira N, Loureiro B, Bizarro S, Correia C, Torres L, Lisboa S, et al. Discontinuation of tyrosine kinase inhibitors in CML patients in real-world clinical practice at a single institution. *BMC Cancer* (2018) 18(1):1245. doi: 10.1186/s12885-018-5167-y

73. Clark RE, Polydoros F, Apperley JF, Milojkovic D, Rothwell K, Pocock C, et al. De-escalation of tyrosine kinase inhibitor therapy before complete treatment discontinuation in patients with chronic myeloid leukaemia (DESTINY): A non-randomised, phase 2 trial. *Lancet Haematol* (2019) 6(7):e375–e83. doi: 10.1016/s2352-3026(19)30094-8

74. Cayssials E, Torregrosa-Diaz J, Gallego-Hernanz P, Tartarin F, Systchenko T, Maillard N, et al. Low-dose tyrosine kinase inhibitors before treatment discontinuation do not impair treatment-free remission in chronic myeloid leukemia patients: Results of a retrospective study. *Cancer* (2020) 126(15):3438–47. doi: 10.1002/cncr.32940

75. Iurlo A, Cattaneo D, Artuso S, Consonni D, Abruzzese E, Binotto G, et al. Treatment-free remission in chronic myeloid leukemia patients treated with low-dose TKIs: A feasible option also in the real-life. *A Campus CML Study Front Oncol* (2022) 12:839915. doi: 10.3389/fonc.2022.839915

76. Ross DM, Pagani IS, Shanmuganathan N, Kok CH, Seymour JF, Mills AK, et al. Long-term treatment-free remission of chronic myeloid leukemia with falling levels of residual leukemic cells. *Leukemia* (2018) 32(12):2572–9. doi: 10.1038/s41375-018-0264-0

77. Legros L, Nicolini FE, Etienne G, Rousselot P, Rea D, Giraudier S, et al. The TKI-free duration after a first discontinuation attempt that failed in CP CML patients is a predictive factor of TKI-free remission after a second attempt. *Blood* (2019) 134:28. doi: 10.1182/blood-2019-123719

78. Kim DDH, Busque L, Forrest DL, Savoie L, Bence-Bruckler I, Couban S, et al. Second attempt of TKI discontinuation with dasatinib for treatment-free remission after failing first attempt with imatinib: Treatment-free remission accomplished by dasatinib (TRAD) trial. *Blood* (2018) 132:787. doi: 10.1182/blood-2018-99-114656

79. Rousselot P, Loiseau C, Delord M, Cayuela JM, Spentchian M. Late molecular recurrences in patients with chronic myeloid leukemia experiencing treatment-free remission. *Blood Adv* (2020) 4(13):3034–40. doi: 10.1182/bloodadvances.2020001772

80. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. *Leukemia* (2020) 34(4):966–84. doi: 10.1038/s41375-020-0776-2

81. Deininger MW, Shah NP, Altman JK, Berman E, Bhatia R, Bhatnagar B, et al. Chronic myeloid leukemia, version 2. *2021 NCCN Clin Pract Guidelines Oncol J Natl Compr Canc Netw* (2020) 18(10):1385–415. doi: 10.6004/jnccn.2020.0047

82. Hijiya N, Suttorp M. How I treat chronic myeloid leukemia in children and adolescents. *Blood* (2019) 133(22):2374–84. doi: 10.1182/blood.2018882233

83. Samis J, Lee P, Zimmerman D, Suttorp M, Hijiya N. The complexity of growth failure in children receiving tyrosine kinase inhibitor therapy for chronic myelogenous leukemia. *Pediatr Blood Cancer* (2017) 64(12). doi: 10.1002/pbc.26703

84. Hijiya N, Millot F, Suttorp M. Chronic myeloid leukemia in children: Clinical findings, management, and unanswered questions. *Pediatr Clin North Am* (2015) 62 (1):107–19. doi: 10.1016/j.pcl.2014.09.008

85. Suttorp M, Schulze P, Glauche I, Göhring G, von Neuhoff N, Metzler M, et al. Front-line imatinib treatment in children and adolescents with chronic myeloid leukemia: Results from a phase III trial. *Leukemia* (2018) 32(7):1657–69. doi: 10.1038/s41375-018-0179-9

86. McCafferty EH, Dhillon S, Deeks ED. Dasatinib: A review in pediatric chronic myeloid leukemia. *Paediatr Drugs* (2018) 20(6):593–600. doi: 10.1007/s40272-018-0319-8

87. Hijiya N, Zwaan CM, Rizzari C, Foà R, Abbink F, Lancaster D, et al. Pharmacokinetics of nilotinib in pediatric patients with Philadelphia chromosome-positive chronic myeloid leukemia or acute lymphoblastic leukemia. *Clin Cancer Res* (2020) 26(4):812–20. doi: 10.1158/1078-0432.Ccr-19-0090

88. Kurosawa H, Tanizawa A, Tono C, Watanabe A, Shima H, Ito M, et al. Leukostasis in children and adolescents with chronic myeloid leukemia: Japanese pediatric Leukemia/Lymphoma study group. *Pediatr Blood Cancer* (2016) 63(3):406–11. doi: 10.1002/pbc.25803

89. de Bruijn CMA, Millot F, Suttorp M, Borisevich M, Brons P, Lausen B, et al. Discontinuation of imatinib in children with chronic myeloid leukaemia in sustained deep molecular remission: Results of the STOP IMAPED study. *Br J Haematol* (2019) 185(4):718–24. doi: 10.1111/bjh.15826

90. Millot F, Suttorp M, Ragot S, Leverger G, Dalle JH, Thomas C, et al. Discontinuation of imatinib in children with chronic myeloid leukemia: A study from the international registry of childhood CML. *Cancers (Basel)* (2021) 13(16):4102. doi: 10.3390/cancers13164102

91. Shima H, Kada A, Tanizawa A, Sato I, Tono C, Ito M, et al. Discontinuation of tyrosine kinase inhibitors in pediatric chronic myeloid leukemia. *Pediatr Blood Cancer* (2022) 69(8):e29699. doi: 10.1002/pbc.29699

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 24 April 2023
DOI 10.3389/fonc.2023.1171568

OPEN ACCESS

EDITED BY
Sherif Farag,
Indiana University Bloomington,
United States

REVIEWED BY
Hamid Sayar,
Indiana University Simon Cancer Center,
United States
Rita Assi,
Indiana University Melvin and Bren Simon
Comprehensive Cancer Center,
United States

*CORRESPONDENCE
Vikram Mathews
vikram@cmcvellore.ac.in

SPECIALTY SECTION
This article was submitted to
Hematologic Malignancies,
a section of the journal
Frontiers in Oncology

RECEIVED 22 February 2023
ACCEPTED 11 April 2023
PUBLISHED 24 April 2023

CITATION
Mathews V, Korula A, Chakrapani A,
Bhurani D, Bhattacharyya J, Sengar M,
Malhotra P, Boyella PK, Singh PK,
Ganesan P, Dhawan R, Melinkeri S,
Damodar S, Dolai TK and Radhakrishnan V
(2023) Management of B-cell lineage acute
lymphoblastic leukemia: expert opinion
from an Indian panel *via* Delphi consensus
method.
Front. Oncol. 13:1171568.
doi: 10.3389/fonc.2023.1171568

Management of B-cell lineage acute lymphoblastic leukemia: expert opinion from an Indian panel *via* Delphi consensus method

Vikram Mathews[1]*, Anu Korula[2], Anupam Chakrapani[3], Dinesh Bhurani[4], Jina Bhattacharyya[5], Manju Sengar[6], Pankaj Malhotra[7], Pavan Kumar Boyella[8], Pawan Kumar Singh[9], Prasanth Ganesan[10], Rishi Dhawan[11], Sameer Melinkeri[12], Sharat Damodar[13], Tuphan Kanti Dolai[14] and Venkatraman Radhakrishnan[15]

[1]Department of Haematology, Christian Medical College, Vellore, Tamil Nadu, India, [2]Department of Haematology, Christian Medical College, Vellore, Tamil Nadu, India, [3]Clinical Hematology, Apollo Gleneagles Hospital, Kolkata, India, [4]Department of Hemato-Oncology & Bone Marrow Transplant (BMT) Unit, Rajiv Gandhi Cancer Institute & Research Centre, New Delhi, India, [5]Department of Clinical Hematology, Gauhati Medical College and Hospital, Guwahati, Assam, India, [6]Medical Oncology Department, Tata Memorial Hospital, Mumbai, Maharashtra, India, [7]Department of Clinical Hematology and Medical Oncology, Nehru Hospital, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India, [8]Department of Medical Oncology, Basavatarakam Indo American Cancer Hospital and Research Centre, Hyderabad, Telangana, India, [9]Haemato-Oncology & Bone Marrow Transplant (BMT), B.L. Kapur (BLK)-Max Center for Bone Marrow Transplant, BLK-Max Superspeciality Hospital, New Delhi, India, [10]Department of Medical Oncology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India, [11]Clinical Hematology, All India Institute of Medical Sciences, Delhi, India, [12]Department of Hematology, Deenanath Mangeshkar Hospital & Research Center, Pune, India, [13]Mazumdar Shaw Medical Center, Narayana Health City, Bengaluru, Karnataka, India, [14]Department of Haematology, Nil Ratan Sarkar (NRS) Medical College and Hospital, Kolkata, India, [15]Department of Medical Oncology and Pediatric Oncology, Cancer Institute (WIA), Chennai, India

Introduction: Currently, there are no guidelines for the management of B-cell lineage acute lymphoblastic leukemia (B-ALL) from an Indian perspective. The diagnostic workup, monitoring, and treatment of B-ALL vary among different physicians and institutes.

Objective: To develop evidence-based practical consensus recommendations for the management of B-ALL in Indian settings.

Methods: Modified Delphi consensus methodology was considered to arrive at a consensus. An expert scientific committee of 15 experts from India constituted the panel. Clinically relevant questions belonging to three major domains were drafted for presentation and discussion: (i) diagnosis and risk assignment; (ii) frontline treatment; and (iii) choice of therapy (optimal vs. real-world practice) in relapsed/refractory (R/R) settings. The questionnaire was shared with the panel members through an online survey platform. The level of consensus was categorized into high (≥ 80%), moderate (60%–79%), and no consensus (< 60%). The process involved 2 rounds of discussion and 3 rounds of Delphi survey. The questions that

received near or no consensus were discussed during virtual meetings (Delphi rounds 1 and 2). The final draft of the consensus was emailed to the panel for final review.

Results: Experts recommended morphologic assessment of peripheral blood or bone marrow, flow cytometric immunophenotyping, and conventional cytogenetic analysis in the initial diagnostic workup. Berlin–Frankfurt–Münster (BFM)–based protocol is the preferred frontline therapy in pediatric and adolescent and young adult patients with B-ALL. BFM/German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia–based regimen is suggested in adult patients with B-ALL. Immunotherapy (blinatumomab or inotuzumab ozogamicin) followed by allogeneic hematopoietic cell transplantation (allo-HCT) is the optimal choice of therapy that would yield the best outcomes if offered in the first salvage in patients with R/R B-ALL. In patients with financial constraints or prior allo-HCT (real-world practice) at first relapse, standard-intensive chemotherapy followed by allo-HCT may be considered. For subsequent relapses, chimeric antigen receptor T-cell therapy or palliative care was suggested as the optimal choice of therapy.

Conclusion: This expert consensus will offer guidance to oncologists/clinicians on the management of B-ALL in Indian settings.

KEYWORDS

B-cell acute lymphoblastic leukemia, relapsed/refractory, India, management, consensus, Delphi

1 Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous hematologic disorder characterized by the neoplastic proliferation of clonal precursor B or T cells in the bone marrow, peripheral blood, and extramedullary locations (1). B-cell lineage acute lymphoblastic leukemia (B-ALL) is the most common subtype of ALL, accounting for 85% of ALL cases (2). The survival outcomes for patients with ALL have improved substantially in the recent decade, especially among children primarily due to an increased understanding of pathogenesis and molecular genetics, the adoption of risk-stratified therapy, and the availability of newer treatment options (3, 4). A review by Arora et al. reported overall survival (OS) between 45% and 81% (follow-up: 4–5 years) in Indian children (median age: 5–10 years) with ALL (4). Radhakrishnan VS et al. reported a 5-year OS of 5.5%–58% and overall relapse rates between 24.3% and 57.1% (median time: 9–24 months) in adolescent and young adult (AYA) and adult patients (aged 10 years and above) with ALL (5). The monthly financial burden of childhood ALL has been reported to be 7.2 times the monthly per capita income of India (5). The burden of ALL in AYA patients appears to be even higher in India because India has a predominately younger patient population (5, 6). This also levies a substantial financial burden on a developing country like India due to the loss of productive years of both the patient and the caregiver, exorbitant treatment costs, and lack of comprehensive health insurance coverage (5). Further, laboratory evaluation of ALL is complex and often relies on advanced laboratory techniques, and financial challenges create significant problems in the timely delivery of treatment (5, 7). These often cause long interruptions or abandoning of treatment, often after successful initiation, which further leads to more resistant forms of the disease (8). It has been shown that intensification of treatment with combination therapies can lead to improvement in OS. However, the intensification of therapy also remains a significant challenge in India (8). This is due to limited resources to manage treatment-related adverse events, high prevalence of multidrug-resistant infections, and prolonged cytopenia with infections that further complicate cancer care (5, 8). Currently, there is a lack of consensus on the diagnostic workup and monitoring of B-ALL, and it varies among different physicians and institutes. In addition, there is a lack of consensus on the utility of different treatment options in frontline and relapsed/refractory (R/R) settings. In recent times, novel targeted immunotherapies, including monoclonal antibodies, antibody–drug conjugates, and cellular therapies, have shown significant promise in R/R adult B-ALL patients (9, 10). In lieu of the gaps identified, a countrywide consensus regarding protocols for diagnosis, treatment, and follow-ups that incorporate recent therapies is the need of the hour to improve treatment outcomes of B-ALL in India (5). Given the changing treatment landscape and the challenges faced in India, a panel of experts assembled to understand the current treatment scenario of B-ALL in India and reach a consensus regarding

diagnostic and treatment approaches best suitable in the Indian setting. In this article, we have summarized expert opinions and recommendations on (i) diagnostic workup and risk assignment of B-ALL; (ii) frontline treatment of B-ALL; and (iii) choice of therapy in R/R B-ALL. Resource availability and cost constraints were considered while drafting consensus recommendations.

2 Methodology

2.1 Panel selection

A panel of 15 experts was selected (Figure 1) based on their academic track records and involvement in clinical research and experience in the field of B-ALL from various areas of the country (Table S1 in Supplementary Material). A chair was identified among the panel members to drive the consensus process.

2.2 Evidence review

A literature review was carried out based on data from the PubMed database to identify relevant articles between January 2001 and September 2022 using keywords such as "B-cell acute lymphoblastic leukemia," "diagnosis," "management," "relapsed/refractory," and "guidelines." The questionnaire was broadly segregated to include relevant questions under:

- Diagnosis and risk assignment
- Frontline treatment
- Choice of therapy in R/R settings (optimal and real-world practice)

Defining optimal choice: Optimal choice is the best possible option supported by evidence and is currently available in India, irrespective of cost or any other constraints. This should consider the absence of chimeric antigen receptor T-cell (CAR-T) therapy for second and subsequent relapse and in patients who had already undergone allogeneic hematopoietic cell transplantation (allo-HCT).

Defining real-world practice: Real-world choice is the best possible option currently available in India, keeping in mind cost and other constraints. This includes the option of a second allo-HCT in patients who have received an allo-HCT upfront or at first relapse.

The questionnaire was finalized in discussion with the chair and was rolled out to the panel members through an online survey platform (Delphi survey—round 1).

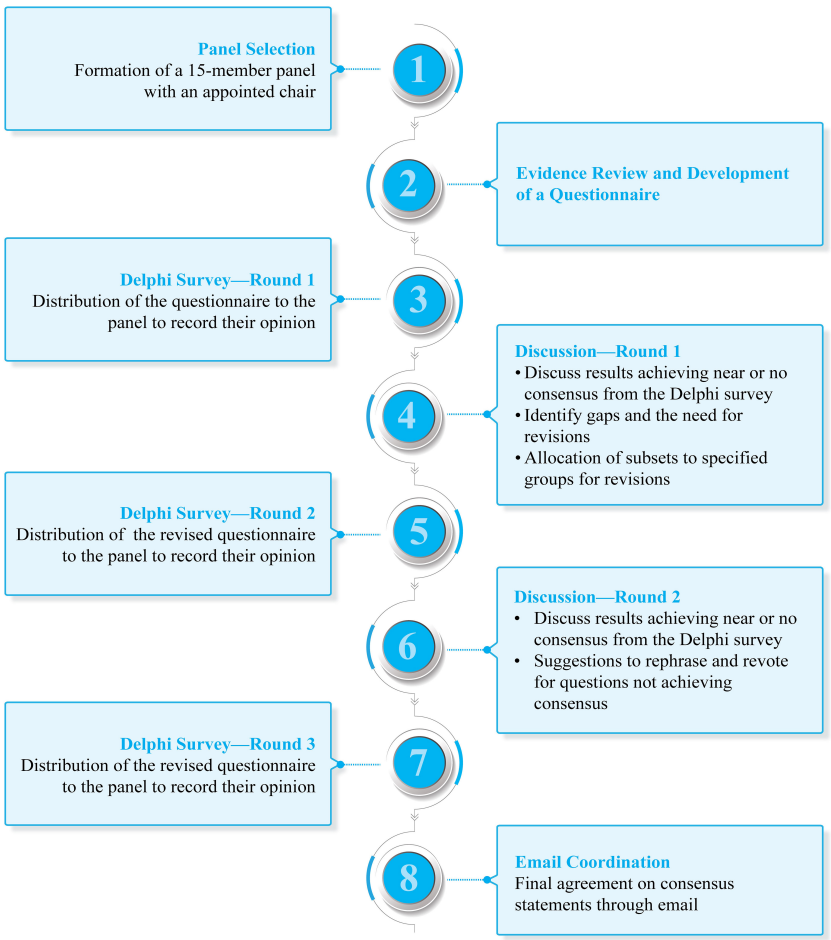

FIGURE 1
Overview of the consensus process used to create the clinical consensus statement.

2.3 Consensus process

The experts discussed the results of the survey during a virtual expert panel meeting on April 22, 2022 (discussion—round 1). Modified Delphi consensus methodology was considered to arrive at a consensus (11). The level of consensus (Table 1) was categorized into high (≥ 80%), moderate (60%–79%), and no consensus (< 60%) (12). The differences in opinions were also discussed for modification of statements for the next round of voting (Delphi survey—round 2). The questions that received near or no consensus in the first round were discussed during the second meeting conducted virtually on August 6, 2022 (discussion—round 2). The recommendations were based on the responses to revised questions. The final round of voting was conducted to determine the definitive acceptance or rejection of a recommendation (Delphi survey—round 3). The final draft of the consensus was emailed to the panel for final review.

3 Results

The experts (N=15) analyzed evidence and guidelines on B-ALL management published between January 2001 and September 2022. Experts made their decisions based on the available evidence and their current practices in India. An effort was made to address optimal vs. real-world management of B-ALL based on loco-regional constraints. This article will first discuss the international guideline (the National Comprehensive Cancer Network [NCCN] and the European Society for Medical Oncology [ESMO]) recommendations followed by expert consensus.

3.1 Diagnostic workup and risk assignment of B-ALL

The diagnosis of ALL generally requires the demonstration of ≥ 20% bone marrow lymphoblasts upon hematopathologist's review of bone marrow aspirate and biopsy materials (13–15). The NCCN and ESMO clinical practice guidelines recommend a comprehensive diagnostic approach in patients with ALL (13–15). This includes the following:

- Morphologic assessment of Wright–Giemsa–stained bone marrow aspirate smears, hematoxylin–eosin–stained core biopsy, and clot sections
- Immunophenotyping
    - o Myeloperoxidase expression
    - o B-lineage markers (CD19, CD79a, CD22, CD10, CD20, CD24, cIgM, and sIg [kappa or lambda])
    - o T-lineage markers (CD3, CD1a, CD2, CD5, CD7, CD4, CD8, and TCR α/β or γ/δ)
- Cytogenetic analysis
- New genetics/genomics (gene expression profiling and next-generation sequencing [NGS])

The NCCN guideline also recommends a computed tomography (CT)/magnetic resonance imaging (MRI) scan of the head with contrast (in patients with major neurologic symptoms), testing for opportunistic infections, and an early allo-HCT evaluation at the time of initial diagnosis (14, 15). Optimal risk stratification and treatment planning require testing marrow or peripheral blood lymphoblasts for specific recurrent gene abnormalities using: (i) fluorescence *in situ* hybridization (FISH) for recurrent genetic abnormalities; (ii) reverse transcriptase-polymerase chain reaction (RT-PCR) testing for the detection of *BCR-ABL1* gene rearrangements, denoting an underlying t (9;22) (q34.1;q11.2)/*BCR-ABL1* chromosomal translocation typical of Philadelphia chromosome-positive (Ph+) ALL; and (iii) NGS for gene fusions and pathogenic mutations (13, 14).

The American Society of Clinical Oncology guidelines recommend testing for (7):

- t(12;21)(p13.2;q22.1) [*ETV6-RUNX1*]; t(9;22)(q34.1;q11.2) [*BCR-ABL1*]; t(v;11q23.3) [*KMT2A (MLL)* translocation]; iAMP21; and trisomy 4 and 10 in pediatric B-ALL.
- t(9;22)(q34.1;q11.2) [*BCR-ABL1*] and t(v;11q23.3) [*KMT2A (MLL)*] translocation in adult B-ALL.

Consensus/recommendations on the diagnostic workup of B-ALL

The initial workup for B-ALL patients should include an evaluation of medical history and physical examination, along with laboratory and imaging studies (Figure 2). Experts recommended complete blood count, morphologic assessment of peripheral blood or bone marrow, flow cytometric immunophenotyping, and conventional cytogenetic analysis in the initial diagnostic workup (high consensus). A minimum panel of markers that includes CD19 plus CD22 for B-ALL is suggested (high consensus). Other recommended tests include hepatitis B/C and HIV evaluations. Female patients in reproductive age may undergo pregnancy testing (moderate consensus), and all male patients should be evaluated for testicular involvement of disease (high consensus). Experts suggested a CT/MRI scan of the head with contrast to detect meningeal disease, chloromas, or central nervous system (CNS) bleeding for patients with major neurologic symptoms at diagnosis. CNS involvement should be evaluated through lumbar puncture at the time of initial scheduled

TABLE 1 Level of consensus.

High	When ≥ 80% of participants agree/strongly agree or disagree/strongly disagree with a statement.
Moderate	When 60%–79% of participants agree/strongly agree or disagree/strongly disagree with a statement.
Low	When < 60% of participants agree/strongly agree or disagree/strongly disagree with a statement.

Level of consensus: Adapted from: Jünger S et al., 2012 (12).

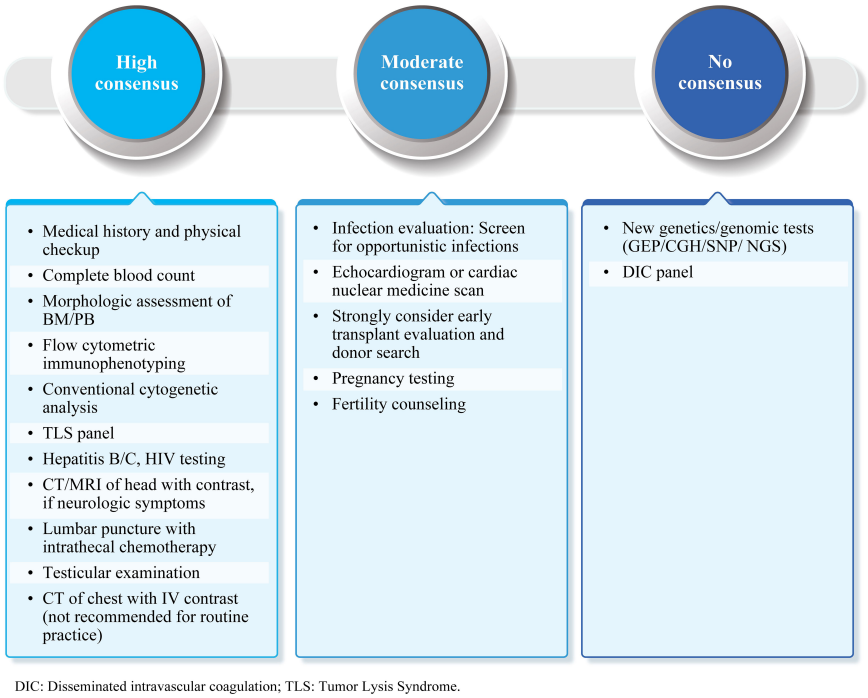

FIGURE 2
Initial diagnostic workup: Summary of expert consensus/recommendations. B-ALL, B-cell lineage acute lymphoblastic leukemia; BM, Bone marrow; PB, Peripheral blood; CT, Computed tomography; MRI, Magnetic resonance imaging; IV, Intravenous; NGS, Next-generation sequencing; CGH, Comparative genomic hybridization; SNP, Single-nucleotide polymorphism; GEP, Gene expression profiling; TLS, Tumor lysis syndrome; DIC, Disseminated intravascular coagulation.

intrathecal therapy (high consensus). Assessment of cardiac function is important for patients with prior cardiac history, cardiac dysfunction, and elderly patients (moderate consensus). Screening for opportunistic infections, early allo-HCT evaluation, and donor search should be considered (moderate consensus).

Risk-directed treatment is an essential aspect of B-ALL management. Thus, it is important to assign risk categories to patients to ensure appropriate treatment decisions. The assignment of risk categories is primarily dependent on the availability of resources. The expert panel group at the 2013 Asian Oncology Summit proposed a four-tier system (basic, limited, enhanced, and maximum) based on which recommendations could be developed (16). In the case of basic resource settings, risk assignment can be based on age, presenting leukocyte count, and early treatment response as assessed by peripheral blood blast cell count. Additional molecular and cytogenetic features can be evaluated with the availability of enhanced resources (16). This stratification was modified and adapted to the Indian setting to evaluate the experts' opinions (Table 2).

Consensus/recommendations on risk assignment criteria

Experts recommended the following risk assignment criteria best suitable in Indian settings (levels 2 and 3; moderate consensus):

- Age, leukocyte count, immunophenotype (T cell vs. B cell), prednisone response or day 8 peripheral blood or bone marrow response, end of induction bone marrow response. If available, RT-PCR for *BCR-ABL1*, cytogenetics for Philadelphia chromosome, or FISH for *BCR-ABL1*

TABLE 2 Risk assignment stratification of B-ALL.

Risk assignment level	Criteria
1	Age, leukocyte count, day 8 peripheral blood response
2	Age, leukocyte count, immunophenotype (T cell vs. B cell), prednisone response or day 8 peripheral blood or bone marrow response, end of induction bone marrow response. If available, RT-PCR for *BCR-ABL1*, cytogenetics for Philadelphia chromosome, or FISH for *BCR-ABL1*
3	RT-PCR for *BCR-ABL1* and *MLL-AFF1*, cytogenetics for hyperdiploid > 50, FISH for *BCR-ABL1*, and flow cytometry for MRD measurements
4	*ABL*-kinase domain mutation analysis, especially the T315I mutation for selection of alternative tyrosine kinase inhibitors, pharmacogenetics, NGS for IgH/TCR rearrangements

B-ALL, B-cell lineage acute lymphoblastic leukemia; MRD, Minimal residual disease; RT-PCR, Real-time reverse transcription-polymerase chain reaction; FISH, Fluorescence in situ hybridization; IgH: Immunoglobulin heavy chain; TCR, T-cell receptor; NGS, Next-generation sequencing.

- RT-PCR for *BCR-ABL1 and MLL-AFF1*, cytogenetics for hyperdiploidy > 50, FISH for *BCR-ABL1*, and flow cytometry for MRD measurements

3.2 Frontline treatment of B-ALL

The ESMO recommends age stratification for appropriate treatment of ALL as the treatment outcome of ALL is often age-associated (AYA: 15/18 to 35/40 years; adults: 35/40 to ≤ 55/60 years; elderly: above 55/60 years), hence necessitating age-based protocols (13). In India, currently there is a lack of consensus regarding age thresholds to categorize pediatric, AYA, and adult ALL (5). Various clinical trials have evaluated the efficacy and safety of chemotherapy regimens (Berlin–Frankfurt–Münster [BFM], Multicenter protocol 841 [MCP-841], Children's Oncology Group [COG], United Kingdom Acute Lymphoblastic Leukemia [UKALL]) in the front line in children with ALL (15). Adapting these protocols in Indian settings has improved patient outcomes in the last decade; however, treatment-related mortality (11%–25%) and disease relapse (relapse rates: 15%–41%) have been reported in children in Indian settings (17–19). In 2013, the Indian Collaborative Childhood Leukaemia group (ICiCLe) developed a risk-stratified treatment protocol for the management of first presentation ALL based on cytogenetics and MRD levels (at the end of induction) in children (aged: 1–18 years) (20). Initial risk classification was based on lymphoblast lineage, age, leucocyte count, disease bulk, CNS disease status, leukaemia cytogenetics and prednisolone response at treatment day 8. The final risk stratification was determined at the end of the induction treatment phase and was based on treatment response, including remission status and the level of bone marrow MRD (20). The protocol is specific to Indian patients with ALL and is designed to (i) decrease toxicity and mortality in induction by shortening the duration of prednisolone therapy in patients with non–high-risk ALL and (ii) improve event-free survival in risk groups by replacing doxorubicin with mitoxantrone in delayed intensification (20). In India, treatment protocols used in AYA and adult ALL include MCP-841, BFM-90, chemotherapy plus tyrosine kinase inhibitor (TKI), German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia (GMALL), and hyper-cyclophosphamide, vincristine, doxorubicin, dexamethasone (hyper-CVAD) (5). A real-world study by Malhotra P et al. found that a modified BFM regimen in adult ALL patients (> 12 years) in resource-limited settings resulted in complete remission (CR) of 85.6% after induction (5-year event-free survival: 21.6%) (21). A retrospective study was done on Indian adult ALL patients, which showed a 5-year OS of 38% and a CR rate of 82.2% with a modified GMALL regimen (8, 22). A more recent report from the Indian Acute Leukaemia Research Database and Hematology Cancer Consortium highlighted that BFM protocol (BFM-90, BFM-95, or BFM-2000) was the most common regimen used in AYA patients (aged 15–29 years) with ALL (23).

Consensus/recommendations on frontline treatment of B-ALL

According to the experts, age, risk stratification, comorbidities, and financial constraints are crucial factors in determining treatment strategy. Patients should be categorized into AYA and adults for the optimal choice of the treatment protocol. However, there was no consensus on the age threshold to be used in practice. Experts recommended BFM-based protocol as frontline therapy in pediatric and AYA patients with B-ALL (high consensus). BFM/GMALL-based regimen is suggested in adult patients with B-ALL (moderate consensus). Figure 3 lists treatment protocols used in pediatric, AYA, and adult patients with B-ALL.

3.2.1 MRD monitoring

The NCCN guidelines state that MRD is an essential component of patient evaluation over the course of sequential therapy (end of induction, consolidation, and surveillance) in pediatric and adult patients with ALL (14, 15). The ESMO guidelines recommend MRD monitoring to guide the decision of chemotherapy or allo-HCT after consolidation in patients with ALL (13). Furthermore, prolonged monitoring of *BCR-ABL1* MRD levels is recommended, associated with resistance mutation screening in patients with persistent MRD detection or re-increasing

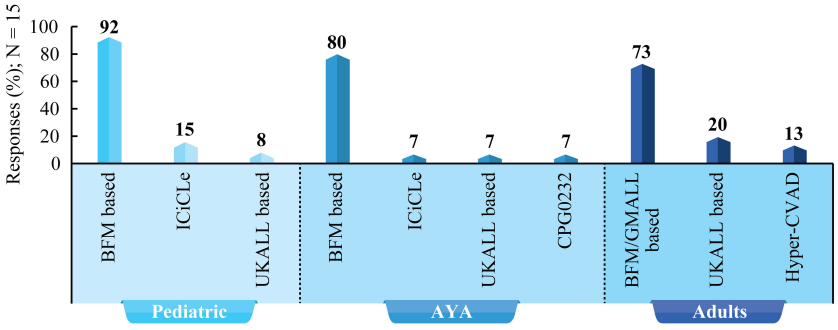

FIGURE 3
Treatment protocols for children, AYA, and adults with B-ALL: Survey results based on experts' clinical practice. BFM, Berlin–Frankfurt–Munster; CVAD, Cyclophosphamide, vincristine sulfate, doxorubicin hydrochloride (Adriamycin), and dexamethasone; UKALL, United Kingdom Acute Lymphoblastic Leukemia; GMALL, German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia; ICiCLe, The Indian Childhood Collaborative Leukemia; AYA, Adolescents and young adults.

MRD levels (13). Table 3 lists different methods for MRD assessment in patients with B-ALL and levels of sensitivity (24–26).

Consensus/recommendations on MRD assessment

MRD is the preferred criterion for determining outcomes in patients with B-ALL. Experts recommended flow cytometry for MRD assessment in patients with B-ALL. The consensus statements on MRD monitoring in patients with B-ALL have been summarized in Table 4.

3.2.2 CNS prophylaxis

CNS prophylaxis aims to prevent relapse or CNS disease and mainly includes intrathecal or systemic chemotherapy. Cranial irradiation is often associated with secondary neoplasms, neurocognitive dysfunction, endocrinopathy, and neurotoxicity (27). A combination of high-dose systemic therapy with CNS penetration (e.g., methotrexate or cytarabine) and intrathecal chemotherapy is quite effective, with CNS recurrence incidence being < 6% (28, 29). The NCCN recommends CNS prophylaxis to be given throughout the entire course of treatment to all patients (15, 30).

Consensus/recommendations

- In pediatric and AYA B-ALL patients, intrathecal methotrexate and systemic therapy is the preferred option for CNS prophylaxis (moderate consensus).
- In adult B-ALL patients, there was no consensus on the choice of therapy.

Experts agreed that CNS prophylaxis is a must in adult B-ALL patients; however, there was no consensus on the choice of therapy. Experts suggested that a combination of systemic and intrathecal chemotherapy may be considered for CNS prophylaxis in adult patients with B-ALL. The use of CNS irradiation in addition to intrathecal methotrexate may be advised based on institutional experience and infrastructure in resource-limited settings.

3.3 Treatment of R/R B-ALL

3.3.1 Prognostic factors

The definitions for "early" and "late" relapse differ among different study groups. The BFM group study categorized time to relapse or length of first CR as (i) very early relapse (less than 18 months from diagnosis); (ii) early relapse (more than 18 months from diagnosis and less than 6 months of completion of frontline therapy); and (iii) late relapse (more than 6 months after the completion of frontline therapy) (31). In contrast, the COG defined "very early time to relapse" as the length of first CR less than 18 months from initial diagnosis; "intermediate" as 18–36 months after initial diagnosis; "early" relapse as within 36 months after initial diagnosis; or (iv) "late" relapse as 36 months or more after diagnosis (31). The ESMO and NCCN guidelines state that age (< 1 year old or ≥10 years) and white blood cell (WBC) count (50 X 10^9 cells/L) on presentation are independent, clinically significant prognostic factors predicting lower CR rate and shorter CR duration in patients with B-ALL (13, 14). Unfavorable cytogenetics, time to relapse, site of relapse, response to first salvage therapy, performance of allo-HCT, and MRD during second CR and before allo-HCT are significant prognostic factors for survival after relapse (14, 31, 32).

Consensus/recommendations on prognostic factors

Experts used the BFM study group definition of "early" and "late" relapse in their clinical practice (high consensus). The expert panel agreed that the response to salvage (high consensus) and performance of allo-HCT (moderate consensus) are two key prognostic factors for CR and survival among relapsed B-ALL

TABLE 3 Different methods for MRD assessment and level of sensitivity in patients with B-ALL.

Techniques	Sensitivity	Applicability
Flow cytometry	10^{-4}	Ph– B-ALL Ph+ B-ALL
RT-PCR of *Ig/TCR* rearrangements	10^{-4}–10^{-5}	Ph– B-ALL Ph+ B-ALL
RT-qPCR of *BCR-ABL1* transcripts	10^{-4}–10^{-5}	Ph+ B-ALL
NGS of *Ig/TCR* rearrangements	10^{-6}	Ph– B-ALL Ph+ B-ALL

B-ALL, B-cell lineage acute lymphoblastic leukemia; RT-PCR, Real-time reverse transcription-polymerase chain reaction; RT-qPCR, Quantitative reverse transcription PCR; Ig, Immunoglobulin; TCR, T-cell receptor; MRD, Minimal residual disease; Ph+, Philadelphia chromosome-positive; Ph–, Philadelphia chromosome-negative; NGS, Next-generation sequencing.
Adapted from: Hein K et al., 2022 (24), Abou Dalle I et al., 2020 (25), and Tierens A et al., 2021 (26).

TABLE 4 Expert consensus/recommendations on MRD monitoring in patients with B-ALL.

High consensus	• Flow cytometry is indicated as the method of choice for MRD assessment. In addition to flow cytometry, RT-PCR may also be used in patients with fusion transcripts. • MRD-stratified protocols assist in decisions regarding the need and timing for allo-HCT. • In patients undergoing allo-HCT, MRD assessments should be conducted before the transplant. For Ph+ B-ALL post-allo-HCT, long-term monitoring with peripheral blood RT-qPCR can be considered once in 3 months.

B-ALL, B-cell lineage acute lymphoblastic leukemia; MRD, Minimal residual disease; RT-PCR, Real-time reverse transcription-polymerase chain reaction; allo-HCT, Allogeneic hematopoietic cell transplantation; RT-qPCR, Quantitative reverse transcription polymerase chain reaction; Ph+, Philadelphia chromosome-positive.

patients. Age, time to relapse, pre-transplant MRD negativity, donor availability, and type are other factors that need to be considered.

3.3.2 Choice of therapy for R/R B-ALL

Salvage treatment after B-ALL relapse involves inducing a complete remission 2 (CR2) with intensive chemotherapy and applying consolidation, re-intensification, and maintenance therapy, or allo-HCT as a further intensification of treatment. Several studies have reported poor survival outcomes (median OS: 4.5–6 months; 5-year OS: 3%–10%) with conventional chemotherapy regimens in relapsed adult B-ALL patients (33–36). The ESMO 2016 guidelines suggest the use of new-generation TKIs, according to the results of mutational analysis of *BCR-ABL1* transcripts in patients with relapsed Ph+ ALL (13). In 2017, blinatumomab (bispecific anti-CD3/CD19 monoclonal antibody) and inotuzumab ozogamicin (InO; calicheamicin-based antibody–drug conjugate targeting CD22) received full approval from the Food and Drug Administration for R/R precursor B-ALL (Ph+ and Ph−) in adults based on promising results from phase II and phase III clinical trials (37–42). Both InO and blinatumomab have shown beneficial outcomes in terms of achieving MRD negativity (39, 43). InO treatment has shown improved rates of CR/CR with incomplete hematologic recovery and OS vs. standard chemotherapy (SC) in adult R/R ALL with high baseline disease burden (bone marrow blast [BMB] > 90%) (44). Consequently, a greater proportion of patients in the InO vs. SC arm proceeded to stem cell transplantation, irrespective of baseline BMB percentage (44).

In pediatric R/R B-ALL patients (Ph+ and Ph−), the NCCN guideline recommend (15):

- Early or late first relapse: Initial treatment with systemic therapy. If patients experience CR2 and are MRD-negative, the options are either to continue chemotherapy and receive maintenance therapy or allo-HCT. In the case of MRD-positive or if the patient experiences the first relapse after a prior allo-HCT, the options are chemotherapy, blinatumomab, CAR-T therapy, or InO before the first or second allo-HCT.
- Multiple relapses: Treatment options include chemotherapy, blinatumomab, CAR-T therapy, or InO and allo-HCT as consolidation therapy.

In Ph+ R/R B-ALL patients (AYA and adults), after *ABL1* kinase domain mutation testing, the more recent NCCN 2021 guideline recommends (14):

- TKI with or without chemotherapy followed by allo-HCT
- Blinatumomab with or without TKI followed by allo-HCT
- InO with or without bosutinib (TKI-intolerant or refractory B-ALL) followed by allo-HCT
- CAR-T therapy (in patients under 26 years with refractory B-ALL or patients with ≥ 2 relapses and failure of 2 TKIs) followed by allo-HCT.

However, in Ph− R/R B-ALL patients (AYA and adults), after MRD assessment, blinatumomab, InO, CAR-T therapy, or chemotherapy may be considered followed by allo-HCT (14). In 2018, InO received permission from Central Drugs Standard Control Organization (CDSCO), India, for the treatment of adults with R/R CD22-positive B-ALL. It is also indicated in patients with Ph+ R/R B-cell precursor ALL who have failed treatment with at least one TKI therapy (45). Currently, the CDSCO has not approved blinatumomab and CAR-T therapy for the management of R/R B-ALL except under a trial setting in India.

Consensus/recommendations

Optimal choice of therapy for early or late first relapse: Experts favored immunotherapy (InO or blinatumomab) followed by allo-HCT for the treatment of R/R Ph+ and Ph− B-ALL patients after the first relapse (early or late; medullary/extramedullary) and to achieve MRD negativity (high consensus). The addition of TKI should always be considered for Ph+ B-ALL patients. Experts agreed that InO would be the optimal treatment of choice in adult patients with R/R B-ALL with BMB ≥ 50% if there are no resource limitations (high consensus). Concurrent use of InO with intrathecal chemotherapy was agreed upon for R/R B-ALL patients with systemic relapse and CNS disease (moderate consensus). To balance the risk of relapse against the potential risk of conditioning regimen-related toxicity, 4–6 weeks was agreed upon between the last dose of InO and allo-HCT (high consensus). Regarding the duration between the last dose of InO and the allo-HCT (where there is a need to start maintenance therapy or another chemotherapy schedule as a bridge for time to transplant), there was an agreement that if the transplant is delayed more than 6 weeks, there is a need to start such therapy as early as possible. However, no consensus was achieved regarding duration with opinions varying between 4 and 8 weeks.

***Real-world practice for early or late first relapse*:** In patients with financial constraints or prior allo-HCT at first relapse, experts recommended standard-intensive chemotherapy (with TKI for Ph+ B-ALL patients) followed by allo-HCT (high consensus). For late relapse, risk stratification and considerations for transplant would depend upon the protocol.

Optimal choice of therapy for early or late second and subsequent relapse: For subsequent relapses, CAR-T therapy (if available) or palliative care (in the absence of CAR-T therapy) was suggested (early or late; medullary/extramedullary), assuming that immunotherapy has already been used in the first relapse (high consensus).

Real-world practice for early or late second and subsequent relapse: No consensus was achieved for the treatment of patients with R/R B-ALL (Ph+ or Ph−) in post-transplant second or subsequent relapse.

Isolated testicular relapse is not treated differently from other relapses if it is an early relapse (high consensus). There was no consensus on whether isolated testicular relapse should be treated differently from other relapses in case of late relapse. There was a divided opinion between palliative care, low-intensity chemotherapy, and immunotherapy regarding the optimal choice of therapy for older R/R B-ALL patients (aged ≥ 60) unfit for standard-intensity chemotherapy (no consensus).

Table 5 summarizes expert recommendations for the management of R/R B-ALL patients.

4 Discussion

The survival rates for patients with ALL have improved in the recent decade. Improvements are largely due to advances in the understanding of disease pathogenesis, molecular genetics, incorporation of MRD testing, advent of new therapeutic agents, adoption of risk-directed treatment, use of allo-HCT, and improvements in supportive care. In recent times, novel targeted immunotherapies, including monoclonal antibodies, antibody–drug conjugates, and cellular therapies, have shown significant promise in R/R settings. The biggest problem for resource-poor countries like India is devising treatment strategies that will enable patients to avail treatment at reasonable costs and obtain substantial treatment benefits. High out-of-pocket expenditures for ALL treatment and the absence of a nationwide comprehensive universal health insurance scheme are some of the biggest constraints in the management of ALL in India.

TABLE 5 Choice of therapy in relapsed/refractory B-ALL: Summary of expert consensus/recommendations.

•Optimal* choice of therapy for R/R Ph+ or Ph− B-ALL patients in the first relapse:
○ Use of immunotherapy agents (InO or blinatumomab) followed by allo-HCT is the optimal choice of therapy for R/R Ph+ or Ph− B-ALL patients in the first relapse. The addition of TKI should always be considered for Ph+ B-ALL patients. The treatment approach remains the same for early and late relapse (medullary and extramedullary) (high consensus).
○ Important determinants of allo-HCT include donor availability, depth of remission, comorbidities, and social support. Immunotherapy (preferably InO) is the recommended choice of therapy that would yield the best outcomes if offered in the first salvage (high consensus).
○ In patients with persistent residual disease, alternative treatment approaches such as immunotherapies can enhance treatment outcomes. MRD negativity has a significant impact on transplant outcomes. The choice of agent to achieve MRD negativity can be InO or blinatumomab (high consensus). Treatment with InO before transplant is associated with both improved CR and MRD negativity (moderate consensus).
○ During treatment with InO, cytoreduction is necessary for those with WBC >10,000/μL (moderate consensus). Monitoring of liver enzymes is essential during treatment with InO (high consensus).
○ Concurrent use of InO with intrathecal chemotherapy is recommended for R/R B-ALL patients with systemic relapse and CNS disease (moderate consensus).
○ The ideal period from the last dose of InO before proceeding with a transplant can be between 4 and 6 weeks. It is important to achieve a balance between preventing VOD and the risk of relapse (high consensus).
○ Conventional maintenance therapy for 2 years is recommended for patients in remission after 6 cycles of InO, who do not undergo transplant (high consensus).
•Real-world** choice of therapy for R/R Ph+ or Ph− B-ALL patients in first relapse (high consensus):
○ Consensus was reached on the use of standard-intensive chemotherapy (with TKI for Ph+ patients) followed by transplant.
○ For late relapse, risk stratification and considerations for transplant would depend upon the protocol.
•Optimal* choice of therapy for R/R Ph+ or Ph− B-ALL patients in second and subsequent relapse (high consensus):
○ CAR-T therapy is preferred if available in clinical trial settings. Palliative care is to be considered in the absence of CAR-T therapy. This is assuming that immunotherapy has already been used in the first relapse. The treatment approach remains the same for early and late relapse (medullary and extramedullary).
•Real-world**choice of therapy for R/R Ph+ or Ph− B-ALL patients in post-transplant second or subsequent relapse (no consensus):
○ For B-ALL patients with early isolated medullary relapse, responses were split between (i) palliative care and (ii) immunotherapy (InO/blinatumomab) followed by allo-HCT.
○ For B-ALL patients with early isolated extramedullary relapse, responses were split between (i) palliative care and (ii) TKI (if Ph+) and/or chemotherapy followed by allo-HCT.
○ For B-ALL patients with late relapse (both isolated medullary and isolated extramedullary), responses were split among (i) palliative care; (ii) TKI (if Ph+) and/or standard-intensive chemotherapy followed by allo-HCT; (iii) TKI (if Ph+) and/or standard-intensive chemotherapy; and (iv) immunotherapy (InO/blinatumomab) followed by allo-HCT.
•Isolated testicular relapse is not treated differently from other relapses if it is an early relapse (high consensus). There was no consensus on whether isolated testicular relapse should be treated differently from other relapses in case of late relapse.
•Optimal* choice of treatment for R/R B-ALL patients with a high disease burden:
○ InO in adult patients with BMB percentage ≥50% (high consensus).
•Real-world** choice of treatment for R/R B-ALL patients with a high disease burden:
○ Standard-intensive chemotherapy (with TKI if Ph+) (high consensus).

Currently, there are no country-specific guidelines/recommendations for the diagnosis and management of B-ALL from an Indian perspective. Moreover, due to the scarcity of well-designed randomized controlled trials conducted in India, oncologists rely on data from the Western world. There is a lack of consensus on the utility of treatment options in frontline and R/R settings. To the best of our knowledge, this is the first practical consensus document to guide clinicians on diagnosis, risk assessment, and treatment approach in line with the latest available evidence and guideline recommendations in Western countries. This consensus document will offer guidance to Indian hematologists/oncologists and help achieve consistency in B-ALL management across various healthcare settings.

Strengths: The panel members were selected to best represent the breadth of knowledge and clinical expertise in the field from all over India. There was no selection bias during the development of the expert committee.

Limitation:

- Hematopathologists were not part of the Delphi consensus panel. The panel was only limited to the clinicians with an active practice in the field.
- The patient's voice was not included in the consensus process.
- Supportive care and follow-up are integral parts of the management of B-ALL In the questions related to the choice of therapy for R/R B-ALL, palliative care was one of the options. The panel did discuss on palliative care; however, the discussions were not elaborate.

5 Conclusion

In this article, we have summarized the Indian consensus on the diagnosis and management of B-ALL. Experts recommended BFM-based protocol in the front line in pediatric and AYA patients with B-ALL. BFM/GMALL-based regimen was suggested in adult patients with B-ALL. In R/R B-ALL patients with residual disease, alternative treatment approaches such as immunotherapies can enhance treatment outcomes. Immunotherapy was agreed upon as the optimal choice of therapy that would yield the best outcomes if offered in the first salvage in R/R B-ALL. InO was recommended in R/R B-ALL patients with high tumor burden and CNS relapse. In patients with financial constraints or prior transplant at first relapse (real-world practice), standard-intensive chemotherapy (with TKI for Ph+ B-ALL patients) followed by allo-HCT may be considered. For older adults, because traditional chemotherapy has been poorly tolerated, current strategies for B-ALL (both Ph+ and Ph−) rely on palliation, low-intensity chemotherapy, or immunotherapy. CAR-T therapy or palliation was suggested after transplant if patients experience recurrent relapses.

Author contributions

All authors contributed to the article and approved the submitted version.VM contributed in the process of concept, design and moderation of the Delphi consensus activity along with drafting, review and finalization of manuscript.

Funding

This study is supported by Pfizer India Ltd.

Acknowledgments

We would like to thank BioQuest Solutions for their editorial support.

Conflict of interest

This study received funding from Pfizer India Ltd. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1171568/full#supplementary-material

References

1. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. *Blood Cancer J* (2017) 7:e577. doi: 10.1038/bcj.2017.53

2. Chiaretti S, Zini G, Bassan R. Diagnosis and subclassification of acute lymphoblastic leukemia. *Mediterr J Hematol Infect Dis* (2014) 6:e2014073. doi: 10.4084/MJHID.2014.073

3. Guru FR, Muzamil J, Bashir S, Mahajan A. Acute lymphoblastic leukemia, the Indian scenario. *MOJ Cell Sci Rep* (2018) 5:33–7. doi: 10.15406/mojcsr.2018.05.00110

4. Arora RS, Arora B. Acute leukemia in children: a review of the current Indian data. *South Asian J Cancer* (2016) 5:155–60. doi: 10.4103/2278-330X.187591

5. Radhakrishnan VS, Agrawal N, Bagal B, Patel I. Systematic review of the burden and treatment patterns of adult and adolescent acute lymphoblastic leukemia in India: comprehending the challenges in an emerging economy. *Clin Lymphoma Myeloma Leuk.* (2021) 21:e85–s98. doi: 10.1016/j.clml.2020.08.023

6. India State-Level Disease Burden Initiative Cancer Collaborators. The burden of cancers and their variations across the states of India: the global burden of disease study 1990-2016. *Lancet Oncol* (2018) 19:1289–306. doi: 10.1016/S1470-2045(18)30447-9

7. de Haas V, Ismaila N, Advani A, Arber DA, Dabney RS, Patel-Donelly D, et al. Initial diagnostic work-up of acute leukemia: ASCO clinical practice guideline endorsement of the college of American pathologists and American society of hematology guideline. *J Clin Oncol* (2019) 37:239–53. doi: 10.1200/JCO.18.01468

8. Jain P, Korula A, Deshpande P, Pn N, Alex AA, Abraham A, et al. Adult acute lymphoblastic leukemia: limitations of intensification of therapy in a developing country. *J Glob Oncol* (2018) 4:1–12. doi: 10.1200/JGO.17.00014

9. Uy N, Nadeau M, Stahl M, Zeidan AM. Inotuzumab ozogamicin in the treatment of relapsed/refractory acute b cell lymphoblastic leukemia. *J Blood Med* (2018) 9:67–74. doi: 10.2147/JBM.S136575

10. Dinner S, Lee D, Liedtke M. Current therapy and novel agents for relapsed or refractory acute lymphoblastic leukemia. *Leuk Lymphoma* (2014) 55:1715–24. doi: 10.3109/10428194.2013.856428

11. Nasa P, Jain R, Juneja D. Delphi Methodology in healthcare research: how to decide its appropriateness. *World J Methodol* (2021) 11:116–29. doi: 10.5662/wjm.v11.i4.116

12. Jünger S, Payne S, Brearley S, Ploenes V, Radbruch L. Consensus building in palliative care: a Europe-wide Delphi study on common understandings and conceptual differences. *J Pain Symptom Manage* (2012) 44:192–205. doi: 10.1016/j.jpainsymman.2011.09.009

13. Hoelzer D, Bassan R, Dombret H, Fielding A, Ribera JM, Buske C, et al. Acute lymphoblastic leukaemia in adult patients: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* (2016) 27:v69–82. doi: 10.1093/annonc/mdw025

14. Brown PA, Shah B, Advani A, Aoun P, Boyer MV, Burke PW, et al. Acute lymphoblastic leukemia, version 2.2021, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw* (2021) 19:1079–109. doi: 10.6004/jnccn.2021.0042

15. Brown P, Inaba H, Annesley C, Beck J, Colace S, Dallas M, et al. Pediatric acute lymphoblastic leukemia, version 2.2020, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw* (2020) 18:81–112. doi: 10.6004/jnccn.2020.0001

16. Yeoh AE, Tan D, Li CK, Hori H, Tse E, Pui C-H. Management of adult and paediatric acute lymphoblastic leukaemia in Asia: resource-stratified guidelines from the Asian oncology summit 2013. *Lancet Oncol* (2013) 14:e508–23. doi: 10.1016/S1470-2045(13)70452-2

17. Magrath I, Shanta V, Advani S, Adde M, Arya LS, Banavali S, et al. Treatment of acute lymphoblastic leukaemia in countries with limited resources; lessons from use of a single protocol in India over a twenty year period [corrected]. *Eur J Cancer* (2005) 41:1570–83. doi: 10.1016/j.ejca.2004.11.004

18. Radhakrishnan V, Gupta S, Ganesan P, Rajendranath R, Ganesan TS, Rajalekshmy KR, et al. Acute lymphoblastic leukemia: a single center experience with Berlin, Frankfurt, and Munster-95 protocol. *Indian J Med Paediatr Oncol* (2015) 36(4):261–4. doi: 10.4103/0971-5851.171552

19. Trehan A, Bansal D, Varma N, Vora A. Improving outcome of acute lymphoblastic leukemia with a simplified protocol: report from a tertiary care center in north India. *Pediatr Blood Cancer* (2017) 64(4). doi: 10.1002/pbc.26281

20. Das N, Banavali S, Bakhshi S, Trehan A, Radhakrishnan V, Seth R, et al. Protocol for ICiCLe-ALL-14 (InPOG-ALL-15-01): a prospective, risk stratified, randomised, multicentre, open label, controlled therapeutic trial for newly diagnosed childhood acute lymphoblastic leukaemia in India. *Trials* (2022) 23(1):102. doi: 10.1186/s13063-022-06033-1

21. Malhotra P, Varma S, Varma N, Kumari S, Das R, Jain S, et al. Outcome of adult acute lymphoblastic leukemia with BFM protocol in a resource-constrained setting. *Leuk Lymphoma* (2007) 48:1173–8. doi: 10.1080/10428190701343255

22. Bajel A, George B, Mathews V, Viswabandya A, Kavitha ML, Srivastava A, et al. Adult ALL: treatment outcome and prognostic factors in an Indian population using a modified German ALL (GMALL) protocol. *Leukemia* (2007) 21:2230–3. doi: 10.1038/sj.leu.2404785

23. Ganesan P, Jain H, Bagal B, Subramanian PG, George B, Korula A, et al. Outcomes in adolescent and young adult acute lymphoblastic leukaemia: a report from the Indian acute leukaemia research database (INwARD) of the hematology cancer consortium (HCC). *Br J Haematol* (2021) 193:e1–4. doi: 10.1111/bjh.17268

24. Hein K, Short N, Jabbour E, Yilmaz M. Clinical value of measurable residual disease in acute lymphoblastic leukemia. *Blood Lymphat Cancer* (2022) 12:7–16. doi: 10.2147/BLCTT.S270134

25. Abou Dalle I, Jabbour E, Short NJ. Evaluation and management of measurable residual disease in acute lymphoblastic leukemia. *Ther Adv Hematol* (2020) 11:2040620720910023. doi: 10.1177/2040620720910023

26. Tierens A, Stockley TL, Campbell C, Fulcher J, Leber B, McCready E, et al. Consensus recommendations for MRD testing in adult b-cell acute lymphoblastic leukemia in Ontario. *Curr Oncol* (2021) 28:1376–87. doi: 10.3390/curroncol28020131

27. Jabbour E, Thomas D, Cortes J, Kantarjian HM, O'Brien S. Central nervous system prophylaxis in adults with acute lymphoblastic leukemia: current and emerging therapies. *Cancer* (2010) 116(10):2290–300. doi: 10.1002/cncr.25008

28. Sancho JM, Ribera JM, Oriol A, Hernandez-Rivas J-M, Rivas C, Bethencourt C, et al. Central nervous system recurrence in adult patients with acute lymphoblastic leukemia: frequency and prognosis in 467 patients without cranial irradiation for prophylaxis. *Cancer* (2006) 106:2540–6. doi: 10.1002/cncr.21948

29. Pui CH. Central nervous system disease in acute lymphoblastic leukemia: prophylaxis and treatment. *Hematol Am Soc Hematol Educ Program* (2006), 142–6. doi: 10.1182/asheducation-2006.1.142

30. *NCCN guidelines for acute lymphoblastic leukemia* . Available at: https://www.nccn.org/patients/guidelines/content/PDF/all-patient.pdf (Accessed 23rd September 23, 2022).

31. Fuster JL. Current approach to relapsed acute lymphoblastic leukemia in children. *World J Hematol* (2014) 3:49–70. doi: 10.5315/wjh.v3.i3.49

32. Gökbuget N, Stanze D, Beck J, Diedrich H, Horst H-A, Hüttmann A, et al. Outcome of relapsed adult lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic factors, and performance of stem cell transplantation. *Blood* (2012) 120:2032–41. doi: 10.1182/blood-2011-12-399287

33. Fielding AK, Richards SM, Chopra R, Lazarus HM, Litzow MR, Buck G, et al. Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG 2993 study. *Blood* (2007) 109:944–50. doi: 10.1182/blood-2006-05-018192

34. Oriol A, Vives S, Hernández-Rivas JM, Tormo M, Heras I, Rivas C, et al. Outcome after relapse of acute lymphoblastic leukemia in adult patients included in four consecutive risk-adapted trials by the PETHEMA study group. *Haematologica* (2010) 95:589–96. doi: 10.3324/haematol.2009.014274

35. Tavernier E, Boiron JM, Huguet F, Bradstock K, Vey N, Kovacsovics T, et al. Outcome of treatment after first relapse in adults with acute lymphoblastic leukemia initially treated by the LALA-94 trial. *Leukemia* (2007) 21:1907–14. doi: 10.1038/sj.leu.2404824

36. Thomas DA, Kantarjian H, Smith TL, Koller C, Cortes J, O'Brien S, et al. Primary refractory and relapsed adult acute lymphoblastic leukemia: characteristics, treatment results, and prognosis with salvage therapy. *Cancer* (1999) 86:1216–30. doi: 10.1002/(sici)1097-0142(19991001)86:7<1216::aid-cncr17>3.0.co;2-o

37. Topp MS, Gökbuget N, Zugmaier G, Klappers P, Stelljes M, Neumann S, et al. Phase II trial of the anti-CD19 bispecific T cell-engager blinatumomab shows hematologic and molecular remissions in patients with relapsed or refractory b-precursor acute lymphoblastic leukemia. *J Clin Oncol* (2014) 32:4134–40. doi: 10.1200/JCO.2014.56.3247

38. Topp MS, Gökbuget N, Stein AS, Zugmaier G, Brien S, Bargou RC, et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory b-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. *Lancet Oncol* (2015) 16:57–66. doi: 10.1016/S1470-2045(14)71170-2

39. Kantarjian H, Stein A, Gökbuget N, Wei AH, Durrant S, Bacon CL, et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. *N Engl J Med* (2017) 376(9):836–47. doi: 10.1080/10428194.2019.1576872

40. Kantarjian H, Thomas D, Jorgensen J, Jabbour E, Kebriaei P, Rytting M, et al. Inotuzumab ozogamicin, an anti-CD22-calecheamicin conjugate, for refractory and relapsed acute lymphocytic leukaemia: a phase 2 study. *Lancet Oncol* (2012) 13:403–11. doi: 10.1016/S1470-2045(11)70386-2

41. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. *N Engl J Med* (2016) 375:740–53. doi: 10.1056/NEJMoa1509277

42. Martinelli G, Boissel N, Chevallier P, Ottmann O, Gökbuget N, Topp MS, et al. Complete hematologic and molecular response in adult patients with relapsed/refractory Philadelphia chromosome-positive b-precursor acute lymphoblastic leukemia following treatment with blinatumomab: results from a phase II, single-arm, multicenter study. *J Clin Oncol* (2017) 35:1795–802. doi: 10.1200/JCO.2016.69.3531

43. Jabbour E, Gökbuget N, Advani A, Stelljes M, Stock W, Liedtke M, et al. Impact of minimal residual disease status in patients with relapsed/refractory acute lymphoblastic leukemia treated with inotuzumab ozogamicin in the phase III INO-VATE trial. *Leuk Res* (2020) 88:106283. doi: 10.1016/j.leukres.2019.106283

44. DeAngelo DJ, Advani AS, Marks DI, Stelljes M, Liedtke M, Stock W, et al. Inotuzumab ozogamicin for relapsed/refractory acute lymphoblastic leukemia: outcomes by disease burden. *Blood Cancer J* (2020) 10(8):81. doi: 10.1038/s41408-020-00345-8

45. *Central drugs standard control organization (CDSCO): list of drugs imported and marketed in India* . Available at: https://cdsco.gov.in/opencms/resources/UploadCDSCOWeb/2018/UploadBiologicalrDNA/jan20Form45.pdf (Accessed November 22, 2022).

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 08 May 2023
DOI 10.3389/fonc.2023.1135160

OPEN ACCESS

EDITED BY
Jean El Cheikh,
American University of Beirut Medical Center, Lebanon

REVIEWED BY
Fabian Beier,
University Hospital RWTH Aachen, Germany
Tim Olson,
Children's Hospital of Philadelphia, United States
Babu Rao Vundinti,
National Institute of Immunohaematology (ICMR), India
Davut Albayrak,
Ondokuz Mayıs University, Türkiye

*CORRESPONDENCE
Riad El Fakih
riadfakih@hotmail.com

RECEIVED 31 December 2022
ACCEPTED 06 April 2023
PUBLISHED 08 May 2023

CITATION
Nassani M, Fakih RE, Passweg J, Cesaro S, Alzahrani H, Alahmari A, Bonfim C, Iftikhar R, Albeihany A, Halkes C, Ahmed SO, Dufour C and Aljurf M (2023) The role of androgen therapy in acquired aplastic anemia and other bone marrow failure syndromes. *Front. Oncol.* 13:1135160. doi: 10.3389/fonc.2023.1135160

The role of androgen therapy in acquired aplastic anemia and other bone marrow failure syndromes

Momen Nassani[1], Riad El Fakih[1]*, Jakob Passweg[2], Simone Cesaro[3], Hazzaa Alzahrani[1], Ali Alahmari[1], Carmem Bonfim[4], Raheel Iftikhar[5], Amal Albeihany[1], Constantijn Halkes[6], Syed Osman Ahmed[1], Carlo Dufour[7] and Mahmoud Aljurf[1]

[1]Department of Hematology, Stem Cell Transplant & Cellular Therapy, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia, [2]Department of Hematology, University Hospital Basel, Basel, Switzerland, [3]Pediatric Hematology Oncology, Azienda Ospedaliera Universitaria Integrata, Verona, Italy, [4]Transplantation Unit, Department of Hematology, Hospital de Clinicas, Federal University of Parana, Curitiba, Brazil, [5]Department of Hematology, Armed Forces Bone Marrow Transplant Centre, Rawalpindi, Pakistan, [6]Department of Hematology, Leiden University Medical Center, Leiden, Netherlands, [7]Hematology Unit, Hemato.Oncology Department, IRCCS, G. Gaslini Children Research Institute, Genova, Italy

Bone marrow failure syndromes are a heterogeneous group of diseases. With the major advancements in diagnostic tools and sequencing techniques, these diseases may be better classified and therapies may be further tailored. Androgens, a historic group of drugs, were found to stimulate hematopoiesis by enhancing the responsiveness of progenitors. These agents have been used for decades to treat different forms of bone marrow failure. With the availability of more effective pathways to treat BMF, androgens are less used currently. Nevertheless, this group of drugs may serve BMF patients where standard therapy is contraindicated or not available. In this article, we review the published literature addressing the use of androgens in BMF patients and we make recommendations on how to best use this class of drugs within the current therapeutic landscape.

KEYWORDS

androgen, aplastic anemia (AA), bone marrow failure (BMF), hematopoiesis, anabolic steroid

Introduction

Aplastic anemia (AA) is a term used to describe a group of heterogeneous syndromes that affect hematopoiesis and result in bone marrow failure (1). It can be inherited, acquired or a result of exposure to certain toxins. This syndrome was first described by the German physician Paul Ehrlich in 1885 (1). Back then, it used to be a fatal condition due to

uncontrolled bleeding or overwhelming infections. The advancement in allogeneic stem cell transplantation and immunosuppressive treatment in the 20th century changed the outcome of the disease dramatically (2, 3). The long term overall survival of bone marrow failure (BMF) syndromes have remarkably improved in the modern era and can exceed 90% (4). Trials comparing transplant to conventional therapies (including androgens) showed clear survival benefit of transplant compared to conventional therapies (5, 6). Nevertheless, limitations and challenges in the management of aplastic anemia and BMF still exist. Allogeneic stem cell transplant may not be feasible for all patients for a number of reasons, especially in countries with limited resources. Alternatives to transplantation include immunosuppressive therapy, growth factors, thrombopoietin agonists and androgen therapy. The objective of this article is to provide a comprehensive and systemic evaluation of the published literature regarding the use of androgens in acquired and to a lesser extent inherited bone marrow failure syndromes.

History of androgen use in aplastic anemia

In the early days, the management of AA patients consisted of supportive transfusions in addition to other interventions with questionable efficacy (steroids, splenectomy, vitamins, etc.) (7). The first indications about the possible efficacy of androgens in bone marrow failure, were the spontaneous remission in two boys upon pubescence and the development of myeloid metaplasia in a patient taking testosterone for breast cancer (7). A small report followed, where five AA patients who failed steroids and transfusion were given testosterone and four of them achieved remarkable responses in hemoglobin levels and transfusion independence along with variable response in neutrophils and platelets (7). A number of publications were reported thereafter supporting the role of testosterone and anabolic steroids in aplastic anemia (8–11). Overall, around 70% of patients on these studies achieved a hemoglobin above 12 g/dl along with improvement in the platelet and neutrophil numbers. Another multicenter trial recruited 45 patients with hypoproliferative or aregenerative anemia treated with oxymetholone for a minimum of 3 months. Patients with hypocellular marrow had the best response (12). In 1976, a prospective randomized clinical trial showed superiority of allogeneic bone marrow transplantation compared to standard of care, which included androgen therapy (5). A clear reduction in mortality was reported in the transplanted group compared to the non-transplant group. Currently, with the consistent encouraging results of transplant over the last decades, HSCT is considered the standard of care for young, fit SAA patients with available donors. Other trials compared different compounds of anabolic steroids. One of these trials, showed better outcome with methandrostenolone compared to other types of anabolic steroids, while methanolone was associated with the worst response and survival rates in this study (13). In another study, where 125 patients with AA were randomized to receive four different androgens (norethandrolone 1 mg/kg/day, fluoxymesterone 1 mg/kg/day, stanozolol 1mg/kg/day and testosterone undecanoate 1.7 mg/kg/day) fluoxymesterone treated group had the best overall survival. The worst survival rate was in patients on stanozolol (14).

Testosterone and anabolic androgenic steroids

The body building action of androgens and their euphoric action on the brain led to widespread illicit use of AAS. Hence, all AAS were designated as class III controlled substances. Nevertheless, these agents have shown significant benefits in a number of disorders. Testosterone was first discovered in 1935 and found to have effects on both reproductive (androgenic) and non-reproductive tissues (anabolic). It has been used in different catabolic states due to its anabolic effects through nitrogen fixation and as such protein synthesis (15). Virilization, on the other hand, is one of the unwanted side effects. Numerous derivatives have been developed aiming to prolong its biological activity, increase its anabolic effects and decrease the androgenic side effects. These derivatives are commonly known as anabolic androgenic steroids (AAS) (15). More than a hundred synthetic products have been developed by different reactions (17 α-alkylation, 17 β-esterification, etc.) to overcome the rapid biotransformation of testosterone and synthesize orally active longer acting compounds (9, 15). Oxandrolone, oxymetholone and nandrolone are commonly used AAS, whereas danazol (2,3-isoxazol-17α-ethynyltestosterone) is a synthetic steroid with antiestrogenic, antigonadotropic, and androgenic activities (15). Hepatotoxicity is a potential side effect of anabolic steroids traditionally observed with the 17 α-alkylated compounds (methyltestosterone, oxymetholone, oxandrolone, norethandrolone, etc.) (16). Around a quarter of patients may experience elevation in liver function tests while on therapy and liver tumors are not uncommon (9). Early studies reported fatality cases from liver disease and jaundice; however, it is unclear if these were due to the anabolic steroids or other potential complications observed in patients with BMF like viral hepatitis, iron overload. The optimal recommended dose of androgens is not well defined; however, it is well known that patients who do not respond to a certain dose may achieve remission using a higher dose of the same product. The recommended doses of the commonly used products are: 2.5 mg/kg/d for oxymetholone and methanolone and 1 mg/kg/d for methandrostenolone and Norethandrolone (9, 13). Given the biological effects of AAS, serious adverse events can happen (masculinization, aggression, liver dysfunction and adenomas among others). Close medical supervision and dose adjustment to the minimal effective dose is recommended. Androgens should be avoided in pregnant women, cancer patients (prostate, breast, etc.), patients with nephrotic syndrome or liver dysfunction and patients with hypercalcemia of malignancy. The androgen side effects (flushing, acne, hirsutism, change in voice, others) usually disappear quickly after discontinuation (17). Table 1 is a summary of side effects and authors' recommendations on how to mitigate these.

TABLE 1 Potential side effects of androgens and authors' recommendations on how to mitigate these.

Side effect	Comments	Mitigation plan
Virilization, Gain of body musculature	Testosterone causes masculinization, flushing of skin and acne, deepening and hoarsening of voice, changes in external genitalia	Use the smallest effective dose whenever possible
Jaundice, hepatotoxicity, hepatoma, hepatocellular carcinoma, Peliosis hepatis	Peliosis hepatis: blood filled enlarged sinusoids and cysts focally or throughout the liver	Monitor Liver enzymes every 3 months Initial Screen for hepatitis B, C, Ferritin Initial screening by liver US and every 6 months thereafter Avoid concurrent hepatotoxic medications Consider Using non 17 alpha alkylated androgens
Polycythemia	Monitor hemoglobin	Slow tapering of androgens once normal hemoglobin is achieved
Hyperlipidemia	Lipid profile return to normal within one month after stopping androgens	Dietary advice to avoid excessive fat intake Avoid other cardiovascular risk factors Exercise Consider statin
Psychiatric and behavioral effects	Monitor for psychiatric symptoms	Use the smallest effective dose whenever possible

Immunosuppression and androgens therapy in aplastic anemia

A randomized trial compared anti-lymphocyte globulin (ALG) (with androgens and haploidentical HSCT vs. Androgens alone) showed 76% vs. 31% survival respectively at two years (p <0.002) (18). Another trial randomized 121 patients to receive anti-thymocyte globulin (ATG) alone or ATG with androgens showed similar response (44% vs 42% respectively) and survival rates (19). In a subsequent trial, 15 patients received ATG and methanolone and 15 patients received ATG alone (20). The response rate was 73% in the combination group with eight complete responses compared to 33% in the ATG alone group with two complete responses (P = 0.01). The difference in survival (87% in the combination arm vs 43%) was not statistically significant. Shahidi et al. treated 23 AA patients with oxymetholone and cyclosporine combination. Thirteen of these patients had already received ATG and did not respond and the remaining 10 did not receive ATG. The response rate was 38% and 70% respectively (21). Further, a randomized controlled trial showed significant difference in responses among males and females to androgens, where females with low neutrophil counts had significant benefit from ATG combined with androgens compared with ATG alone (22). Bacigalupo et al. randomized 134 patients to ALG and methylprednisolone with or without oxymetholone. At 4 months, the response rate with significantly higher in patients who received oxymetholone (56% vs 40%; P < 0.04) (22). In a relatively recent report, Jaime-Pérez et al. reported the outcomes of fifty AA patients (23). Thirteen patients were transplanted and 37 patients were not eligible to transplant, had no access to IST, and as such were treated with danazol (median dose 400 mg) and supportive measures. The five-year OS was in favor of the transplant group (92% vs 41%, p = 0.001). The ORR in the danazol group was 46% with a median time to respond of 3 months. Although, transplant and IST are highly efficacious in AA, androgens continue to be an option, even in the frontline, for transplant ineligible patients with no access to modern IST.

Androgens for other BMF syndromes

Congenital bone marrow failure syndromes are a heterogeneous group of cytopenias associated with various congenital defects and cancer predisposition. HSCT is the only curative treatment for the hematological complications related to these diseases, but many patients are ineligible and androgens are considered the main non-transplant modality to treat these patients. Early studies of using androgens in these patients showed favorable responses (8). Oxymetholone and danazol are frequently used for Fanconi anemia (FA) and dyskeratosis congenita (DC) patients with responses reaching up to 80% (24–26). Diamond-Blackfan anemia patients are usually treated with steroids but many of them fail steroids (tolerance, side effects, relapse) and eventually receive androgens (24). Oxymetholone is the androgen of choice for congenital anemias with a starting dose of 0.5-2 mg/kg/day. Expectedly, response starts within 4-8 weeks and once a hemoglobin concentration of 12 g/dl is reached the dose is reduced gradually to the minimum effective dose to maintain hemoglobin between 10-12 g/dl (25). In paroxysmal nocturnal hemoglobinuria (PNH), a rare acquired clonal stem cell disorder, androgens were efficacious to treat the anemia part of the disease especially in patients with hypoplasia (27–29). However, androgens have no effect on hemolysis and their impact on thrombogenesis need to be watched closely in these patients.

Fanconi anemia

Fanconi anemia is the prototype of inherited bone marrow failure syndromes characterized by a number of mutations leading to genetic instability and multiple cancer susceptibility with frequent incidence of marrow failure and myelodysplasia (30–32). Clinically, FA patients are characterized by diverse congenital malformations (small stature, skeletal malformations, hyperpigmentation, urogenital abnormalities, etc.) (32). In a small cohort of FA patients, seven out of eight patients responded to danazol 5 mg/kg/day and had stable counts up to 3 years (33). In another cohort, seven out nine patients treated with oxandrolone had a hematologic response with major side effects being elevated liver function tests and virilzation (34). A retrospective trial analyzed 70 patients who received androgens for FA. Out of 70 patients, 37 were evaluable. Oxymetholone was the most frequently used androgen. Hematologic response was seen in 25 out 37 evaluable patients (68%) with a median of 6.5 g/dl improvement in hemoglobin (median time to respond of 14 weeks), a median of 70000 platelet count increase (median time to respond of 11.5 weeks) and a median of 1350/ml improvement in neutrophils (median time to respond of 12 weeks) (35). Virilization, liver toxicity, liver adenomas and clonal evolution were the most frequently reported adverse events. In the largest retrospective trial addressing androgen use in FA patients, 66 patients were reported; 49 received oxymetholone and 17 received danazol (36). Danazol was started at a dose of 2-4 mg/kg/day and oxymetholone was started at as dose of 0.5-1 mg/kg/day. After a median duration of therapy of 18 months, 52 patients (78%) achieved hematologic response and 30 patients (45%) had trilineage response. There was no difference in response rates between danazol and oxymetholone. Seven patients (11%) developed grade 3 liver toxicity that was noticed more in patients on oxymetholone. Peliosis hepatis developed in one patient on oxymetholone. The majority of patients developed virilzation signs. These reports included patients from different age groups (age range: 3 – 22 years).

Androgens for telomeropathies

Telomeres are essential for genomic stability but their length decreases with each cellular division. Dyskeratosis congenita (DC) is the prototype of telomeropathies. Androgens improve blood counts and reduce transfusion frequency in telomeropathies. Some studies have shown improvement in telomere length as well (37–41), although other reports did not confirm this finding (42). Apparently, the improvement in telomere length depends on the underlying mutational profile (41). In a phase 1/2 study, danazol (800 mg/day) was administered to patients with telomeropathies. In 12 evaluable patients, telomere elongation was achieved in all. Hematologic response was seen in 19 of 24 evaluable patients (79%) after 3 months of therapy. Liver toxicity (41%) and muscle cramps (33%) were the most frequently reported side effects (37). In another study, 26 DC patients were followed prospectively. Ten patients received androgens and 16 were not treated. There was no statistical difference in telomere length in the two groups (42). Seven out of the 10 treated patients had a consistent RBC and platelets response to androgens. In a more recent report, seven patients were treated with androgens (danazol, oxymetholone). All patients had hematological response as well as significant increase in telomere length (41). An ongoing trial (clinicaltrials.gov NCT02055456) is evaluating nandrolone decanoate (parenteral androgen with no first hepatic pass) in patients with telomeropathies. These reports included patients from different age groups (age range: 3 – 66 years). Table 2 is a summary of androgen studies in AA and BMF syndromes.

Mechanism of action and effects on hematopoiesis

The exact mechanism of action of androgens in stimulating hematopoiesis remains unknown, however it seems that the use of supra-physiological doses of androgens cause erythropoiesis expansion. This is in contrast to hematinic supplements (iron, folate) where excess supply will not cause excessive response in hematopoiesis. Earlier studies showed that androgens enhance the responsiveness of erythroid progenitors to erythropoietin and possibly enhance the growth of pluripotent and committed granulocyte/macrophage progenitors (19). Some of the anabolic steroids have unique properties, for example, danazol inhibits interleukin-1 and TNF-α production (a property of corticosteroids) and has myelosuppressive effects (43). More recent studies showed that androgens stimulate erythropoietin production and release, activation of the erythropoietin receptor on progenitor cells and increase iron incorporation into the red cells (44, 45). Additionally, androgens increase telomerase activity in hematopoietic cells (39). Lately, a study showed that the chronic use of oxymetholone improves hematological parameters by diminution of quiescence and promotion of proliferation of hematopoietic progenitors and stem cells. Oxymetholone down regulated the transcription of osteopontin, a cytokine that up-regulates the expression of certain interferons and interleukins, which inhibits cellular proliferation. Hence, it was proposed that oxymetholone suppression of osteopontin transcription induces hematopoietic stem cell cycling (46). The earliest phase of erythroid response is characterized by erythrocytosis that happens shortly after initiating androgen therapy followed by delayed improvement in hemoglobin. This delay is probably related to an initial increase in erythrocyte destruction while patients are still transfusion dependent. Usually, the improvement of hemoglobin may be observed as early as 3 months or as late as 6 months after starting androgen therapy (13). The increase in neutrophils usually mirrors the response of hemoglobin. The response rate of neutrophils was found to be 35.8% in one case series (8, 13). Platelets are the latest to improve and usually show less prominent improvement (13). The response rate of platelets is observed in about one-third of cases. In one study, normal platelets were achieved in 18 out of 67 patients (8, 13). Of note that despite the minimal improvement in the numbers of

TABLE 2 Important studies about androgens in BMF patients.

Author and date	Number of patients	Disease	Androgen	Response	Comments
Camitta 1983 (18)	13	Severe acquired aplastic anemia	Oxymetholone vs. Antithoracic duct lymphocyte globulin (ATDLG)	31% OS in androgen arm Vs. 76% in ATG group	ATG compared to androgen
Champlin R 1985 (19)	52	Moderate to severe Aplastic anemia	ATG+Androgen (oral androgen oxymetholone or fluoxymesterone) Vs ATG+placebo	ATG+Androgen ORR 42% ATG+Placebo ORR 44% P:>0.9 OS: 55 vs 50% P:.65	
Kaltwasser J 1988 (20)	30	Aplastic anemia	ATG with or without oral androgen (Methenolone)	ATG+androgen ORR73% ATG alone ORR =31% P=0.01 OS=87% vs 43% p=0.15	
Shahidi N 1990 (21)	23	Aplastic anemia	oxymetholone and cyclosporine	Post ATG failure ORR: 38% Not exposed to ATG ORR 70%	
Bacigalupo A 1993 (22)	134 (69/65)	Acquired aplastic anemia	HALG + methylprednisolone with or without oxymetholone (RCT)	ORR: 68% vs. 48% For females: 78% vs. 27%	RCT Response to androgen is more prominent in females with low ANC
Shahidi NT 1961 (8)	7	Inherited aplastic anemia Fanconi anemia	Testosterone + steroid	Increase reticulocytosis 7/7 Transfusion independence 6/7	Most patients needed to stay on low dose therapy
Scheckenbach K 2012 (33)	8	Fanconi anemia	Danazol	ORR: 7/8	
Rose SR 2014 (34)	9	Fanconi Anemia	Oxandrolone	ORR 7/9	
Paustian L 2016 (35)	37	Fanconi anemia	Oxymetholone Danazol Methonolone Enanthate Norethandrolone	ORR: 68%	Median time to response 14 weeks
Ribeiro L 2015 (36)	66	Fanconi anemia	Oxymetholone, danazol	ORR 78%	
Townsley DM 2016 (37)	27	telomere diseases	Danazol	ORR 79%	All evaluable 12 patients had a gain in telomere length at 24 months as compared with baseline
HARTMANN RC 1966 (28)	6	PNH	flyoxymesterone	ORR 5/6	Improved anemia in responders with persistent low grade hemolysis
Halder R 2020 (29)	20 pediatric patients	Classical PNH anemia with hemolysis, normal platelets and neutrophils	Danazol or stanazolol	ORR 80% CR 30%	
Jaime-Pérez 2011 (23)	50 patients	Aplastic anemia	Danazol Vs Allogenic transplantation	ORR in danazol group =46% OS (92% vs 41%, p = 0.001). in favor of transplant	

(Continued)

TABLE 2 Continued

Author and date	Number of patients	Disease	Androgen	Response	Comments
Payal P. Khincha 2018 (42)	10 patients treated with androgen vs 16 untreated	dyskeratosis congenita	oxymetholone danazol, halotestin	ORR 7/10	There was no statistical difference in telomere length among two groups
Martin Kirschner 2021 (41)	7 patients	dyskeratosis congenita	danazol, oxymetholone	ORR 7/7	All patients had hematological response as well as significant increase in telomere length

neutrophils and platelets, the clinical benefit, in terms of less bleeding and infections is disproportionately higher (9). Responding patients can achieve a normal hemoglobin level and a protective platelets and neutrophils levels (8). The bone marrow examination of patients receiving androgens show groups of growing stromal cells, reduction of fat cells and the appearance of erythroid hyperplasia foci. These changes are usually seen within 3 months of treatment initiation (7). In summary, response to androgens starts 3 to 6 months after treatment initiation and responding patients may enjoy durable response in around 50% of cases while the other 50% will relapse with relapse being higher in rapidly tapered patients (< 3 months) (9, 17). A second remission can be achieved with re-treatment but these patients will need continuous androgen therapy thereafter (17). Upon withdrawal of androgens, the reticulocytes and hemoglobin drop within the first month, and then stabilize during the second month with similar effects in the neutrophils and platelets.

Practical author's recommendations and clinical scenarios

The diagnostic armamentarium is expanding with the use of new and more comprehensive sequencing and molecular techniques. These new diagnostic tools help to better delineate these syndromes and to identify more patients with cryptic alterations who may not benefit from immunosuppression. A number of innovative ideas and approaches (gene therapy, leucine, quercetin, etc.) to address the unmet needs of these patients are in ongoing trials. Bone marrow failure patients who are not candidates for transplant (elderly, comorbidities, no donors, etc.) and patients with the non-severe forms are usually defaulted to non-transplant medical interventions. In affluent countries, a number of non-transplant options are available for this group of patients. However, in restricted resources countries these novel options are not readily available. Androgens are considered a classic old group of medications that can stimulate hematopoiesis and as such used for this group of disorders when modern resources are not available or have failed already. Androgens result in hematologic responses (transient in some patients however) in most patients with FA and telomeropathies but does not alter nor affect the risk of clonal evolution. A number of pros and cons have to be considered when a decision is made to treat a BMF patient with non-transplant modalities including the use of androgens. Factors linked to a potential better response to androgens include higher residual cellularity, mild to moderate cytopenia, toxin induced BMF, women with lower absolute neutrophil count, the use of higher dose of androgens and rapid improvement of counts after androgen initiation (9, 13, 14). The presence of a number of these factors in a patient may sway the managing team to consider androgens, while the presence of contraindications may push the team to consider alternatives. Below are selected scenarios in which the authors' believe androgen therapy should be considered.

- As a bridge to transplant in patients with symptomatic FA and telomeropathies
- Older SAA patients, not candidate for HSCT and failed standard immunosuppression therapy (IST) and thrombopoietin mimetic (TPO) or TPO inaccessible
- Patients with renal failure precluding the use of calcineurin inhibitors and failed TPO mimetic or TPO inaccessible
- Multiply relapsed patients after failing standard lines of therapy
- PNH patients with no access to complement inhibitors and parallel existing AA (AA/PNH overlap)

Currently, there are no published trials looking into the safety, efficacy and different dosages of various AAS formulations to guide the clinicians' choice when treating BMF patients. New trials looking into these issues are warranted.

Author contributions

MA, RE, and MN designed and performed the research. MN wrote the first draft. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Young NS. Aplastic anemia. *New Engl J Med* (2018) 379(17):1643–56. doi: 10.1056/NEJMra1413485

2. El Fakih R, Alfraih F, Alhayli S, Ahmed SO, Shaheen M, Chaudhri N, et al. Frontline-matched sibling donor transplant of aplastic anemia patients using primed versus steady-state bone marrow grafts. *Ann Hematol* (2022) 101(2):421–8. doi: 10.1007/s00277-021-04708-4

3. Bacigalupo A, Hows J, Gluckman E, Nissen C, Marsh J, Van Lint M, et al. Bone marrow transplantation (BMT) versus immunosuppression for the treatment of severe aplastic anaemia (SAA): a report of the EBMT* SAA working party. *Br J haematol* (1988) 70(2):177–82. doi: 10.1111/j.1365-2141.1988.tb02460.x

4. Scheinberg P. Aplastic anemia: therapeutic updates in immunosuppression and transplantation. *Hematol 2010 Am Soc Hematol Educ Program Book.* (2012) 2012 (1):292–300. doi: 10.1182/asheducation.V2012.1.292.3798310

5. Camitta BM, Thomas ED, Nathan DG, Santos G, Gordon-Smith E, Gale RP, et al. Severe aplastic anemia: a prospective study of the effect of early marrow transplantation on acute mortality. *Blood.* (1976) 48:63–70. doi: 10.1182/blood.V48.1.63.bloodjournal48163

6. Camitta BM, Thomas ED, Group IAAS. Severe aplastic anaemia: a prospective study of the effect of androgens or transplantation on haematological recovery and survival. *Clinics haematol* (1978) 7(3):587–95. doi: 10.1016/S0308-2261(21)00051-5

7. Shahidi NT, Diamond LK. Testosterone-induced remission in aplastic anemia. *AMA J Dis Children* (1959) 98(3):293–302. doi: 10.1001/archpedi.1959.02070020295002

8. Shahidi NT, Diamond LK. Testosterone-induced remission in aplastic anemia of both acquired and congenital types. *Further observations 24 cases N Engl J Med* (1961) 264:953–67. doi: 10.1056/NEJM196105112641901

9. Sanchez-Medal L, Gomez-Leal A, Duarte L, Rico MG. Anabolic androgenic steroids in the treatment of acquired aplastic anemia. *Blood.* (1969) 34(3):283–300. doi: 10.1182/blood.V34.3.283.283

10. Daiber A, Herveé L, Con I, Donoso A. Treatment of aplastic anemia with nandrolone decanoate. *Blood.* (1970) 36(6):748–53. doi: 10.1182/blood.V36.6.748.748

11. Allen DM, Fine MH, Necheles TF, Dameshek W. Oxymetholone therapy in aplastic anemia. *Blood.* (1968) 32(1):83–9. doi: 10.1182/blood.V32.1.83.83

12. Skarberg K, Engstedt L, Jameson S, Killander A, Lundh B, Pers B, et al. OXYMETHOLONE TREATMENT IN HYPOPROLIFERATIVE ANEMIA. 1. FREQUENCY OF RESPONSE. *Acta Haematologica* (1973) 49(6):321–30. doi: 10.1159/000208422

13. Najean Y, Pecking A, Danvic ML, CGftSo A, Secretaries RA. Androgen therapy of aplastic anaemia-a prospective study of 352 cases. *Scandinavian J Haematol* (1979) 22(4):343–56. doi: 10.1111/j.1600-0609.1979.tb00430.x

14. Najean Y. Aplastic fcgftso, anaemias r. androgen therapy in aplastic anaemia: a comparative study of high and low-doses and of 4 different androgens. *Scandinavian J Haematol* (1986) 36(4):346–52.

15. Shahidi NT. A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. *Clin Ther* (2001) 23(9):1355–90. doi: 10.1016/S0149-2918(01)80114-4

16. Al-Sharefi A, Mohammed A, Abdalaziz A, Jayasena CN. Androgens and anemia: current trends and future prospects. *Front Endocrinol* (2019) 10:754. doi: 10.3389/fendo.2019.00754

17. Najean Y. Long-term follow-up in patients with aplastic anemia: a study of 137 androgen-treated patients surviving more than two years. *Am J Med* (1981) 71(4):543–51. doi: 10.1016/0002-9343(81)90204-7

18. Camitta B, O'Reilly RJ, Sensenbrenner L, Rappeport J, Champlin R, Doney K, et al. Antithoracic duct lymphocyte globulin therapy of severe aplastic anemia. *Blood.* (1983) 62:883–8. doi: 10.1182/blood.V62.4.883.bloodjournal624883

19. Champlin R, Ho W, Feig S, Winston D, Lenarsky C, Gale R. Do androgens enhance the response to antithymocyte globulin in patients with aplastic anemia? a prospective randomized trial. *Blood.* (1985) 66(1):184–8. doi: 10.1182/blood.V66.1.184.184

20. Kaltwasser J, Dix U, Schalk K, Vogt H. Effect of androgens on the response to antithymocyte globulin in patients with aplastic anaemia. *Eur J haematol* (1988) 40 (2):111–8. doi: 10.1111/j.1600-0609.1988.tb00806.x

21. Shahidi N, Wang W, Shurin S, Finlay J, Sondel P, Dinndorf P. Treatment of acquired aplastic anemia with cyclosporine and androgens. In: Shahidi NT ed. *Aplastic anemia and other bone marrow failure syndromes*. Springer-Verlag New York Berlin Heidelberg London Paris Tokyo (1990). p. 155–65.

22. Bacigalupo A, Chaple M, Hows J, Van Lint M, McCann S, Milligan D, et al. Treatment of aplastic anaemia (AA) with antilymphocyte globulin (ALG) and methylprednisolone (MPred) with or without androgens: a randomized trial from the EBMT SAA working party. *Br J haematol* (1993) 83(1):145–51. doi: 10.1111/j.1365-2141.1993.tb04645.x

23. Jaime-Pérez JC, Colunga-Pedraza PR, Gómez-Ramírez CD, Gutiérrez-Aguirre CH, Cantú-Rodríguez OG, Tarín-Arzaga LC, et al. Danazol as first-line therapy for aplastic anemia. *Ann Hematol* (2011) 90(5):523–7. doi: 10.1007/s00277-011-1163-x

24. Calado RT, Clé DV. Treatment of inherited bone marrow failure syndromes beyond transplantation. *Hematol 2014 Am Soc Hematol Educ Program Book.* (2017) 2017(1):96–101. doi: 10.1182/asheducation-2017.1.96

25. Glader BE, Guinan E, Lipton JM, Boxer L. Congenital bone marrow failure syndromes: diagnosis and therapeutic strategies. *Am Soc Hematol Educ Program Book Washington DC: Am Soc Hematol* (1998) 1998:384–403.

26. Khincha PP, Wentzensen IM, Giri N, Alter BP, Savage SA. Response to androgen therapy in patients with dyskeratosis congenita. *Br J Haematol* (2014) 165 (3):349–57. doi: 10.1111/bjh.12748

27. Rosse WF. Treatment of paroxysmal nocturnal hemoglobinuria. *Blood.* (1982) 60(1):20–3. doi: 10.1182/blood.V60.1.20.20

28. Hartmann Rc, Jenkins DeJr, Mckee Lc, Heyssel Rm. Paroxysmal nocturnal hemoglobinuria: clinical and laboratory studies relating to iron metabolism and therapy with androgen and iron. *Medicine* (1966) 45(5):331–63. doi: 10.1097/00005792-196609000-00001

29. Halder R, Mishra P, Aggarwal M, Mannivanan P, Dhawan R, Seth T, et al. Outcomes of paroxysmal nocturnal hemoglobinuria in the pediatric age group in a resource-constrained setting. *Pediatr Blood Cancer.* (2020) 67(4):e27712. doi: 10.1002/pbc.27712

30. Antoniou AC, Casadei S, Heikkinen T, Barrowdale D, Pylkäs K, Roberts J, et al. Breast-cancer risk in families with mutations in PALB2. *New Engl J Med* (2014) 371 (6):497–506. doi: 10.1056/NEJMoa1400382

31. D'Andrea AD. Susceptibility pathways in fanconi's anemia and breast cancer. *New Engl J Med* (2010) 362(20):1909–19. doi: 10.1056/NEJMra0809889

32. Auerbach AD. Fanconi anemia and its diagnosis. *Mutat Research/Fundamental Mol Mech Mutagenesis* (2009) 668(1-2):4–10. doi: 10.1016/j.mrfmmm.2009.01.013

33. Scheckenbach K, Morgan M, Filger-Brillinger J, Sandmann M, Strimling B, Scheurlen W, et al. Treatment of the bone marrow failure in fanconi anemia patients with danazol. *Blood Cells Molecules Diseases* (2012) 48(2):128–31. doi: 10.1016/j.bcmd.2011.11.006

34. Rose SR, Kim MO, Korbee L, Wilson KA, Douglas Ris M, Eyal O, et al. Oxandrolone for the treatment of bone marrow failure in fanconi anemia. *Pediatr Blood Cancer.* (2014) 61(1):11–9. doi: 10.1002/pbc.24617

35. Paustian L, Chao MM, Hanenberg H, Schindler D, Neitzel H, Kratz CP, et al. Androgen therapy in fanconi anemia: a retrospective analysis of 30 years in Germany. *Pediatr Hematol Oncol* (2016) 33(1):5–12. doi: 10.3109/08880018.2015.1129567

36. Ribeiro L, Pilonetto D, Bitencourt M. Treatment of bone marrow failure in 49 fanconi anemia patients with oxymetholone. *Rev Bras Hematol Hemoter* (2015) 37 (suppl 1):220.

37. Townsley DM, Dumitriu B, Liu D, Biancotto A, Weinstein B, Chen C, et al. Danazol treatment for telomere diseases. *New Engl J Med* (2016) 374(20):1922–31. doi: 10.1056/NEJMoa1515319

38. Ziegler P, Schrezenmeier H, Akkad J, Brassat U, Vankann L, Panse J, et al. Telomere elongation and clinical response to androgen treatment in a patient with aplastic anemia and a heterozygous hTERT gene mutation. *Ann hematol* (2012) 91:1115–20. doi: 10.1007/s00277-012-1454-x

39. Calado RT, Yewdell WT, Wilkerson KL, Regal JA, Kajigaya S, Stratakis CA, et al. Sex hormones, acting on the TERT gene, increase telomerase activity in human primary hematopoietic cells. *Blood J Am Soc Hematol* (2009) 114(11):2236–43. doi: 10.1182/blood-2008-09-178871

40. Bär C, Huber N, Beier F, Blasco MA. Therapeutic effect of androgen therapy in a mouse model of aplastic anemia produced by short telomeres. *Haematologica.* (2015) 100(10):1267. doi: 10.3324/haematol.2015.129239

41. Kirschner M, Vieri M, Kricheldorf K, Ferreira MSV, Wlodarski MW, Schwarz M, et al. Androgen derivatives improve blood counts and elongate telomere length in adult cryptic dyskeratosis congenita. *Br J Haematol* (2021) 193(3):669–73. doi: 10.1111/bjh.16997

42. Khincha PP, Bertuch AA, Gadalla SM, Giri N, Alter BP, Savage SA. Similar telomere attrition rates in androgen-treated and untreated patients with dyskeratosis congenita. *Blood advances* (2018) 2(11):1243–9. doi: 10.1182/bloodadvances.2018016964

43. Chuhjo T, Yamazaki H, Omine M, Nakao S. Danazol therapy for aplastic anemia refractory to immunosuppressive therapy. *Am J hematol* (2008) 83(5):387–9. doi: 10.1002/ajh.21118

44. Shahani S, Braga-Basaria M, Maggio M, Basaria S. Androgens and erythropoiesis: past and present. *J endocrinological Invest* (2009) 32(8):704–16. doi: 10.1007/BF03345745

45. Maggio M, Snyder P, Ceda G, Milaneschi Y, Luci M, Cattabiani C, et al. Is the haematopoietic effect of testosterone mediated by erythropoietin? the results of a clinical trial in older men. *Andrology*. (2013) 1(1):24–8. doi: 10.1111/j.2047-2927.2012.00009.x

46. Zhang Q-S, Benedetti E, Deater M, Schubert K, Major A, Pelz C, et al. Oxymetholone therapy of fanconi anemia suppresses osteopontin transcription and induces hematopoietic stem cell cycling. *Stem Cell Rep* (2015) 4(1):90–102. doi: 10.1016/j.stemcr.2014.10.014

frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 06 September 2023
DOI 10.3389/fonc.2023.1193978

OPEN ACCESS

EDITED BY
Giuseppe Maurizio Campo,
University of Messina, Italy

REVIEWED BY
Kevin Dzobo,
University of Cape Town, South Africa

*CORRESPONDENCE
Rong Sun
doctorsunrong818@163.com
Yang Wu
surgeonyang@outlook.com
Xiaoyan Zhang
877307375@qq.com

†These authors have contributed equally to this work

RECEIVED 27 March 2023
ACCEPTED 14 August 2023
PUBLISHED 06 September 2023

CITATION
Ding Z, Shi R, Hu W, Tian L, Sun R, Wu Y and Zhang X (2023) Cancer-associated fibroblasts in hematologic malignancies: elucidating roles and spotlighting therapeutic targets.
Front. Oncol. 13:1193978.
doi: 10.3389/fonc.2023.1193978

Cancer-associated fibroblasts in hematologic malignancies: elucidating roles and spotlighting therapeutic targets

Ziyang Ding[1†], Run Shi[2†], Weikang Hu[3†], Lei Tian[3], Rong Sun[4*], Yang Wu[3*] and Xiaoyan Zhang[5*]

[1]The Second Clinical School of Nanjing Medical University, Nanjing, China, [2]Department of Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China, [3]Pancreas Center, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China, [4]Department of Radiation Oncology, Jinling Hospital, Nanjing, China, [5]Department of Hematology, Tongren Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Hematologic malignancies comprise a diverse range of blood, bone marrow, and organ-related disorders that present significant challenges due to drug resistance, relapse, and treatment failure. Cancer-associated fibroblasts (CAFs) represent a critical component of the tumor microenvironment (TME) and have recently emerged as potential therapeutic targets. In this comprehensive review, we summarize the latest findings on the roles of CAFs in various hematologic malignancies, including acute leukemia, multiple myeloma, chronic lymphocytic leukemia, myeloproliferative neoplasms, and lymphoma. We also explore their involvement in tumor progression, drug resistance, and the various signaling pathways implicated in their activation and function. While the underlying mechanisms and the existence of multiple CAF subtypes pose challenges, targeting CAFs and their associated pathways offers a promising avenue for the development of innovative treatments to improve patient outcomes in hematologic malignancies.

KEYWORDS

cancer associated fibroblast (CAF), hematologic malignancies, crosstalk, chemoresistance, therapeutic target

Introduction

Hematologic malignancies encompass a diverse array of blood, bone marrow, and organ-related disorders. Presently, leukemias and lymphomas can be treated using drugs or drug combinations, such as chemotherapy, targeted therapies, immunotherapy, immune checkpoint inhibitors, and chimeric antigen receptor-T (CAR-T) cells. These treatments have significantly enhanced patient prognoses. However, emerging drug resistance poses a major challenge, leading to relapse and treatment failure (1).

CAFs constitute the largest proportion of stromal cells in the tumor microenvironment (TME) (2). The origin of CAFs remains a subject of debate, with fibroblasts and mesenchymal stem cells (MSCs) from bone marrow (BM) and adipose tissue reservoirs believed to be their primary source (3). No specific markers exist for CAFs, although elevated alpha-smooth muscle actin (αSMA) expression is considered indicative of activated CAFs (4). Exhibiting enhanced proliferative and migratory capabilities, CAFs significantly influence tumor progression (5). Numerous studies have established the critical role of CAFs in solid tumors such as pancreatic, breast, colon, gastric, and liver malignancies (6–10). Further research has also explored targeting mechanisms like the TGFβ signaling pathway and the JAK/STAT signaling pathway (11, 12). The significance of CAFs in tumor progression and drug resistance is increasingly acknowledged, making them a focal point of recent research. Promisingly, several CAF-targeting therapies have entered clinical trials (5, 13).

The intricate interplay between CAFs and cancer cells is crucial for their interaction and is evident in hematologic tumors as well (Figure 1). For instance, bone marrow stromal cells can adopt CAF phenotypes, with the latter secreting various cytokines to stimulate tumor cell growth, infiltration, and endosteal niche reconstruction. Concurrently, TME remodeling provides tumor stem cells additional time for clonal reproduction, resulting in the continuous emergence of new genetic mutations that drive disease progression. This CAF-mediated remodeling also contributes to drug resistance, relapse, and tumor cell progression. In this article, we provide a comprehensive review of recent literature and summarize the roles of CAFs in hematologic tumors, as well as their potential value in disease treatment.

CAFs in acute leukemia (AL)

AL is characterized by the abnormal differentiation and proliferation of hematopoietic stem cells, which impedes normal hematopoiesis (14). Zhai et al. found that the presence of abundant reticulin fibers was associated with poor outcomes in acute myeloid leukemia (AML) (15). Their study showed that CAFs expressing elevated levels of FSP1, αSMA, or FAP protein were extensively distributed within the bone marrow (BM) of AML patients. They also proposed that CAFs could potentially shield leukemia cell lines (THP-1/K-562) from chemotherapy (15). By targeting growth differentiation factor-15 (GDF15) or suppressing GDF15 expression, the sensitivity of leukemic cells to chemotherapy increased, suggesting that GDF15 secretion by CAFs may play a crucial role in mediating the chemoprotective effects of CAFs (15).

Pan et al. carried out a series of investigations on CAFs in B-cell acute lymphoblastic leukemia (B-ALL) (16). They discovered that in newly diagnosed and relapsed B-ALL patients, bone marrow mononuclear cells had a higher percentage of CAF markers αSMA and FAP (16). Additionally, when BM-MSCs were co-cultured with leukemia cells, they adopted a CAF phenotype, which led to increased production of tumor-promoting growth factors and reduced daunorubicin-induced damage to B-ALL cells (16). Notably, while the chemoprotective effects of MSCs and CAFs on B-ALL were somewhat similar, CAFs proved to be more effective than MSCs in promoting the aggressiveness of B-ALL cells (16).

Subsequent research indicated that the overexpression of TGF-β plays a critical role in promoting the differentiation of BM-MSCs into CAFs, which may be dependent on the SDF-1/CXCR4 pathway (16, 17). The TGF-β receptor inhibitor LY2109761 and the CXCR4 antagonist AMD3100 both reduce CAF activation, offering a novel approach for chemotherapeutic regimens in AL (16–18). Li et al. isolated and cultured the first fibroblast tumor cell line, HXWMF-1, derived from CAFs in a 6-year-old B-ALL patient. They found compelling evidence that leukemic cells could potentially induce the malignant transformation of CAFs in a BALB/c nude mouse model (19).

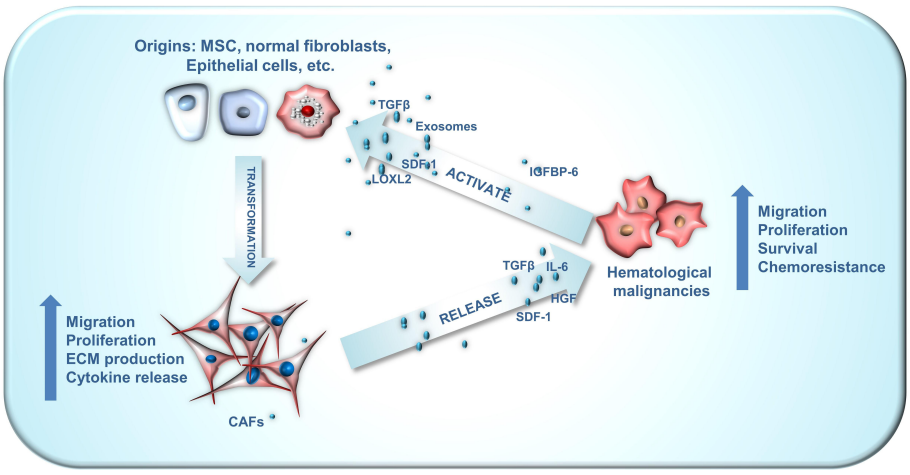

FIGURE 1
The crosstalk between CAF origins, CAFs and hematological malignancies. CAFs can emanate from a wide array of origins, encompassing mesenchymal stem cells, normal fibroblasts, myofibroblasts, endothelial cells, adipocyte pericytes, monocytes and macrophages, each exhibiting distinct phenotypes. The crosstalk between CAFs and hematological malignancies plays an important role in the development of blood cancer. Hematological malignancies are capable of facilitating the conversion of these diverse CAF origins into activated CAFs via numerous paracrine pathways. Subsequently, these activated CAFs can enhance the malignant phenotype of hematological malignancies through paracrine routes.

CAFs in multiple myeloma (MM)

MM is a disorder characterized by malignant plasma cell proliferation. The TME plays a substantial role in MM pathophysiology by secreting various cytokines that promote plasma cell survival, proliferation, and treatment resistance (20). Notably, the expression of CAF markers (FSP1, αSMA, FAP) in the bone marrow (BM) of patients with active MM was significantly higher (21). MM cells were found to induce CAF proliferation and enhance MM cell adhesion, proliferation, and apoptosis inhibition (21). The interaction between the two may be mediated through the SDF-1α/CXCR4 axis and integrins (21). Ciavarella et al. discovered that the activation levels of CAFs in MM patients at different clinical stages correlated with the expression of the fibrinolytic system (22). Compared to patients in the quiescent phase, CAFs in active MM patients exhibited higher transcriptional levels of u-PAR and u-PA. Selectively silencing u-PAR significantly suppressed CAF phenotype and function (22). Meanwhile, Kanehira et al. demonstrated that lysophosphatidic acid receptors 1 and 3 influenced the transition of MSCs to CAF differentiation, resulting in distinct outcomes (23).

Several targeted therapies for MM have emerged, but most have encountered drug resistance. For example, bortezomib, the first protease inhibitor approved by the FDA for MM treatment, has demonstrated limited efficacy in most patients due to the development of drug resistance (24). Several studies have investigated the vital role CAFs play in this issue. *In vitro* experiments indicated that CAFs from bortezomib-resistant patients inhibited bortezomib-induced apoptosis in MM cells. It is well-known that cellular autophagy contributes to drug resistance. When bortezomib-resistant CAFs are exposed to bortezomib, the autocrine TGF-pathway, which fosters autophagy, may become activated. Conversely, using TβR-I/II inhibitors to block Smad2/3 and autophagic pathways may help counteract MM resistance (25). CAR-T treatments targeting BCMA can detect and eradicate malignant plasma cells in MM patients, making them a promising therapeutic option. A study by Sakemura et al. revealed through ex vivo experiments that MM-CAFs inhibited antigen-specific proliferation of BCMA CAR-T cells via TGF-β secretion, consequently dampening their anti-myeloma activity (26). Simultaneously, targeting both MM cells and their CAFs with CAR-T cells reduced drug resistance development and slowed tumor progression, suggesting a new treatment approach (26).

CAFs in chronic lymphocytic leukemia (CLL)

CLL is a cancer characterized by the uncontrolled growth of mature lymphocytes in the blood, bone marrow, lymph nodes, and spleen (27). In the context of CLL, CAFs play a critical role in disease progression and interaction with the tumor microenvironment. CLL cells have the ability to activate the AKT pathway and stimulate the proliferation of MSCs via platelet-derived growth factor (PDGF) receptors (28). Furthermore, both bone marrow-derived MSCs and endothelial cells (ECs) can adopt a CAF phenotype when exposed to CLL-derived exosomes (29). These exosomes contain various molecular signals that can influence the behavior of recipient cells. Recent research has shown that CLL cells can trigger the transformation of BM-MSCs into CAFs by releasing exosomes containing miR-146a, which in turn inhibits USP16 (30). Additionally, a significant presence of αSMA(+) stromal cells was identified in infiltrating lymph nodes, further confirming the existence of numerous CAFs in CLL patients (29). CAFs play a crucial role in shaping the CLL microenvironment by influencing various immune cell functions. They release cytokines and chemokines that contribute to T cell and myeloid cell immunosuppression, and activate the AKT and NF-κB pathways, all of which promote tumor progression (31).

CAFs in myeloproliferative neoplasms (MPNs)

MPNs are malignant diseases characterized by excessive proliferation within the myeloid lineage, and αSMA, a CAF marker, is significantly elevated in MPN patients (32). Research suggests that αSMA expression levels influence the self-renewal and differentiation potential of MSCs, indicating a possible connection between αSMA expression and MPN development and prognosis (33). Primary myelofibrosis (PMF) is an MPN subtype characterized by progressive myelofibrosis. The development of myelofibrotic processes in PMF is currently believed to be associated with excessive stimulation of MSCs by growth factors (34).

In MPNs, lysyl oxidase (LOX), a stromal cross-linking protein, contributes to increased bone marrow stromal deposition. The use of a LOX inhibitor (BAPN) to decrease reticulin fibers supports LOX's role in myelofibrosis development (35). LOXL2 expression is found to be elevated in MPN patients, especially those with PMF (36). Higher levels of LOXL2 may contribute to MPN progression by modulating the function of peripheral stromal cells that display a cancer-associated fibroblast phenotype (36). Furthermore, LOXL2 is considered a key factor in driving the differentiation of mesenchymal stem cells (MSCs) into CAFs (37). These discoveries provide novel perspectives for targeted MPN treatments. Simtuzumab, a monoclonal antibody that inhibits LOXL2, is currently being tested in phase II clinical trials (38).

In PMF patients, there is a significant expansion of clonal tumorigenic fibroblasts, a particular type of CAFs, which are functionally different from normal fibroblasts. This difference may be associated with JAK2 signaling, and these fibroblasts contribute to the progression of myelofibrosis (34). On the other hand, the fibroblast differentiation inhibitor SAP (PRM-151) substantially increases the survival rate of NSG mice transplanted with PMF bone marrow cells and reduces the development of myelofibrosis (34). Longhitano et al. found that exposure to IGFBP-6 leads to an increased expression of CAF markers (αSMA, FAP, TGF-β) in HS5 cells. Their research suggests that IGFBP-6 triggers the differentiation of MSCs into CAFs and indicates a connection

between the IGFBP-6/SHH/TLR4 axis and alterations in the PMF microenvironment. This offers new perspectives on the pathogenesis of fibrosis in PMF patients (39).

CAFs in lymphoma

Lymphoma is the most prevalent hematologic malignancy, divided mainly into non-Hodgkin's lymphoma (90%) and Hodgkin's lymphoma (10%) (40). CAF-like cells and their precursors are present in secondary lymphoid organs (SLOs) before lymphoma onset, playing a crucial role in the progression of malignancies. For example, fibroblastic reticular cells (FRCs) form the structural foundation of SLOs and are essential for organ development, T and B cell compartmentalization, and adaptive immune response involvement. This provides a supportive microenvironment for the proliferation of malignant B cells (41). Numerous studies indicate that CAFs can aid lymphocyte survival by enhancing glycolysis (42, 43). Metabolic analyses have shown that elevated concentrations of CAF-secreted pyruvate decrease intracellular ROS production in primary lymphoma cells, augment tumor cell dependence on the citric acid cycle, and boost tumor cell survival (44). Furthermore, CAFs modulate the expression of the pyrimidine transporter protein ENT2 in tumor cells by secreting exosomes containing miR-4717-5p, resulting in chemoresistance (43).

Diffuse large B-cell lymphoma (DLBCL), the most prevalent lymphoma type, triggers the activation process of CAFs. Activated CAFs display a compensatory suppressive response by increasing PD-L1 expression and reducing the lytic-killing activity of CD8 T cells against tumor cells (45, 46). These findings offer a fresh perspective on the disease's initiation. Two CAF subtypes have been identified in adult T-cell leukemia/lymphoma (ATLL): CAFs/EGR_{high} and CAFs/EGR_{low}. CAFs in ATLL were found to significantly contribute to CD4 T-cell proliferation (47). Additionally, CAF/EGR_{high} influences CD8 and NKT cell expansion through EGFR (47). These findings suggest potential avenues for targeted therapy.

CAF-related targets and pathways in hematologic malignancies

CAFs have emerged as critical contributors to hematologic malignancies, influencing tumor progression and drug resistance. While the underlying mechanisms of CAF activation and function in hematologic malignancies are not yet fully understood, recent research has highlighted several potential therapeutic targets and pathways (48). Targeting CAFs and their associated pathways could provide an innovative approach to treating hematologic malignancies and enhancing patient outcomes. As research into the interplay between CAFs and hematologic malignancies continues, there is a promising prospect for developing novel treatments that target CAFs and improve clinical outcomes for patients.

Table 1 provides a summary of the latest research advancements in CAF-related targets and pathways within the context of hematologic malignancies.

Discussion

In recent years, the understanding of cancer biology has expanded, leading to the identification of various cellular and molecular players involved in tumor progression. One such player is CAFs, which have been implicated in the progression of solid tumors. However, their role in blood cancers remains underexplored. In this discussion, we will delve into the potential

TABLE 1 Passways/targets associated with CAFs in hematological malignancies.

Target/ Pathway	Hematologic Malignancy	Discovery/Advancement
TGF-β signaling pathway	AL, MM	Overexpression of TGF-β induces differentiation of BM-MSCs into CAFs; TGF-β receptor inhibitors reduce CAF activation (16–18)
JAK/STAT signaling pathway	AL, PMF	Clonal tumorigenic fibroblasts in PMF patients have functional differences associated with JAK2 signaling; targeting the JAK/STAT pathway may provide a new approach for AL treatment (34)
SDF-1/CXCR4 pathway	AL, MM	The activation of BM-MSCs into CAFs is dependent on the SDF-1/CXCR4 pathway; CXCR4 antagonists may reduce CAF activation (16, 21)
Autophagy	MM	Bortezomib-resistant CAFs may foster drug resistance through the autocrine TGF-β pathway and autophagy; blocking the TGF-β pathway may counteract drug resistance (25)
LOX/LOXL2	MPN	LOXL2 drives MSC differentiation into CAFs and contributes to MPN progression by modulating peripheral stromal cells; LOX inhibitors and LOXL2 inhibitors are being tested for targeted MPN treatment (35–37).
PD-1/PD-L1 pathway	DLBCL	Activated CAFs increase PD-L1 expression and reduce CD8 T cell lytic-killing activity against tumor cells (45, 46)
SHH/TLR4 axis	MPN	IGFBP-6 may trigger the differentiation of MSCs into CAFs via the IGFBP-6/SHH/TLR4 axis (39)
Exosomal miR-4717-5p	Lymphoma	CAFs modulate ENT2 expression in tumor cells by secreting exosomes containing miR-4717-5p, resulting in chemoresistance (43)

involvement of CAFs in blood cancers and evaluate their suitability as a promising therapeutic target.

To begin, it is essential to understand the role of CAFs in the tumor microenvironment. CAFs are key stromal cells that modulate the extracellular matrix, support angiogenesis, and produce a myriad of growth factors and cytokines. These actions contribute to the tumor-promoting milieu, ultimately enhancing cancer cell survival, proliferation, and metastasis. Given their critical role in solid tumors, it is plausible to assume that CAFs may have similar functions in blood cancers.

Blood cancers, such as leukemia, lymphoma, and myeloma, arise from the malignant transformation of cells in the blood, bone marrow, or lymphatic system. Although these cancers lack the solid tumor architecture, they still interact with the surrounding microenvironment, which may include CAFs. For instance, interactions between leukemia cells and bone marrow stromal cells, including fibroblasts, have been reported to support leukemia cell survival and contribute to therapeutic resistance. This suggests that CAFs could be critical players in the pathogenesis of blood cancers.

Targeting CAFs as a therapeutic strategy in blood cancers may have several advantages. First, as stromal cells, CAFs are genetically more stable than cancer cells, making them less likely to develop resistance to targeted therapies. Second, by disrupting the crosstalk between CAFs and cancer cells, the tumor-promoting microenvironment could be altered, potentially enhancing the efficacy of existing treatments. Finally, targeting CAFs may have a synergistic effect when combined with other therapies, leading to improved clinical outcomes.

However, it is important to consider the challenges and limitations associated with targeting CAFs in blood cancers. One of the primary challenges lies in the heterogeneity of CAFs, as they can originate from various cell types and exhibit diverse phenotypes and functions. This complexity may hinder the development of specific CAF-targeted therapies and could necessitate the identification of common signaling pathways or markers that can be targeted across different CAF subpopulations (4, 5). Cancer boasts a multifaceted biological composition and structure, encompassing cancerous cells, stromal cells, and the extracellular matrix (49). Historically, the majority of treatments have primarily aimed at cancer cells themselves (49). However, recent research has shed light on the significant influence the TME has on the behavior of cancer cells and their response to therapies (49, 50). Notably, CAFs, which constitute the most prevalent type of stromal cells within the TME, play a crucial yet understated role in the inception, progression, and metastasis of cancer (49). Consequently, focusing research on TME and CAF markers has emerged as a pivotal component of innovative strategies for the design and discovery of next-generation cancer drugs (49). However, unlike the case with solid tumors, the study of the tumor microenvironment and CAF markers in fibroblasts associated with hematological malignancies is still in its early stages (49, 51, 52). This remains an important area for future exploration and research.

Another challenge is the potential for off-target effects, given that CAFs share similarities with normal fibroblasts. Developing therapies that selectively target CAFs without affecting healthy fibroblasts is essential to minimize adverse side effects. Furthermore, the dynamic nature of the tumor microenvironment and the reciprocal interactions between CAFs and cancer cells may result in compensatory mechanisms that limit the efficacy of CAF-targeted therapies. Therefore, understanding the molecular mechanisms underlying these interactions is crucial for the development of effective treatment strategies (53).

In light of these challenges, future research should focus on elucidating the molecular and cellular mechanisms that govern CAFs' involvement in blood cancers. High-throughput screening technologies, such as single-cell RNA sequencing, could provide valuable insights into the heterogeneity of CAF populations and identify potential therapeutic targets (54–56). Additionally, the development of advanced *in vitro* and *in vivo* models that more closely mimic the tumor microenvironment will be essential for evaluating the safety and efficacy of novel CAF-targeted therapies. Moreover, the potential synergistic effects of combining CAF-targeted therapies with other treatment modalities, such as chemotherapy, immunotherapy, and targeted therapies, should be investigated. This combinatorial approach may help overcome potential resistance mechanisms and improve clinical outcomes for patients with blood cancers.

In summary, the targeting of CAFs in blood cancers presents a promising therapeutic strategy, but it is not without challenges. Future research should address the limitations and obstacles associated with CAF-targeted therapies and explore the potential benefits of combining these treatments with existing therapies. By deepening our understanding of CAFs' role in blood cancers and overcoming the hurdles associated with their targeting, we may be able to unlock new, more effective treatment options for patients suffering from these malignancies.

Author contributions

Investigating and writing the manuscript, ZYD and RUS. Editing and revising, WKH and ROS. Literature reading and review, LT. Project administration, YW and XYZ. All authors contributed to the article and approved the submitted version.

Funding

The project was funded by the Science and Technology Innovation Project for Nanjing Overseas Chinese (YW, No. B42), the Incubation Program of National Natural Science Foundation of Jiangsu Provincial People's Hospital (YW, No. 39) and Jiangsu Province "Double-Creation Doctorate" Talent Program (YW).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Auberger P, Tamburini-Bonnefoy J, Puissant AMDPI. *Drug resistance in hematological Malignancies*. Switzerland: MDPI (2020).

2. Chen X, Song E. Turning foes to friends: targeting cancer-associated fibroblasts. *Nat Rev Drug Discovery* (2019) 18(2):99–115. doi: 10.1038/s41573-018-0004-1

3. De Wever O, Van Bockstal M, Mareel M, Hendrix A, Bracke M. *Carcinoma-associated fibroblasts provide operational flexibility in metastasis*. In: *Seminars in cancer biology*. Elsevier, Amsterdam (2014).

4. Biffi G, Tuveson DA. Diversity and biology of cancer-associated fibroblasts. *Physiol Rev* (2021) 101(1):147–76. doi: 10.1152/physrev.00048.2019

5. Saw PE, Chen J, Song E. Targeting CAFs to overcome anticancer therapeutic resistance. *Trends Cancer* (2022) 8:527–55. doi: 10.1016/j.trecan.2022.03.001

6. Richards KE, Zeleniak AE, Fishel ML, Wu J, Littlepage LE, Hill R. Cancer-associated fibroblast exosomes regulate survival and proliferation of pancreatic cancer cells. *Oncogene* (2017) 36(13):1770–8. doi: 10.1038/onc.2016.353

7. Wen S, Hou Y, Fu L, Xi L, Yang D, Zhao M, et al. Cancer-associated fibroblast (CAF)-derived IL32 promotes breast cancer cell invasion and metastasis via integrin β3–p38 MAPK signalling. *Cancer Lett* (2019) 442:320–32. doi: 10.1016/j.canlet.2018.10.015

8. Herrera M, Berral-González A, López-Cade I, Galindo-Pumariño C, Bueno-Fortes S, Martín-Merino M, et al. Cancer-associated fibroblast-derived gene signatures determine prognosis in colon cancer patients. *Mol Cancer* (2021) 20(1):1–6. doi: 10.1186/s12943-021-01367-x

9. Li X, Sun Z, Peng G, Xiao Y, Guo J, Wu B, et al. Single-cell RNA sequencing reveals a pro-invasive cancer-associated fibroblast subgroup associated with poor clinical outcomes in patients with gastric cancer. *Theranostics* (2022) 12(2):620. doi: 10.7150/thno.60540

10. Song M, He J, Pan QZ, Yang J, Zhao J, Zhang YJ, et al. Cancer-associated fibroblast-mediated cellular crosstalk supports hepatocellular carcinoma progression. *Hepatology* (2021) 73(5):1717–35. doi: 10.1002/hep.31792

11. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, et al. A framework for advancing our understanding of cancer-associated fibroblasts. *Nat Rev Cancer* (2020) 20(3):174–86. doi: 10.1038/s41568-019-0238-1

12. Vicent S, SaylesL C, Vaka D, Khatri P, Gevaert O, Chen R, et al. Cross-species functional analysis of cancer-associated fibroblasts identifies a critical role for Clcf1 and Il-6 in non–small cell lung cancer *in vivo*. *Cancer Res* (2012) 72(22):5744–56. doi: 10.1158/0008-5472.CAN-12-1097

13. Van Cutsem E, Tempero MA, Sigal D, Oh D-Y, Fazio N, Macarulla T, et al. Randomized phase III trial of pegvorhyaluronidase alfa with nab-paclitaxel plus gemcitabine for patients with hyaluronan-high metastatic pancreatic adenocarcinoma. *J Clin Oncol* (2020) 38(27):3185. doi: 10.1200/JCO.20.00590

14. Devine SM, Larson RA. Acute leukemia in adults: recent developments in diagnosis and treatment. *CA: Cancer J Clin* (1994) 44(6):326–52. doi: 10.3322/canjclin.44.6.326

15. Zhai Y, Zhang J, Wang H, Lu W, Liu S, Yu Y, et al. Growth differentiation factor 15 contributes to cancer-associated fibroblasts-mediated chemo-protection of AML cells. *J Exp Clin Cancer Res* (2016) 35(1):1–12. doi: 10.1186/s13046-016-0405-0

16. Pan C, Liu P, Ma D, Zhang S, Ni M, Fang Q, et al. Bone marrow mesenchymal stem cells in microenvironment transform into cancer-associated fibroblasts to promote the progression of B-cell acute lymphoblastic leukemia. *Biomedicine Pharmacotherapy* (2020) 130:110610. doi: 10.1016/j.biopha.2020.110610

17. Xu Y, Tabe Y, Jin L, Watt J, McQueen T, Ohsaka A, et al. TGF-beta receptor kinase inhibitor LY2109761 reverses the anti-apoptotic effects of TGF-beta1 in myelo-monocytic leukaemic cells co-cultured with stromal cells. *Br J Haematol* (2008) 142 (2):192–201. doi: 10.1111/j.1365-2141.2008.07130.x

18. Cooper TM, Sison EAR, Baker SD, Li L, Ahmed A, Trippett T, et al. A phase 1 study of the CXCR4 antagonist plerixafor in combination with high-dose cytarabine and etoposide in children with relapsed or refractory acute leukemias or myelodysplastic syndrome: A Pediatric Oncology Experimental Therapeutics Investigators' Consortium study (POE 10-03). *Pediatr Blood Cancer* (2017) 64(8): e26414. doi: 10.1002/pbc.26414

19. Li Y, Gu L. Establishment and characterization of HXWMF-1: the first mouse fibroblastic tumor cell line derived from leukemia-associated fibroblasts. *Cancer Cell Int* (2021) 21(1):1–10. doi: 10.1186/s12935-021-01870-7

20. Roccaro AM, Sacco A, Maiso P, Azab AK, Tai Y-T, Reagan M, et al. BM mesenchymal stromal cell–derived exosomes facilitate multiple myeloma progression. *J Clin Invest* (2013) 123(4):1542–55. doi: 10.1172/JCI66517

21. Frassanito MA, Rao L, Moschetta M, Ria R, Di Marzo L, De Luisi A, et al. Bone marrow fibroblasts parallel multiple myeloma progression in patients and mice: *in vitro* and *in vivo* studies. *Leukemia* (2014) 28(4):904–16. doi: 10.1038/leu.2013.254

22. Ciavarella S, Laurenzana A, De Summa S, Pilato B, Chillà A, Lacalamita R, et al. u-PAR expression in cancer associated fibroblast: new acquisitions in multiple myeloma progression. *BMC Cancer* (2017) 17(1):1–11. doi: 10.1186/s12885-017-3183-y

23. Kanehira M, Fujiwara T, Nakajima S, Okitsu Y, Onishi Y, Fukuhara N, et al. A lysophosphatidic acid receptors 1 and 3 axis governs cellular senescence of mesenchymal stromal cells and promotes growth and vascularization of multiple myeloma. *Stem Cells* (2017) 35(3):739–53. doi: 10.1002/stem.2499

24. Ruschak AM, Slassi M, Kay LE, Schimmer AD. Novel proteasome inhibitors to overcome bortezomib resistance. *J Natl Cancer Institute* (2011) 103(13):1007–17. doi: 10.1093/jnci/djr160

25. Frassanito M, De Veirman K, Desantis V, Di Marzo L, Vergara D, Ruggieri S, et al. Halting pro-survival autophagy by TGFβ inhibition in bone marrow fibroblasts overcomes bortezomib resistance in multiple myeloma patients. *Leukemia* (2016) 30 (3):640–8. doi: 10.1038/leu.2015.289

26. Sakemura R, Hefazi M, Siegler EL, Cox MJ, Larson DP, Hansen MJ, et al. Targeting cancer-associated fibroblasts in the bone marrow prevents resistance to CART-cell therapy in multiple myeloma. *Blood J Am Soc Hematol* (2022) 139 (26):3708–21. doi: 10.1182/blood.2021012811

27. Hallek M, Shanafelt TD, Eichhorst B. Chronic lymphocytic leukaemia. *Lancet* (2018) 391(10129):1524–37. doi: 10.1016/S0140-6736(18)30422-7

28. Raffaghello L, Vacca A, Pistoia V, Ribatti D. Cancer associated fibroblasts in hematological Malignancies. *Oncotarget* (2015) 6(5):2589. doi: 10.18632/oncotarget.2661

29. Paggetti J, Haderk F, Seiffert M, Janji B, Distler U, Ammerlaan W, et al. Exosomes released by chronic lymphocytic leukemia cells induce the transition of stromal cells into cancer-associated fibroblasts. *Blood J Am Soc Hematol* (2015) 126 (9):1106–17. doi: 10.1182/blood-2014-12-618025

30. Yang Y, Li J, Geng Y. Exosomes derived from chronic lymphocytic leukaemia cells transfer miR-146a to induce the transition of mesenchymal stromal cells into cancer-associated fibroblasts. *J Biochem* (2020) 168(5):491–8. doi: 10.1093/jb/mvaa064

31. van Attekum MH, Eldering E, Kater AP. Chronic lymphocytic leukemia cells are active participants in microenvironmental cross-talk. *haematologica* (2017) 102 (9):1469. doi: 10.3324/haematol.2016.142679

32. Schmitt-Graeff AH, Nitschke R, Zeiser R. The hematopoietic niche in myeloproliferative neoplasms. *Mediators Inflammation* (2015) 2015. doi: 10.1155/2015/347270

33. Talele NP, Fradette J, Davies JE, Kapus A, Hinz B. Expression of α-smooth muscle actin determines the fate of mesenchymal stromal cells. *Stem Cell Rep* (2015) 4 (6):1016–30. doi: 10.1016/j.stemcr.2015.05.004

34. Verstovsek S, Manshouri T, Pilling D, Bueso-Ramos CE, Newberry KJ, Prijic S, et al. Role of neoplastic monocyte-derived fibrocytes in primary myelofibrosis. *J Exp Med* (2016) 213(9):1723–40. doi: 10.1084/jem.20160283

35. Eliades A, Papadantonakis N, Bhupatiraju A, Burridge KA, Johnston-Cox HA, Migliaccio AR, et al. Control of megakaryocyte expansion and bone marrow fibrosis by lysyl oxidase. *J Biol Chem* (2011) 286(31):27630–8. doi: 10.1074/jbc.M111.243113

36. Xu N, Li Y, Zhou X, Li L, Lu Q, Liu Q, et al. *LOXL2 highly-expressed induce the transition of stromal cells into cancer-associated fibroblasts which maybe involve in myeloproliferative neoplasms progession*. Washington, DC: American Society of Hematology. (2015).

37. Liu X, Xu N, Pan C, Zhou X, Liu Q, Feng R, et al. *Emerging potential roles of lysyl-oxidase-like 2 stimulates cancer associated fibroblasts promoting myelofibrosis of myeloproliferative neoplasms*. Washington, DC: American Society of Hematology. (2016).

38. Verstovsek S, Savona MR, Mesa RA, Dong H, Maltzman JD, Sharma S, et al. A phase 2 study of simtuzumab in patients with primary, post-polycythaemia vera or post-essential thrombocythaemia myelofibrosis. *Br J haematology* (2017) 176(6):939–49. doi: 10.1111/bjh.14501

39. Longhitano L, Tibullo D, Vicario N, Giallongo C, La Spina E, Romano A, et al. IGFBP-6/sonic hedgehog/TLR4 signalling axis drives bone marrow fibrotic transformation in primary myelofibrosis. *Aging (Albany NY)* (2021) 13(23):25055. doi: 10.18632/aging.203779

40. Mugnaini EN, Ghosh N. Lymphoma. *Primary Care: Clinics Office Pract* (2016) 43(4):661–75. doi: 10.1016/j.pop.2016.07.012

41. Höpken UE, Rehm A. Targeting the tumor microenvironment of leukemia and lymphoma. *Trends Cancer* (2019) 5(6):351–64. doi: 10.1016/j.trecan.2019.05.001

42. Aoki T, Shimada K, Sakamoto A, Sugimoto K, Morishita T, Kojima Y, et al. Emetine elicits apoptosis of intractable B-cell lymphoma cells with MYC rearrangement through inhibition of glycolytic metabolism. *Blood* (2016) 128 (22):3019. doi: 10.1182/blood.V128.22.3019.3019

43. Kunou S, Shimada K, Takai M, Sakamoto A, Aoki T, Hikita T, et al. Exosomes secreted from cancer-associated fibroblasts elicit anti-pyrimidine drug resistance through modulation of its transporter in Malignant lymphoma. *Oncogene* (2021) 40 (23):3989–4003. doi: 10.1038/s41388-021-01829-y

44. Sakamoto A, Kunou S, Shimada K, Tsunoda M, Aoki T, Iriyama C, et al. Pyruvate secreted from patient-derived cancer-associated fibroblasts supports survival of primary lymphoma cells. *Cancer Sci* (2019) 110(1):269–78. doi: 10.1111/cas.13873

45. Apollonio B, Jarvis P, Phillips B, Kuhnl A, Salisbury J, Zacharioudakis G, et al. Diffuse large B-cell lymphoma remodels the fibroblastic reticular network that acquires aberrant immunosuppressive capabilities; implications for the regulation of anti-tumor immunity in the immuno-oncology era. *Blood* (2018) 132:675. doi: 10.1182/blood-2018-99-116409

46. Apollonio B, Jarvis P, Phillips B, Kuhnl A, Salisbury J, Zacharioudakis G, et al. Diffuse large B-cell lymphoma (DLBCL) tumor cells reprogram lymphatic fibroblasts into cancer-associated fibroblasts (CAFs) that contribute to tumor microenvironment (TME)-driven immune privilege. *Blood* (2015) 126(23):1474. doi: 10.1182/blood.v126.23.1474.1474

47. Joo E-H, Bae JH, Park J, Bang YJ, Han J, Gulati N, et al. Deconvolution of adult T-cell leukemia/lymphoma with single-cell RNA-seq using frozen archived skin tissue reveals new subset of cancer-associated fibroblast. *Front Immunol* (2022) 13. doi: 10.3389/fimmu.2022.856363

48. Glabman RA, Choyke PL, Sato N. Cancer-associated fibroblasts: tumorigenicity and targeting for cancer therapy. *Cancers (Basel)* (2022) 14(16). doi: 10.3390/cancers14163906

49. Dzobo K, Dandara C. Broadening drug design and targets to tumor microenvironment? Cancer-associated fibroblast marker expression in cancers and relevance for survival outcomes. *OMICS* (2020) 24(6):340–51. doi: 10.1089/omi.2020.0042

50. Dzobo K. Taking a full snapshot of cancer biology: deciphering the tumor microenvironment for effective cancer therapy in the oncology clinic. *OMICS* (2020) 24 (4):175–9. doi: 10.1089/omi.2020.0019

51. Rimal R, Desai P, Daware R, Hosseinnejad A, Prakash J, Lammers T, et al. Cancer-associated fibroblasts: Origin, function, imaging, and therapeutic targeting. *Adv Drug Delivery Rev* (2022) 189:114504. doi: 10.1016/j.addr.2022.114504

52. Dzobo K, Senthebane DA, Dandara C. The tumor microenvironment in tumorigenesis and therapy resistance revisited. *Cancers (Basel)* (2023) 15(2). doi: 10.3390/cancers15020376

53. Gu L, Liao P, Liu H. Cancer-associated fibroblasts in acute leukemia. *Front Oncol* (2022) 12:1022979. doi: 10.3389/fonc.2022.1022979

54. Lavie D, Ben-Shmuel A, Erez N, Scherz-Shouval R. Cancer-associated fibroblasts in the single-cell era. *Nat Cancer* (2022) 3(7):793–807. doi: 10.1038/s43018-022-00411-z

55. Luo H, Xia X, Huang L-B, An H, Cao M, Kim GD, et al. Pan-cancer single-cell analysis reveals the heterogeneity and plasticity of cancer-associated fibroblasts in the tumor microenvironment. *Nat Commun* (2022) 13(1):6619. doi: 10.1038/s41467-022-34395-2

56. Dzobo K, Dandara C. Architecture of cancer-associated fibroblasts in tumor microenvironment: mapping their origins, heterogeneity, and role in cancer therapy resistance. *OMICS* (2020) 24(6):314–39. doi: 10.1089/omi.2020.0023

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 20 October 2023
DOI 10.3389/fonc.2023.1275800

OPEN ACCESS

EDITED BY
Felix Seyfried,
Ulm University Medical Center, Germany

REVIEWED BY
Walter Hanel,
The Ohio State University, United States
Kiana Shahzamani,
Lorestan University of Medical Sciences, Iran

*CORRESPONDENCE
Pierluigi Porcu
pierluigi.porcu@jefferson.edu

RECEIVED 10 August 2023
ACCEPTED 09 October 2023
PUBLISHED 20 October 2023

CITATION
Rosenberg M, Poluch M, Thomas C, Sindaco P, Khoo A and Porcu P (2023) Hepatitis B Virus and B-cell lymphoma: evidence, unmet need, clinical impact, and opportunities.
Front. Oncol. 13:1275800.
doi: 10.3389/fonc.2023.1275800

Hepatitis B Virus and B-cell lymphoma: evidence, unmet need, clinical impact, and opportunities

Maya Rosenberg[1,2], Maria Poluch[2], Colin Thomas[3,4], Paola Sindaco[4,5], Alan Khoo[4] and Pierluigi Porcu[4,5]*

[1]Department of Internal Medicine, New York University Langone Health, New York, NY, United States, [2]Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, United States, [3]Department of Medical Oncology, Hospital of the University of Pennsylvania, Philadelphia, PA, United States, [4]Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, United States, [5]Department of Medical Oncology, Thomas Jefferson University, Philadelphia, PA, United States

Nearly a billion people worldwide are infected with the hepatitis B Virus (HBV) and about a third of them have chronic infection. HBV is an important cause of morbidity and mortality, including acute and chronic hepatitis and hepatocellular carcinoma (HCC). Screening and control of primary HBV infection through vaccination represent a major advance in global public health, but large sections of the world population, in both developed and underdeveloped countries, remain unscreened and unvaccinated. In addition to being a global cause of liver disease, an important role of HBV in lymphoma has also emerged. First, the high risk of HBV reactivation in previously infected patients receiving chemo-immunotherapy necessitates the systematic evaluation of HBV serological status in all non-Hodgkin's lymphoma (NHL) cases and preemptive antiviral therapy for those who may have chronic or occult HBV infection. Second, HBV has been shown to infect lymphocytes, namely B-cells, and has been associated with a higher risk of developing B-cell lymphoma, most clearly in countries where HBV is endemic. While the risk of HBV reactivation with chemoimmunotherapy in NHL is well known, the role and the impact of HBV as a global lymphoma risk factor and potential oncogenic driver in B-cells are very poorly understood. Here, we review the clinical and scientific evidence supporting an association between HBV and B-cell lymphoma, with a particular focus on diffuse large B-cell lymphoma (DLBCL) and provide an overview of the estimated impact of HBV infection on the biology and clinical course of DLBCL. We also discuss ways to gain a better insight into the unmet need posed by HBV in lymphoma and whether assessing immune responses to HBV, measuring viral loads, and detecting the presence of HBV-encoded proteins in tumor tissue could be integrated into the molecular and clinical risk stratification of patients with DLBCL.

KEYWORDS

hepatitis B, B-cell, lymphoma, DLBCL, double hit, viral oncogenesis

1 Introduction

Viral infections are associated with several types of cancer with widely different levels of risk in different populations and geographical areas and the global role of viruses as oncogenic drivers is widely recognized. Six viruses (HBV, Hepatitis C Virus [HCV], Epstein-Barr Virus [EBV], Human papillomavirus [HPV], Human T-Cell Lymphotropic Virus [HTLV-1], and Human Herpes Virus-8 [HHV-8]) are classified as class I carcinogenic agents by the International Agency for Research on Cancer (IARC) (1) and about 10% of all cancers worldwide can be attributed to viruses (2). Global vaccination campaigns for HPV and HBV have been implemented, and efforts to test the efficacy and safety of vaccines for EBV are ongoing in clinical trials (NCT04645147, NCT05164094) (3). In addition, ongoing studies show the potential of targeted therapies for virus-associated cancers. EBV-targeting adoptive cellular immunotherapies (4) and new treatments that use the presence of oncogenic viruses as an intrinsic tumor-specific vulnerability are being investigated. For example, a small molecule inhibiting the EBV protein EBNA-1 (VK-2019) has shown promising anti-tumor efficacy in preclinical models of EBV-positive cancers (5) and a Phase I clinical trial of VK-2019 in nasopharyngeal carcinoma (NPC) is ongoing (NCT04925544). Additionally, the combination of the histone deacetylase (HDAC) inhibitor Nanatinostat and the nucleoside analog valganciclovir (VGCV) was recently granted orphan drug designation (ODD) for EBV-positive lymphomas by the FDA, based on initial Phase 1/2 data (6), and an international multi-cohort Phase 2 clinical trial (Naval-1) is ongoing (NCT05011058) (7). These studies show the potential of developing targeted therapies for virus-associated cancers.

Awareness of the linkage between carcinogenic viruses and cancers remains inadequate in the public and in parts of the medical and advocacy communities. This is the case of the association between prior infection with HBV and risk of B-cell lymphoma, in particular diffuse large B-cell lymphoma (DLBCL), the most common type of aggressive B-cell lymphoma worldwide (1). While the importance of chronic HBV infection in the development of HCC is well established, its role as a risk factor for DLBCL is less known. Consequently, education efforts to increase awareness of symptoms of DLBCL among HBV seropositive patients are inadequate, potentially leading to a delay in diagnosis of DLBCL in this patient population. Likewise, the impact of HBV vaccination and antiviral therapy on the risk of developing DLBCL remains unknown.

The goal of this paper is to provide a background on HBV infection and DLBCL, and then critically review the evidence supporting a role for chronic HBV infection in DLBCL, outline the criteria currently defining HBV-associated DLBCL, provide available estimates of its frequency and distribution globally and in the U.S., and review the distinctive aspects of HBV-associated DLBCL that have been identified. We will also offer an assessment of the unmet need and opportunity in terms of scientific discovery and public health impact.

2 Background

2.1 Hepatitis B Virus

2.1.1 HBV as a global health problem

In the United States, 850,000 individuals are estimated to be living with HBV (8). The prevalence of past or present HBV infection amongst people in the U.S. is 4.3% (9). However, populations of foreign-born minorities, with a higher prevalence of HBV, are likely underrepresented in this calculation. This pathogen is thought to be responsible for over 296 million chronic infections worldwide (10). Further, in 2021, the World Health Organization (WHO) reported that only 30.4 million people living with HBV knew their HBV status, accounting for only 10% of the total people in the world living with chronic HBV infection (10).

HBV infection carries a heavy financial toll for patients and healthcare systems, with total HBV hospitalization charges in the U.S. increasing from $357 million in 1990 to $1.5 billion in 2003 (11). In 2019, the total mean all-cause annual healthcare costs for HBV patients with Medicare who had decompensated cirrhosis, HCC, received liver transplants, or had compensated liver disease was $479,595 (12). This financial burden is felt globally (13). The significant financial and health impact of HBV makes understanding this virus and its sequelae important.

2.1.2 HBV's life cycle

HBV is an enveloped virus with a circular, partially double-stranded DNA genome, which belongs to the *Hepadnaviridae* family. The infectious virion consists of a lipid envelope containing the HBV surface antigen (HBsAg). This surrounds an inner nucleocapsid composed of the HBV core antigen (HBcAg) complexed with virally encoded polymerase (14). The viral genome contains 4 overlapping open reading frames that encode proteins essential for viral replication (14). HBV entry into host cells is mediated by low-affinity binding to heparan sulfate proteoglycans (HSPGs), followed by high-affinity binding to sodium taurocholate co-transporting polypeptides (NTCPs). Glypican 5 is an HSPG that preferentially binds HBV (15, 16). HSPGs are found at the surface and in the extracellular matrix of most human cells. At least some of the virus' hepatotropic nature is thought to be due to the prevalence of glypican 5 in the liver.

2.1.3 Infection, clinical manifestations, and outcomes

Worldwide, the most common route of HBV transmission is perinatal transmission, especially in endemic areas (15). HBV is also transmitted through percutaneous and mucous membrane exposures and sexual intercourse with infected individuals (8). Once the infection is acquired, the host can experience an acute infection with complete recovery, a fulminant course with hepatic failure, or a chronic infection (17).

The infection is diagnosed by detecting HBsAg and anti-hepatitis B core IgM antibody (HBcAb) in plasma. Chronic HBV

is characterized by the persistent presence of HBsAg in the serum for greater than 6 months, in addition to HBcAb (18). Chronic HBV infections are typically characterized by four phases, varying in length and defined by laboratory results and clinical symptoms.

Mortality among adults with chronic HBV far exceeds that of uninfected individuals. One study including 39,206 patients concluded that those with chronic HBV infection had a 1.9-fold (95% CI 1.1–3.3) increased hazard of all-cause mortality compared to uninfected people, and a 13.3-fold (95% CI 3.9–45.5) increased hazard of liver-related mortality (19). Hepatocellular carcinoma (HCC) is closely associated with HBV, with studies showing that chronically infected individuals have 100 times the risk of developing HCC than non-carriers (20). Unfortunately, deaths from chronic HBV infection are increasing, with a rising incidence of HCC of particular concern in endemic areas like Africa and the Western Pacific (21). Professional medical societies, such as World Health Organization (WHO) and the American Association for the Study of Liver Diseases (AASLD), vary in their recommendations for screening and treatment (22). There are significant barriers to effective HBV screening, prevention, and treatment, with an impact that may not be limited to chronic disease and HCC, but may include B-cell lymphoma, specifically diffuse large B-cell lymphoma (DLBCL).

2.2 Diffuse large B-cell lymphoma

Non-Hodgkin's Lymphomas (NHLs) are hematologic malignancies of mature lymphocytes and one of the more common cancers in the United States, accounting for about 4% of all cancers (23). DLBCL is an aggressive NHL that comprises about 30% of all lymphoma cases. It is the most common subtype of NHL in the U.S (24) and worldwide (25). DLBCL is most prevalent in elderly patients, with a median age at diagnosis in the 7th decade of life. The incidence of DLBCL varies by race, with racial differences in age and gender distribution (26–28). However, the incidence of DLBCL increases with age for all races (27).

In clinical practice, DLBCL is frequently classified based on immunohistochemistry (IHC)-defined cell-of-origin (COO). There are two major subtypes, a germinal center B-cell (GCB) type and a non-GCB type, corresponding to the activated B-cell (ABC) type defined by gene expression profiling (29). The use of cytogenetics and fluorescence *in situ* hybridization (FISH) further classify DLBCL by identifying chromosomal translocations in tumor cells, in particular rearrangements involving *MYC* (8q24), BCL-2 (18q21), and BCL-6 (3q27). DLBCL carrying genetic rearrangements of both *MYC* and *BCL-2* genes, with or without a rearranged BCL-6 gene, were formerly known as double-hit (or triple-hit if BCL-6 is also involved) lymphomas. Double hit and triple hit (DH/TH) DLBCL represent approximately 10% of all DLBCL and are particularly aggressive and chemotherapy-resistant. Data are emerging about the impact of deletions or inactivating mutations of *TP53*, a tumor suppressor gene involved in cell cycle arrest and apoptosis. *TP53* mutations are present in about 10% of DLBCL cases and are an independent predictor of poor prognosis (30, 31). More recently, next generation sequencing (NGS) studies of whole genomes and transcriptomes in untreated DLBCL have identified several genetic clusters and molecular subgroups characterized by specific cancer-driving signatures and epigenetic pathways, with distinct outcomes (32–34).

The International Prognostic Index (IPI) is a risk stratification tool for DLBCL patients taking into account age, performance status, serum lactate dehydrogenase (LDH), number of extranodal sites, and stage (35). This scale, or one of its modifications, is the mainstay of risk stratification in DLBCL (36). While multiple studies report inferior outcomes in patients with EBV-positive (37), HCV-positive (38), and HBV-positive lymphomas (39), serological status and quantitative viral load measurements have not been included in any of the risk-stratification tools for lymphoma, except for plasma EBV DNA in extranodal NK/T-cell lymphoma (ENKTL) (40).

The standard first-line therapy for most DLBCL patients is the combination of the chemotherapy regimen CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), with rituximab, a monoclonal antibody that targets the pan-B cell surface antigen CD20. This regimen is referred to as R-CHOP. With this regimen, 60-65% of patients are cured. The prognosis is poor for 35-40% of patients who relapse following R-CHOP or have refractory disease. Current research is focused on finding better risk-stratification tools, to identify patients who will do well with R-CHOP versus those who may require more aggressive regimens up-front.

Patients with DH/TH DLBCL generally have more aggressive clinical courses, with advanced-stage presentation, extranodal involvement, higher serum LDH, and a high IPI score. DH/TH DLBCL carries a particularly poor prognosis, with a 5-year survival of <30% (41). *TP53* is also commonly mutated in DH/TH DLBCL and Double-Hit Signature (DHITsig)-positive DLBCL, which adds an unfavorable feature to these patients (42). Therapeutic approaches more aggressive than R-CHOP are often used for DH DLBCL, but overall survival rates remain poor (43).

3 Association between hepatitis B Virus and B-cell lymphomas

3.1 Hepatitis B Virus and non-Hodgkin's lymphoma

It is well known that HBV increases the risk of HCC. Studies show that chronically HBV-infected individuals have 100 times the risk of developing HCC than non-infected individuals (20). Epidemiological and seroprevalence studies have shown that HBV also increases the risk of other types of cancer, such as NHL. This association, particularly between HBV and B-cell lymphomas, has been documented in studies from both endemic and non-endemic areas. A 2007 case-control study conducted in the U.S. showed that patients (N=3,888) with chronic HBV infection were 2.8 times more likely to develop NHL than matched controls (N=205,203; HR = 2.80, 95% CI = 1.16-6.75). This study controlled for age, race, sex, income, Charlson comorbidity index, study site, and HCV infection (44). A recent 2018 meta-analysis of 58 published studies included

data on 53,714 NHL cases and 1,778,591 controls. The studies were from Asia (N=46), Europe (N=8), North America (N=2), Africa (N=1) and Oceania (N=1). The meta-analysis found that HBV-infected individuals were 2.5 times more likely to develop NHL than non-infected individuals (45). These findings have been replicated (46, 47). A recent cohort study by Spradling (47) used data from the U.S. National Cancer Institute (NCI) and the National Program of Cancer Registries to assess the incidence of specific cancers in patients ≥20 years old with HBV compared to non-HBV-infected patients of the same age range. Patients with previous or current HCV or Human Immunodeficiency Virus (HIV) coinfection were excluded. Results showed, with a 95% confidence interval, that patients with HBV had over 2.5 times the risk of developing NHL compared to the general population. Thus, multiple case-control and cohort studies from endemic and non-endemic areas show that HBV infection is associated with a 2-3 fold higher risk of developing NHL, compared to controls, leading to the question of the mechanism behind this association (Table 1, Figure 1).

TABLE 1 Summary of studies analyzing HBV and its effect on Non-Hodgkin Lymphoma (NHL) and Diffuse Large B-Cell Lymphoma (DLBCL).

Study (Year)	Location	Sample	Key Findings
An J, Kim JW, Shim JH, et al. (2018) (48)	South Korea	95,034 patients with non-hepatocellular malignancy, 118,891 controls	HBV was positively associated with DLBCL (AOR 1.75, p = 0.003 for men, AOR 4.37, p < 0.001 for women) when compared to other B-NHLs
Cheng C-L, Huang S-C, Chen J-H, et al. (2020) (49)	Taiwan	416 DLBCL cases	Compared with DLBCL patients who were HBsAg-negative, HBsAg-positive patients had a lower overall response rate (ORR) (76.5% vs. 85.5%, p = .043), poorer 5-year overall survival (OS) rate (57.2% vs. 73.5%, p <.001), and shorter 5-year progression-free survival (PFS) rate (47.2% vs. 60.7%, p = .013).
Dalia S, Chavez J, Castillo JJ, Sokol L. (2013) (50)	Asia, Australia, Europe, U.S.	1,377 NHL cases and 2,633,274 controls	HBV was positively associated with all NHL subsets when compared with the control population (OR 2.24; 95% CI 1.80 – 2.78; p ¾ 0.001). HBV was positively associated with DLBCL specifically (OR 2.05; 95% CI 1.25 – 3.35; p ¾ 0.001)
Kim M, Lee YK, Park B, Oh DJ, Choi HG. (2020) (51)	South Korea	929 NHL cases, 3716 controls	HBV rates were higher in the NHL group than in the control group (OR 3.25; 95% CI 1.99 – 5.31; p < 0.001)
Lai Y-R, Chang YL, Lee CH, Tsai TH, Huang KH, Lee CY. (2022) (52)	Taiwan	54,157 HBV or HCV cases and 270,785 controls	Incidence of NHL was significantly higher in patients with HBV than in patients from the general population (HR 1.49; 95% CI 1.94 – 3.19)
Li M, Gan Y, Fan C, et al. (2018) (45)	Africa, Asia, Europe, North America, Oceania	53,714 NHL cases and 1,778,591 controls.	HBV infected individuals were 2.5 times more likely to develop NHL than non-infected individuals. (95% CI 2.20 – 2.83)
Li M, Shen Y, Chen Y, et al. (2020) (53)	China	411 NHL cases, 957 controls	Positive rates of HBsAg (OR 3.11; 95% CI 2.20 – 4.41) and HBeAg (OR 3.99; 95% CI 1.73 0 9.91) were significantly higher in patients with NHL. Prevalence of HBsAg was significantly increased in B NHL (OR 3.36; 95% CI 2.33 – 4.84) but not in T-cell NHL.
Mahale P, Engels EA, Koshiol J. (2019) (46)	U.S.	1,825,316 first cancer diagnoses and 200,000 controls	HBV was positively associated with DLBCL (OR 1.24; 95% CI 1.06 – 1.46)
Nath A, Agarwal R, Malhotra P, Varma S. (2010) (54)	China, Egypt, Italy, Japan, Romania, Saudi Arabia, Singapore, South Korea, Turkey	3,262 NHL patients with 1,523,205 controls and 3,888 HBV patients with 205,203 controls	HBV was positively associated with NHL when compared with the control population (OR 2.56; 95% CI 2.24 – 2.92)
Spradling PR, Xing J, Zhong Y, et al. (2022) (47)	U.S.	5,773 HBV cases	Compared with the general population, substantially higher incidence among HBV-infected patients was observed for NHL (SRR 2.52)
Ulcickas Yood M, Quesenberry CP, Guo D, et al. (2007) (44)	U.S.	3,888 chronic HBV patients and 205,203 controls	Patients with chronic HBV infection were 2.8 times more likely to develop NHL than matched controls (HR = 2.80, 95% CI = 1.16-6.75)
Yi H, Chen JJ, Cen H, Yan W, Tan XH. (2014) (55)	China, Denmark, France, Germany, Greece, Italy, Netherlands, Norway, South Korea, Spain, Sweden, United Kingdom	5,396 B NHL cases and 20,671 controls	HBV was positively associated with B-NHL when compared with control population (OR 2.98; 95% CI 2.30 – 3.86)

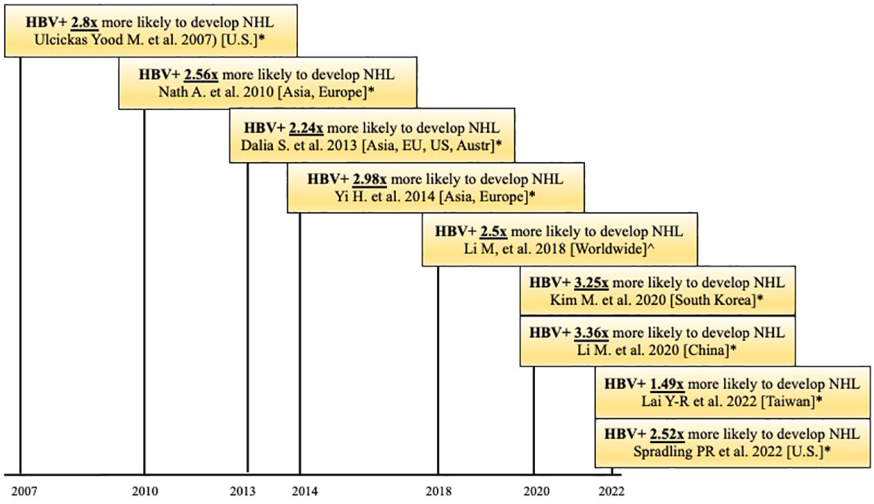

FIGURE 1
Timeline of published case-control and cohort studies (*) and meta-analyses (^) that observed that HBV patients are more likely to develop Non-Hodgkin Lymphoma (NHL). HBV+ = positive for HBsAg.

3.2 HBV's Lymphotropism

The ability of HBV to infect human lymphocytes has been reported in multiple studies but remains poorly understood (Table 1). A 2011 study used human bone marrow (BM) from the iliac crest of healthy volunteers aged 18-36 years, who had no serologic evidence of current or previous HBV infection. BM cells were exposed to HBV *in vitro* for 24 hours. Following a ten-week incubation period, the BM stem cells were harvested, and their DNA was extracted. HBsAg and HBeAg levels were then measured using electrochemiluminescence (56). This approach assessed the efficiency with which HBV productively infected bone marrow stem cells *in vitro*, based on the expression of HBV-encoded proteins. Results showed that the infection efficiency was comparable to its ability to infect primary human hepatocytes and human hepatoma cell lines (56). Later studies confirmed this finding (57). It was further shown that the HBV residing in a patient's BM stem cells could infect HBV-naïve hepatocytes in transplanted livers. This finding was important as it presented a possible source of graft re-infection following stem cell transplantation in patients with chronic HBV (58, 59).

During HBV's life cycle, viral DNA can be integrated into the genome of infected cells (60). Integrated genomes can serve as templates for RNAs coding for viral proteins. HBV genome integration is a primary driver of HCC. Known cancer genes such as the telomerase reverse transcriptase (TERT) (61), mixed-lineage leukemia 4 (MLL4) (62), and Cyclin E1 (CCNE1) (63) are preferential integration sites in HCC and about one-third of the genes recurrently targeted by HBV integration are cancer-related genes (64). Recently, Svicher et al. published an overview of the mechanisms of HBV DNA integration into immune cells, highlighting the hypothesis that the oncogenic effect of HBV in lymphoma is driven by the integration of HBV DNA into lymphocytes (65). It was noted that peripheral blood mononuclear cells (PBMCs) may act as a extrahepatic reservoir for HBV infection.

HBV DNA integration has been shown to affect multiple gene sites (65), as shown in Figure 2. A 2020 study identified the integration of HBV DNA in the lymphoma cells of 34 individuals with NHL (53). In total, 313 integration sites were identified. Half of the integration occurred in intergenic regions (49.5%), and the remaining took place in introns (44.7%), 3'-untranslated region (1.6%), gene upstream region (1.3%), and gene downstream region (1.3%). In the NHL samples, HBV integration had preferential targets. Seven genes, *ANKS1B*, *CAPZB*, *CTNNA3*, *EGFLAM*, *FHOD3*, *HDAC4*, and *OPCML* were found to be repeatedly targeted by HBV DNA integration. HBV DNA is regarded as a strong cis activator of flanking genes, so integration can influence the expression of target genes over a long distance (60). Six of the seven genes were found to be overexpressed in NHL, based on publicly available databases such as TCGA. KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis of the HBV-targeted genes in NHL revealed that terms related to developmental process and cell differentiation, signal transduction, cell junction, and transcriptional regulation were significantly enriched ($p<.05$). Axon guidance was the most impacted pathway ($p<0.0001$), followed by Ras signaling, glycosaminoglycan biosynthesis, and cytokine-cytokine receptor interaction ($p<.05$). These pathways are also enriched in studies of HBV-targeted genes in HCC which implies that some pathways are commonly affected by HBV integration in HCC and NHL. This study is important, as it demonstrates that HBV DNA can integrate into the genome of NHL cells, affecting genes that have been reported to play an oncogenic role in other cancers.

Numerous *in vitro* and *in vivo* studies have shown the lymphotropism of HBV. *In vitro*, HBV was shown to be able to infect bone marrow progenitor cells and inhibit their growth (66). Mature lymphocytes were also shown to be infected with HBV, with HBV mRNA found in B-cells and T-cells (67). *In vivo* studies, most

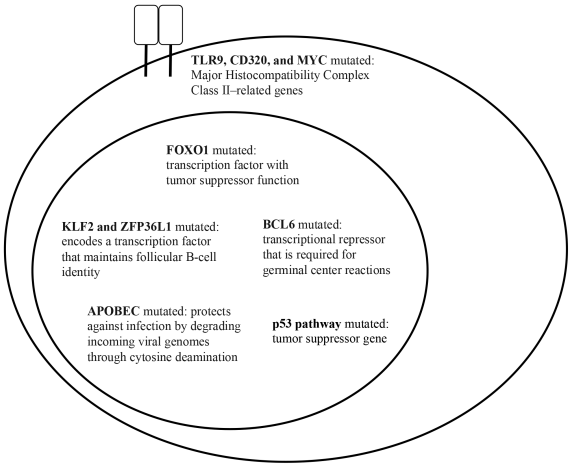

FIGURE 2
HBsAg+ DLBCL key gene mutations (data taken from Ren W. et al. (39)).

notably with chimpanzees, showed that chimpanzees with chronic HBV infection showed HBV DNA in PMBCs (68). Svicher et. al. further discusses the evidence that HBV infection is persistent in hematopoietic and lymphoid cells, which may act as a site for HBV reactivation and genome changes (65).

3.3 HBV and DLBCL

Multiple retrospective studies and meta-analyses have shown that in patients chronically infected with HBV, the most common type of NHL is DLBCL (16, 39, 50, 54, 55). HBV-associated DLBCL has been shown to have an incidence of 14.3% in West Africa and, according to a meta-analysis, has poor prognosis (69, 70). A 2018 study by An et al. (48) revealed a significantly positive link between HBV infection and DLBCL (adjusted odds ratio [AOR] 1.75, p = 0.003 for men, and AOR 4.37, p < 0.001 for women) when compared to other B-NHL. A 2020 study by Cheng et al. (49), including 426 patients with DLBCL at the National Taiwan University Hospital in Taipei, Taiwan, found that 23.6% of the patients were positive for HBsAg. When compared to HBsAg- patients, HBsAg+ patients were younger, diagnosed more frequently with advanced-stage disease, had lower overall response rates to R-CHOP (57.2% vs 73.5%, p < 0.001), and had shorter 5-year progression-free survival rates (47.2% vs 60.7%, p=0.013) (49). Another 2020 study, including data from 929 patients with NHL and 3716 healthy subjects in Korea, found that HBV rates were higher in the NHL group than in the control group (p < 0.001). The adjusted OR of HBV infection in patients with NHL was 3.25 (95% CI, 1.99 – 5.31) (51). Lastly, a 2022 retrospective cohort study used the nationally representative database in Taiwan to investigate the correlation between HBV and NHL. The study showed that the incidence rate of NHL was significantly higher in patients with HBV than in patients from the general population (HR, 2.49; 95% CI, 1.94 – 3.19) (52) (Table 2).

HBV proteins can be detected in tissue biopsies from patients with DLBCL and chronic HBV infection. Huang et al. assessed tumor biopsies from 96 HBsAg+ and 10 HBsAg- DLBCL patients treated at five Chinese centers (16). The HBV antigen HBx, a protein essential for viral replication, was present in the lymphoma cells in 48.9% of HBsAg+ DLBCL patients; additionally, the HBV antigen Pre-S2, a component of HBsAg, was detected in the lymphoma cells of 57.2% HBsAg+ DLBCL patients. The authors also showed that the presence of HBx antigen in DLBCL cells was associated with high MYC expression (Table 3).

The mutational landscape of HBV-associated DLBCL in a cohort of 275 Chinese patients was assessed in a landmark 2018 study by Ren and colleagues (39). This study showed that DLBCL patients with concomitant HBV infection were characterized by a younger age (median age, 42 vs. 60 years; p <.0001), more advanced disease stage at diagnosis, and shorter overall survival. GC-type and ABC-type DLBCL were equally frequent. An enhanced rate of mutagenesis and an increased total mutation load were observed in HBsAg+ DLBCL genomes (median, 15,036 vs. 9,902 mutations). In addition, more non-silent mutations were observed in HbsAg+ DLBCLs (median, 99 vs 66). The genome-wide mutational signatures of 60 DLBCL cases were characterized based on the 96 possible mutation types. Seven mutational signatures were extracted from the cohort and three were significantly enriched in HBsAg+ tumors. One of the signatures was linked to APOBEC enzymes, a family of proteins with anti-viral functions (71), suggesting that HBV-associated DLBCLs are associated with distinct mutational signatures. Additionally, *TMSB4X, FAS, UBE2A, DDX3X, CXCR4, KLF2*, and *SGK1*, were significantly more mutated in the HBsAg+ group. Some of these genes are potential targets for activation-induced cytidine deaminase (AID), the driver of somatic

TABLE 2 Summary of studies showing differential outcomes in HBV-positive and HBV-negative DLBCL patients.

Study (Year)	Location	Sample	Key Findings
Cheng C-L, Huang S-C, Chen J-H, et al. (2020) (49)	Taiwan	416 DLBCL cases	HBsAg+ patients were younger and diagnosed more frequently with advanced stage disease; these patients had lower ORR to R-CHOP (57.2% vs 73.5%, p < 0.001) and shorter 5-year PFS rates (47.2% vs 60.7%, p=0.013), compared to HBsAg- patients
Li M, Shen Y, Chen Y, et al. (2020) (53)	China	411 NHL cases, 957 controls	HBV patients had a significantly higher level of serum LDH (p < 0.001), a more advanced stage of NHL (p = 0.001), a worse ECOG performance status (p = 0.029), and a less favorable prognosis (p = 0.023).
Ren W, Ye X, Su H, et al. (2018) (39)	China	275 DLBCL cases	DLBCL patients with concomitant HBV infection were characterized by a younger age (median age, 42 vs 60 years; p <.0001), a more advanced disease stage at diagnosis (p = 0.0002), higher international prognostic index (p = 0.007) and reduced overall survival.

TABLE 3 Summary of studies showing integration of HBV genome/expression of HBV genes in lymphoma.

Study (Year)	Location	Sample	Key Finding
Lau KC, Joshi SS, Gao S, et al. (2020) (57)	China, U.S.	52 HBV cases	The replicative potential of HBV within lymphoid cells was evidenced by up-regulation of viral DNA in peripheral blood mononuclear cells (PBMC) supernatant after ex vivo mitogen-stimulation. Increased viral replication was evidenced by increased levels of HBV cccDNA and enhanced viral mRNA expression.
Li M, Shen Y, Chen Y, et al. (2020) (53)	China	411 NHL cases, 957 controls	HBsAg, HBcAg, and HBV DNA were detected in 34.4%, 45.2%, and 47.0% of the NHL tissues, respectively. There was a total of 313 HBV integration sites isolated from the NHL tissues. Terms related to developmental process and cell differentiation, signal transduction, cell junction, and transcriptional regulation were significantly enriched ($p<.05$). Axon guidance was the most impacted pathway ($p<0.0001$), followed by Ras signaling, glycosaminoglycan biosynthesis, and cytokine-cytokine receptor interaction ($p<.05$). These pathways are also enriched in studies of HBV-targeted genes in HCC which implies that some pathways are commonly affected by HBV integration in HCC and NHL.
Ma R, Xing Q, Shao L, et al. (2011) (56)	---	---	Results showed that the ability of HBV to infect bone marrow stem cells *in vitro* was comparable to its ability to infect primary human hepatocytes and human hepatoma cell lines.

hypermutation (SHM) in the immunoglobulin (Ig) genes during B-cell maturation and selection in the germinal center. The study also showed that the frequency of chromosomal translocations involving *BCL6* was significantly increased in HBsAg+ DLBCL genomes (57% vs 28%; p = .0472), suggesting that *BCL6* dysregulation plays a role in HBV-associated DLBCL. Finally, antigen processing and p53 signaling pathway-associated genes were significantly upregulated in HBsAg+ DLBCLs (Figure 3). These mutations or deletions carry a particularly poor prognosis for DLBCL. Interestingly, most of the genes that were mutated in HBV-associated DLBCLs were not mutated in HBV-associated HCC or HBV-positive lung adenocarcinoma, suggesting that genetic alterations found in HBV-associated DLBCL may be B-cell specific. The finding of APOBEC signatures and the lack of sequence homology of the CD3 region on characterization of the V(D)J region of immunoglobulin heavy chain (IgH) suggest that the lymphoma was due to direct carcinogenic effects of the virus rather than due to chronic antigen stimulation. Based on these observations, the authors proposed that HBsAg+ DLBCL should be considered and classified as a distinct subtype of DLBCL (Table 4).

4 Open questions and future directions

Most of the sero-epidemiological studies that have assessed the link between B-cell NHL and HBV, and in particular the impact of chronic HBV infection on clinical outcomes, have been performed in East Asian countries, where HBV is endemic. Several environmental and host- or tumor-specific factors, however, may

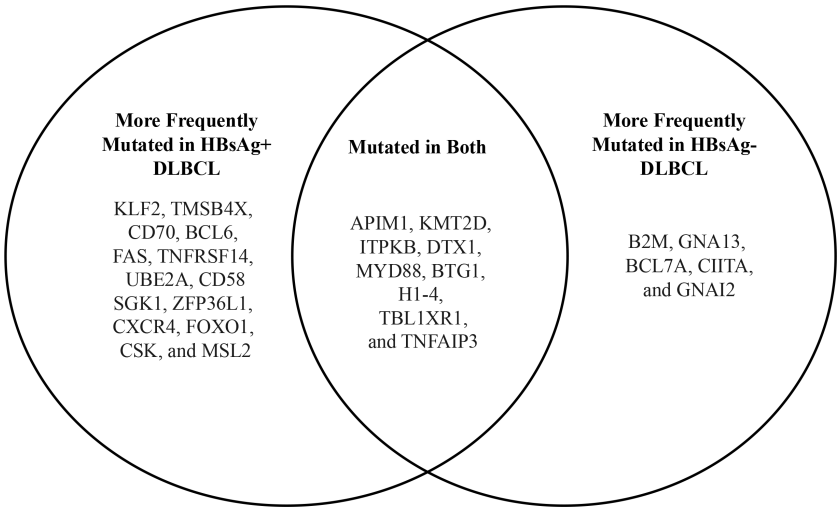

FIGURE 3
Comparison of mutated genes from HBsAg+ and HBsAg- DLBCL (data from Ren W. et al. (39)).

TABLE 4 Summary of major genomic findings in HBV-positive DLBCL.

Study (Year)	Location	Sample	Key Findings
Huang X, Young KH, Guo W, et al. (2020) (16)	China	96 HBsAg + and 10 HBsAg- DLBCL cases	The HBV antigen HBx, a protein essential for viral replication, was present in the lymphoma cells in 48.9% of HBsAg+ DLBCL patients; additionally, the HBV antigen Pre-S2, a component of HBsAg, was detected in the lymphoma cells of 57.2% HBsAg+ DLBCL patients. Notably, the authors also showed that the presence of HBx antigen in DLBCL cells was associated with high MYC expression ($p = 0.0302$). The frequency of MYC gene rearrangement was significantly higher in HBV+ DLBCL cases than in the HBV- group.
Ren W, Ye X, Su H, et al. (2018) (39)	China	275 DLBCL cases	An enhanced rate of mutagenesis and an increased total mutation load were observed in HBsAg+ DLBCL genomes. More non-silent mutations were observed in HbsAg+ DLBCLs ($p = 0.048$). *TMSB4X, FAS, UBE2A, DDX3X, CXCR4, KLF2*, and *SGK1*, were significantly more mutated in the HBsAg+ group ($p < 0.05$). The frequency of chromosomal translocations involving *BCL6* was significantly increased in HBsAg+ DLBCL genomes (57% vs 28%; $p = .0472$) Antigen processing and p53 signaling pathway-associated genes were significantly upregulated in HBsAg+ DLBCLs ($p = 0.015$ and 0.036, respectively), as were BCL6-trageted, ZFP36L1-bound, and FOXO1-bound genes ($p = 0.008$, 0.000, 0.002, respectively).

confound the strength of the association between HBV infection and clinical outcome in B-cell NHL, including DLBCL. In addition, while two positive meta-analyses have been published, the relatively limited sample size of each individual study (the largest study by Ren et al. had 275 patients) and the bias inevitably present in retrospective datasets make any final conclusion about the impact of HBV on survival and response to treatment in DLBCL premature. It is therefore of significant interest to determine if such findings can be confirmed in non-endemic countries, in populations of different racial and ethnic backgrounds, and ideally in larger studies. In terms of ascertaining relative risk, considering that the prevalence of HBV infection is not as high in the U.S., it will be more challenging to validate the higher prevalence of B-NHL among patients with HBV infection, compared to endemic countries. However, focusing on subsets of U.S. patients with a higher prevalence of HBV infection but not from endemic areas may mitigate this challenge. A large enough multi-center retrospective study in North America or Europe in an unselected DLBCL population could provide a dataset addressing whether the higher prevalence and inferior survival outcomes of patients with concomitant HBV and DLBCL observed in Asia can be confirmed. If a study in non-endemic areas were to strongly suggest or confirm the inferior clinical outcome of DLBCL patients with concomitant HBV, it would also be important to determine if these disparities affect specific subsets of DLBCL patients, based on patient characteristics (ethnicity, race, age, comorbidities) or subtype of DLBCL (cell-of-origin, double hit, or double expressor status) defined by the standard of care methods (IHC, FISH). These questions can be addressed by a retrospective study, since all patients diagnosed with B-NHL in the U.S., including those with DLBCL, are screened for prior HBV infection prior to initiating therapy, with serologies for HBsAg, HBsAb, and HBcAB.

Of even greater interest is whether the mutational spectrum and the prevalence and specificity of the genomic signatures described by Ren and colleagues in HBV-associated DLBCL can be confirmed, although such studies will require more resources and coordination among centers. Given the increase in mutagenesis observed in HBsAg+ DLBCL (39), it would be important to determine if there is an association between HBV and one or more of the genetic subtypes of DLBCL defined by Schmitz et al. (34) and Chapuy et al. (32) The off-target AID-associated mutagenesis observed in lymphoma cases with HBV integration and the BCL6 chromosomal translocations in HBV associated DLBCL (39) could potentially lead to a higher prevalence of DH/TH lymphomas among HBV associated DLBCL. By specifying the DLBCL subtypes most impacted by HBV, it might also be possible to propose a mechanism by which HBV infection contributes to the development of DLBCL, and more broadly, B-cell NHL. This could also elucidate new prognostic factors and aid in earlier detection, treatment, or prevention of NHL in chronic HBV patients. Finally, it remains unclear, even from the retrospective studies conducted in Asia, whether *past* HBV exposure (HBsAg-, HBcAb+) confers an increased risk of B-NHL and worse clinical outcomes for B-NHL, or whether the presence of chronic HBV infection (HBsAg+, HBcAb+) is what ultimately confers these risks (discussed below).

Because HBV virus antigens can be detected in DLBCL cells via IHC in HBsAg+ patients using formalin-fixed paraffin-embedded (FFPE) tissue (16), the clinical relevance of such findings can be assessed in retrospective studies, to determine whether the presence of HBV antigens in the lymphoma cells of HBV associated DLBCL leads to distinct clinical characteristics or outcomes. For example: assuming that a linkage between chronic (or past) HBV infection is confirmed, is the presence of HBV antigens in the lymphoma cells necessary to confer worse clinical outcomes? This will be essential in the design of prognostic tools based on HBV status and for developing personalized therapies for these patients.

In need of further investigation is also the issue of occult HBV infection (OBI), defined as a condition where replication-competent HBV DNA is present in the liver or other tissues, with or without detectable HBV DNA in blood or plasma, in *HBsAg-negative* patients. HBV genome sequences were recently detected by NGS in plasma, normal B-cells, and tumor tissues from 40 HBsAg-negative DLBCL patients, 27 of which (68%) had OBI (72). Sequencing of these gene segments revealed a high frequency of viral DNA variants, including a T1762A/A1764G missense mutation in the basal core promoter and an HBsAg missense mutation that could account for HBsAg negativity and therefore

OBI status. The accumulation of viral variants could provide HBV with a survival advantage and drive lymphomagenesis in DLBCL in the absence of clinically overt chronic HBV infection. Additional support for the clinical impact of OBI in patients with lymphoma comes from studies showing that HBV reactivation in HBsAg-negative lymphoma patients receiving chemo-immunotherapy can occur (73–75), suggesting the need for antiviral prophylaxis in this group, and from a case-control study from Japan showing that patients with OBI had a higher prevalence of DLBCL than other groups (76).

Lastly, the association of HBV with indolent B-cell NHL might also be of interest, considering that HBV-associated follicular lymphoma (FL) was recently reported to also have distinct clinical and genetic features and worse outcomes (77–79).

5 Conclusions

The available evidence shows that chronic HBV infection is associated with a higher risk of developing B-cell NHL, particularly DLBCL, with odds ratios consistently ranging between 2.0 and 4.0. The relative risk of lymphoma associated with HBV infection is significantly lower compared to that of HCC, but, considering the global prevalence of HBV, it amounts to a very high burden of aggregate lymphoma risk. There is also evidence that DLBCL patients with chronic HBV infection have more aggressive disease, greater frequency of high-risk IPI, and inferior outcomes with R-CHOP, compared to patients without chronic HBV infection. Differential risk, clinical presentation, and outcome studies come almost exclusively from endemic areas, such as China, Taiwan, and Japan, and need to be confirmed in non-endemic settings. HBV-positive DLBCL showed a distinct gene expression profile, spectrum of somatic mutations, and genetic signatures enriched in pathways associated with high mutagenesis, suggesting that HBV-associated DLBCL are a distinct subtype. Finally, the risk of developing B-NHL in patients with OBI needs to be better studied considering the magnitude of the population at risk.

Author contributions

MR: Conceptualization, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. MP: Conceptualization, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. CT: Conceptualization, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. PS: Conceptualization, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. AK: Conceptualization, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. PP: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We would like to acknowledge Dr. Chari Cohen, President of the Hepatitis B Foundation in Philadelphia, and Dr. Amy Leader, Associate Professor in the Division of Population Science in the Department of Medical Oncology at Thomas Jefferson University, for their guidance in writing this report.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sehn LH, Salles G. Diffuse large B-cell lymphoma. *N Engl J Med* (2021) 384 (9):842–58. doi: 10.1056/NEJMra2027612

2. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S. Global burden of cancers attributable to infections in 2012: a synthetic analysis. *Lancet Glob Health* (2016) 4(9):e609–16. doi: 10.1016/S2214-109X(16)30143-7

3. *NIH launches clinical trial of Epstein-Barr virus vaccine* . National Institutes of Health (NIH. Available at: https://www.nih.gov/news-events/news-releases/nih-launches-clinical-trial-epstein-barr-virus-vaccine (Accessed December 6, 2022).

4. Prockop S, Doubrovina E, Suser S, Heller G, Barker J, Dahi P, et al. Off-the-shelf EBV-specific T cell immunotherapy for rituximab-refractory EBV-associated lymphoma following transplantation. *J Clin Invest* (2020) 130(2):733–47. doi: 10.1172/JCI121127

5. Messick TE, Smith GR, Soldan SS, McDonnell ME, Deakyne JS, Malecka KA, et al. Structure-based design of small-molecule inhibitors of EBNA1 DNA binding blocks Epstein-Barr virus latent infection and tumor growth. *Sci Transl Med* (2019) 11(482). doi: 10.1126/scitranslmed.aau5612

6. *Search orphan drug designations and approvals*. Available at: https://www.accessdata.fda.gov/scripts/opdlisting/oopd/detailedIndex.cfm?cfgridkey=847721 (Accessed December 7, 2022).

7. Haverkos BM, Alpdogan O, Baiocchi R, Brammer JE, Feldman TA, Capra M, et al. Nanatinostat (Nstat) and valganciclovir (VGCV) in relapsed/refractory (R/R) epstein-barr virus-positive (EBV +) lymphomas: final results from the phase 1b/2 VT3996-201 study. *Blood* (2021) 138(Supplement 1):623–3. doi: 10.1182/blood-2021-152603

8. Alter MJ. Epidemiology and prevention of hepatitis B. *Semin Liver Dis* (2003) 23 (1):39–46. doi: 10.1055/s-2003-37583

9. Kruszon-Moran D, Paulose-Ram R, Martin CB, Barker LK, McQuillan G. Prevalence and trends in hepatitis B virus infection in the United States, 2015-2018. *NCHS Data Brief* (2020) 361):1–8.

10. *Global progress report on HIV, viral hepatitis and sexually transmitted infections* (2021). Available at: https://www.who.int/publications/i/item/9789240027077 (Accessed December 6, 2022).

11. Kim WR. Epidemiology of hepatitis B in the United States. *Hepatology* (2009) 49 (5 Suppl):S28–34. doi: 10.1002/hep.22975

12. Nguyen MH, Burak Ozbay A, Liou I, Meyer N, Gordon SC, Dusheiko G, et al. Healthcare resource utilization and costs by disease severity in an insured national sample of US patients with chronic hepatitis B. *J Hepatol* (2019) 70(1):24–32. doi: 10.1016/j.jhep.2018.09.021

13. Kavosi Z, Zare F, Jafari A, Fattahi MR. Economic burden of hepatitis B virus infection in different stages of disease; a report from southern Iran. *Middle East J Dig Dis* (2014) 6(3):156–61.

14. Liang TJ. Hepatitis B: the virus and disease. *Hepatology* (2009) 49(5 Suppl):S13–21. doi: 10.1002/hep.22881

15. Chang M-H. Hepatitis B virus infection. *Semin Fetal Neonatal Med* (2007) 12 (3):160–7. doi: 10.1016/j.siny.2007.01.013

16. Huang X, Young KH, Guo W, Wang Y, Wang X, Xi Y, et al. Identification of hepatitis B virus aetiologic antigens, HBx and Pre-S2, in diffuse large B-cell lymphoma. *J Viral Hepat* (2020) 27(9):948–50. doi: 10.1111/jvh.13301

17. Croagh CMN, Lubel JS. Natural history of chronic hepatitis B: phases in a complex relationship. *World J Gastroenterol* (2014) 20(30):10395–404. doi: 10.3748/wjg.v20.i30.10395

18. de Almeida Pondé RA. Detection of the serological markers hepatitis B virus surface antigen (HBsAg) and hepatitis B core IgM antibody (anti-HBcIgM) in the diagnosis of acute hepatitis B virus infection after recent exposure. *Microbiol Immunol* (2022) 66(1):1–9. doi: 10.1111/1348-0421.12943

19. Zhou K, Dodge JL, Grab J, Poltavskiy E, Terrault NA. Mortality in adults with chronic hepatitis B infection in the United States: a population-based study. *Aliment Pharmacol Ther* (2020) 52(2):382–9. doi: 10.1111/apt.15803

20. Ganem D, Prince AM. Hepatitis B virus infection–natural history and clinical consequences. *N Engl J Med* (2004) 350(11):1118–29. doi: 10.1056/NEJMra031087

21. McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of hepatocellular carcinoma. *Hepatology* (2021) 73 Suppl 1(Suppl 1):4–13. doi: 10.1002/hep.31288

22. Nguyen MH, Wong G, Gane E, Kao J-H, Dusheiko G. Hepatitis B virus: advances in prevention, diagnosis, and therapy. *Clin Microbiol Rev* (2020) 33(2). doi: 10.1128/CMR.00046-19

23. Siegel RL, Miller KD, Fuchs HE, Jemal A. CA: a cancer journal for clinicians. *CA: A Cancer J Clin* (2022) 72(1):7–33. doi: 10.3322/caac.21708

24. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood* (2016) 127(20):2375–90. doi: 10.1182/blood-2016-01-643569

25. Li S, Young KH, Medeiros LJ. Diffuse large B-cell lymphoma. *Pathology* (2018) 50(1):74–87. doi: 10.1016/j.pathol.2017.09.006

26. Shenoy PJ, Malik N, Nooka A, Sinha R, Ward KC, Brawley OW, et al. Racial differences in the presentation and outcomes of diffuse large B-cell lymphoma in the United States. *Cancer* (2011) 117(11):2530–40. doi: 10.1002/cncr.25765

27. Li Y, Wang Y, Wang Z, Yi D, Ma S. Racial differences in three major NHL subtypes: descriptive epidemiology. *Cancer Epidemiol* (2015) 39(1):8–13. doi: 10.1016/j.canep.2014.12.001

28. Ayers AA, Lyu L, Dance K, Ward KC, Flowers CR, Koff JL, et al. Characterizing lymphoma incidence and disparities for a cancer center catchment region. *Clin Lymphoma Myeloma Leuk* (2019) 19(11):699–708.e5. doi: 10.1016/j.clml.2019.06.009

29. Nowakowski GS, Feldman T, Rimsza LM, Westin JR, Witzig TE, Zinzani PL. Integrating precision medicine through evaluation of cell of origin in treatment planning for diffuse large B-cell lymphoma. *Blood Cancer J* (2019) 9(6):48. doi: 10.1038/s41408-019-0208-6

30. Chiappella A, Diop F, Agostinelli C, Novo M, Nassi L, Evangelista A, et al. Prognostic impact of TP53 mutation in newly diagnosed diffuse large B-cell lymphoma patients treated in the FIL-DLCL04 trial. *Br J Haematol* (2022) 196(5):1184–93. doi: 10.1111/bjh.17971

31. Dodero A, Guidetti A, Marino F, Tucci A, Barretta F, Re A, et al. Dose-adjusted EPOCH and rituximab for the treatment of double expressor and double-hit diffuse large B-cell lymphoma: impact of TP53 mutations on clinical outcome. *Haematologica* (2022) 107(5):1153–62. doi: 10.3324/haematol.2021.278638

32. Chapuy B, Stewart C, Dunford AJ, Kim J, Kamburov A, Redd RA, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat Med* (2018) 24(5):679–90. doi: 10.1038/s41591-018-0016-8

33. Wright GW, Huang DW, Phelan JD, Coulibaly ZA, Roulland S, Young RM, et al. A probabilistic classification tool for genetic subtypes of diffuse large B cell lymphoma with therapeutic implications. *Cancer Cell* (2020) 37(4):551–568.e14. doi: 10.1016/j.ccell.2020.03.015

34. Schmitz R, Wright GW, Huang DW, Johnson CA, Phelan JD, Wang JQ, et al. Genetics and pathogenesis of diffuse large B-cell lymphoma. *N Engl J Med* (2018) 378 (15):1396–407. doi: 10.1056/NEJMoa1801445

35. International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. *N Engl J Med* (1993) 329 (14):987–94. doi: 10.1056/NEJM199309303291402

36. Sehn LH, Berry B, Chhanabhai M, Fitzgerald C, Gill K, Hoskins P, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. *Blood* (2007) 109(5):1857–61. doi: 10.1182/blood-2006-08-038257

37. Bourbon E, Maucort-Boulch D, Fontaine J, Mauduit C, Sesques P, Safar V, et al. Clinicopathological features and survival in EBV-positive diffuse large B-cell lymphoma not otherwise specified. *Blood Adv* (2021) 5(16):3227–39. doi: 10.1182/bloodadvances.2021004515

38. Hosry J, Miranda RN, Samaniego F, Economides MP, Torres HA. Clinicopathologic characteristics and outcomes of transformed diffuse large B-cell lymphoma in hepatitis C virus-infected patients. *Int J Cancer* (2018) 142(5):940–8. doi: 10.1002/ijc.31110

39. Ren W, Ye X, Su H, Li W, Liu D, Pirmoradian M, et al. Genetic landscape of hepatitis B virus-associated diffuse large B-cell lymphoma. *Blood* (2018) 131(24):2670–81. doi: 10.1182/blood-2017-11-817601

40. Kim SJ, Yoon DH, Jaccard A, Chng WJ, Lim ST, Hong H, et al. A prognostic index for natural killer cell lymphoma after non-anthracycline-based treatment: a multicentre, retrospective analysis. *Lancet Oncol* (2016) 17(3):389–400. doi: 10.1016/S1470-2045(15)00533-1

41. Landsburg DJ, Petrich AM, Abramson JS, Sohani AR, Press O, Cassaday R, et al. Impact of oncogene rearrangement patterns on outcomes in patients with double-hit non-Hodgkin lymphoma. *Cancer* (2016) 122(4):559–64. doi: 10.1002/cncr.29781

42. Song JY, Perry AM, Herrera AF, Chen L, Skrabek P, Nasr MR, et al. Double-hit signature with TP53 abnormalities predicts poor survival in patients with germinal center type diffuse large B-cell lymphoma treated with R-CHOP. *Clin Cancer Res* (2021) 27(6):1671–80. doi: 10.1158/1078-0432.CCR-20-2378

43. Dunleavy K. Double-hit lymphoma: optimizing therapy. *Hematol Am Soc Hematol Educ Program* (2021) 2021(1):157–63. doi: 10.1182/hematology.2021000247

44. Ulcickas Yood M, Quesenberry CP, Guo D, Caldwell C, Wells K, Shan J, et al. Incidence of non-Hodgkin's lymphoma among individuals with chronic hepatitis B virus infection. *Hepatology* (2007) 46(1):107–12. doi: 10.1002/hep.21642

45. Li M, Gan Y, Fan C, Yuan H, Zhang X, Shen Y, et al. Hepatitis B virus and risk of non-Hodgkin lymphoma: An updated meta-analysis of 58 studies. *J Viral Hepat* (2018) 25(8):894–903. doi: 10.1111/jvh.12892

46. Mahale P, Engels EA, Koshiol J. Hepatitis B virus infection and the risk of cancer in the elderly US population. *Int J Cancer* (2019) 144(3):431–9. doi: 10.1002/ijc.31643

47. Spradling PR, Xing J, Zhong Y, Rupp LB, Moorman AC, Lu M, et al. Incidence of Malignancies among patients with chronic hepatitis B in US health care organizations, 2006-2018. *J Infect Dis* (2022) 226(5):896–900. doi: 10.1093/infdis/jiac011

48. An J, Kim JW, Shim JH, Han S, Yu CS, Choe J, et al. Chronic hepatitis B infection and non-hepatocellular cancers: A hospital registry-based, case-control study. *PloS One* (2018) 13(3):e0193232. doi: 10.1371/journal.pone.0193232

49. Cheng C-L, Huang S-C, Chen J-H, Wei C-H, Fang W-Q, Su T-H, et al. Hepatitis B surface antigen positivity is an independent unfavorable prognostic factor in diffuse large B-cell lymphoma in the rituximab era. *Oncologist* (2020) 25(9):793–802. doi: 10.1634/theoncologist.2019-0756

50. Dalia S, Chavez J, Castillo JJ, Sokol L. Hepatitis B infection increases the risk of non-Hodgkin lymphoma: a meta-analysis of observational studies. *Leuk Res* (2013) 37 (9):1107–15. doi: 10.1016/j.leukres.2013.06.007

51. Kim M, Lee YK, Park B, Oh DJ, Choi HG. Hepatitis virus B and C infections are associated with an increased risk of non-Hodgkin lymphoma: A nested case-control study using a national sample cohort. *J Med Virol* (2020) 92(8):1214–20. doi: 10.1002/jmv.25653

52. Lai Y-R, Chang Y-L, Lee C-H, Tsai T-H, Huang K-H, Lee C-Y. Risk of non-hodgkin lymphoma among patients with hepatitis B virus and hepatitis C virus in Taiwan: A nationwide cohort study. *Cancers (Basel)* (2022) 14(3):583. doi: 10.3390/cancers14030583

53. Li M, Shen Y, Chen Y, Gao H, Zhou J, Wang Q, et al. Characterization of hepatitis B virus infection and viral DNA integration in non-Hodgkin lymphoma. *Int J Cancer* (2020) 147(8):2199–209. doi: 10.1002/ijc.33027

54. Nath A, Agarwal R, Malhotra P, Varma S. Prevalence of hepatitis B virus infection in non-Hodgkin lymphoma: a systematic review and meta-analysis. *Intern Med J* (2010) 40(9):633–41. doi: 10.1111/j.1445-5994.2009.02060.x

55. Yi H, Chen J, Cen H, Yan W, Tan X. Association between infection of hepatitis B virus and onset risk of B-cell non-Hodgkin's lymphoma: a systematic review and a meta-analysis. *Med Oncol* (2014) 31(8):84. doi: 10.1007/s12032-014-0084-7

56. Ma R, Xing Q, Shao L, Wang D, Hao Q, Li X, et al. Hepatitis B virus infection and replication in human bone marrow mesenchymal stem cells. *Virol J* (2011) 8:486. doi: 10.1186/1743-422X-8-486

57. Lau KC, Joshi SS, Gao S, Giles E, Swidinsky K, van Marle G, et al. Oncogenic HBV variants and integration are present in hepatic and lymphoid cells derived from chronic HBV patients. *Cancer Lett* (2020) 480:39–47. doi: 10.1016/j.canlet.2020.03.022

58. Coffin CS, Mulrooney-Cousins PM, Osiowy C, Michalak TI. Virological characteristics of occult hepatitis B virus in a North American cohort of human immunodeficiency virus type 1-positive patients on dual active anti-HBV/HIV therapy. *J Clin Virol* (2014) 60(4):347–53. doi: 10.1016/j.jcv.2014.04.021

59. Coffin CS, Mulrooney-Cousins PM, Michalak TI. Hepadnaviral lymphotropism and its relevance to HBV persistence and pathogenesis. *Front Microbiol* (2021) 12:695384. doi: 10.3389/fmicb.2021.695384

60. Zhao K, Liu A, Xia Y. Insights into Hepatitis B Virus DNA Integration-55 Years after Virus Discovery. *Innovation (Camb)* (2020) 1(2):100034. doi: 10.1016/j.xinn.2020.100034

61. Paterlini-Bréchot P, Saigo K, Murakami Y, Chami M, Gozuacik D, Mugnier C, et al. Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene. *Oncogene* (2003) 22(25):3911–6. doi: 10.1038/sj.onc.1206492

62. Saigo K, Yoshida K, Ikeda R, Sakamoto Y, Murakami Y, Urashima T, et al. Integration of hepatitis B virus DNA into the myeloid/lymphoid or mixed-lineage leukemia (MLL4) gene and rearrangements of MLL4 in human hepatocellular carcinoma. *Hum Mutat* (2008) 29(5):703–8. doi: 10.1002/humu.20701

63. Li W, Zeng X, Lee NP, Liu X, Chen S, Guo B, et al. HIVID: an efficient method to detect HBV integration using low coverage sequencing. *Genomics* (2013) 102(4):338–44. doi: 10.1016/j.ygeno.2013.07.002

64. Li X, Zhang J, Yang Z, Kang J, Jiang S, Zhang T, et al. The function of targeted host genes determines the oncogenicity of HBV integration in hepatocellular carcinoma. *J Hepatol* (2014) 60(5):975–84. doi: 10.1016/j.jhep.2013.12.014

65. Svicher V, Salpini R, D'Anna S, Piermatteo L, Iannetta M, Malagnino V, et al. New insights into hepatitis B virus lymphotropism: Implications for HBV-related lymphomagenesis. *Front Oncol* (2023) 13:1143258. doi: 10.3389/fonc.2023.1143258

66. Zeldis JB, Mugishima H, Steinberg HN, Nir E, Gale RP. *In vitro* hepatitis B virus infection of human bone marrow cells. *J Clin Invest* (1986) 78(2):411–7. doi: 10.1172/JCI112591

67. Stoll-Becker S, Repp R, Glebe D, Schaefer S, Kreuder J, Kann M, et al. Transcription of hepatitis B virus in peripheral blood mononuclear cells from persistently infected patients. *J Virol* (1997) 71(7):5399–407. doi: 10.1128/JVI.71.7.5399-5407.1997

68. Wieland SF. The chimpanzee model for hepatitis B virus infection. *Cold Spring Harb Perspect Med* (2015) 5(6). doi: 10.1101/cshperspect.a021469

69. Jaquet A, Boni SP, Boidy K, Tine J, Tchounga B, Touré SA, et al. Chronic viral hepatitis, HIV infection and Non-Hodgkin lymphomas in West Africa, a case-control study. *Int J Cancer* (2021) 149(8):1536–43. doi: 10.1002/ijc.33709

70. Rong X, Wang H, Ma J, Pan S, Wang H, Jing S, et al. Chronic hepatitis B virus infection is associated with a poorer prognosis in diffuse large B-cell lymphoma: a meta-analysis and systemic review. *J Cancer* (2019) 10(15):3450–8. doi: 10.7150/jca.31033

71. Vile RG, Melcher A, Pandha H, Harrington KJ, Pulido JS. APOBEC and cancer viroimmunotherapy: thinking the unthinkable. *Clin Cancer Res* (2021) 27(12):3280–90. doi: 10.1158/1078-0432.CCR-20-1888

72. Sinha M, Sundar K, Premalata CS, Asati V, Murali A, Bajpai AK, et al. Pro-oncogenic, intra host viral quasispecies in Diffuse large B cell lymphoma patients with occult Hepatitis B Virus infection. *Sci Rep* (2019) 9(1):14516. doi: 10.1038/s41598-019-51157-1

73. Yeo W, Chan TC, Leung NWY, Lam WY, Mo FKF, Chu MT, et al. Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. *J Clin Oncol* (2009) 27(4):605–11. doi: 10.1200/JCO.2008.18.0182

74. Pei S-N, Chen C-H, Lee C-M, Wang M-C, Ma M-C, Hu T-H, et al. Reactivation of hepatitis B virus following rituximab-based regimens: a serious complication in both HBsAg-positive and HBsAg-negative patients. *Ann Hematol* (2010) 89(3):255–62. doi: 10.1007/s00277-009-0806-7

75. Hsu C, Tsou H-H, Lin S-J, Wang M-C, Yao M, Hwang W-L, et al. Chemotherapy-induced hepatitis B reactivation in lymphoma patients with resolved HBV infection: a prospective study. *Hepatology* (2014) 59(6):2092–100. doi: 10.1002/hep.26718

76. Tajima K, Takahashi N, Ishizawa K, Murai K, Akagi T, Noji H, et al. High prevalence of diffuse large B-cell lymphoma in occult hepatitis B virus-infected patients in the Tohoku district in Eastern Japan. *J Med Virol* (2016) 88(12):2206–10. doi: 10.1002/jmv.24584

77. Ren W, Wang X, Yang M, Wan H, Li X, Ye X, et al. Distinct clinical and genetic features of hepatitis B virus-associated follicular lymphoma in Chinese patients. *Blood Adv* (2022) 6(9):2731–44. doi: 10.1182/bloodadvances.2021006410

78. Fernández-Rodríguez C, Rodríguez-Sevilla JJ, Fernández-Ibarrondo L, Sánchez-González B, Gibert J, Bento L, et al. Worse outcome and distinct mutational pattern in follicular lymphoma with anti-HBc positivity. *Blood Adv* (2022) 6(1):82–6. doi: 10.1182/bloodadvances.2021005316

79. Cheng C-L, Fang W-Q, Lin Y-J, Yuan C-T, Ko B-S, Tang J-L, et al. Hepatitis B surface antigen positivity is associated with progression of disease within 24 months in follicular lymphoma. *J Cancer Res Clin Oncol* (2022) 148(5):1211–22. doi: 10.1007/s00432-021-03719-y

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 08 November 2023
DOI 10.3389/fonc.2023.1275327

OPEN ACCESS

EDITED BY
Sophia Yohe,
University of Minnesota Twin Cities,
United States

REVIEWED BY
Zhiming Li,
Sun Yat-sen University Cancer Center (SYSUCC), China
Xianhuo Wang,
Tianjin Medical University Cancer Institute and Hospital, China

*CORRESPONDENCE
Victor Tomacinschii
victor.tomacinschii@usmf.md
Adrian Mosquera Orgueira
Adrian.Mosquera.Orgeira@sergas.es

RECEIVED 09 August 2023
ACCEPTED 27 October 2023
PUBLISHED 08 November 2023

CITATION
Tomacinschii V, Mosquera Orgueira A, Santos CA, Robu M, Buruiana S and Fraga Rodriguez MF (2023) The implication of next-generation sequencing in the diagnosis and clinical management of non-Hodgkin lymphomas.
Front. Oncol. 13:1275327.
doi: 10.3389/fonc.2023.1275327

The implication of next-generation sequencing in the diagnosis and clinical management of non-Hodgkin lymphomas

Victor Tomacinschii[1,2]*, Adrian Mosquera Orgueira[3,4]*, Carlos Aliste Santos[3], Maria Robu[1], Sanda Buruiana[1] and Maximo Francisco Fraga Rodriguez[3,4,5]

[1]Department of Hematology, Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Moldova, [2]Department of Hematology, Public Medical Sanitary Institution (PMSI) Institute of Oncology, Chisinau, Moldova, [3]University Hospital of Santiago de Compostela, Servizo Galego de Saude (SERGAS), Santiago de Compostela, Spain, [4]Health Research Institute of Santiago de Compostela, Santiago de Compostela, Spain, [5]Department of Forensic Sciences, Pathology, Ginecology and Obstetrics and Pediatrics, Faculty of Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain

Next generation sequencing (NGS) is a technology that broadens the horizon of knowledge of several somatic pathologies, especially in oncological and oncohematological pathology. In the case of NHL, the understanding of the mechanisms of tumorigenesis, tumor proliferation and the identification of genetic markers specific to different lymphoma subtypes led to more accurate classification and diagnosis. Similarly, the data obtained through NGS allowed the identification of recurrent somatic mutations that can serve as therapeutic targets that can be inhibited and thus reducing the rate of resistant cases. The article's purpose is to offer a comprehensive overview of the best ways of integrating of next-generation sequencing technologies for diagnosis, prognosis, classification, and selection of optimal therapy from the perspective of tailor-made medicine.

KEYWORDS

lymphomas, non-Hodgkin lymphomas, genomics, diagnosis, next-generation sequencing

1 Introduction

Non-Hodgkin's lymphomas (NHL) are hematopoietic tumors that develop from the malignant proliferation of the lymphatic tissue. NHLs are the most common hematological neoplasms, accounting for roughly 3% of cancer cases worldwide. According to the most recent GLOBOCAN data, 544,352 new cases of NHL were diagnosed worldwide in 2020 (1, 2).

Classifications of non-Hodgkin's lymphomas have undergone numerous refinements and completions over time, ranging from classifications based on the histological and immunological profile of tumors (Rappoport classification (3), Kiel classification (4), Lukes and Collins classification (5), etc.) to current classifications systems (World Health Organization Classifications from 2016 and 2022 and The International Consensus Classification of Mature Lymphoid Neoplasms (6–8)) which divides non-Hodgkin's lymphomas according to histological, immunohistochemical and gene expression profiles (GEP).

Healthcare practitioners prefer also to group NHL subtypes based on the speed of disease progression. For example, indolent B-cell lymphomas, such as follicular lymphomas, marginal zone lymphoma, small lymphocytic lymphoma, proceed as chronic incurable diseases, in which the clinical course is slow-progressing and oligosymptomatic for a long period of time but for the treatment of which, however, regular exposure to toxic cytostatic drugs and/or radiation therapy is required (9).

On the other hand, aggressive B-cell lymphomas represent a heterogeneous category of lymphomas that may involve precursor lymphoid neoplasms (B-lymphoblastic leukaemia/lymphoma NOS and B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities) as well as a variety of mature B-cell lymphomas, like Burkitt lymphoma, mantle cell lymphoma, primary effusion lymphoma, and diffuse large B-cell lymphoma. They have aggressive behavior, with frequent extranodal involvement and require immediate treatment, otherwise, resulting in patient's rapid desmise. Although modern treatment regimens can increase survival in certain patients with aggressive large-cell lymphomas (approximately 60% in diffuse B-large-cell lymphoma, about 30% in peripheral T-cell lymphomas), disease progression remains the leading cause of death (1, 9–11).

The notable progress in recent years should be attributed to the advances in molecular genetics. These advancements have enabled a shift from analyzing individual genes and markers to conducting comprehensive studies on multiple genes or their expressed products concurrently, particularly in the context of cancer research (12–14). The emergence of high-tech genome-wide research methods and their integration into publicly available databases make it possible to obtain more detailed information about the mechanisms of oncogenesis, explain the division of tumors by histological types, differentiate gene networks that determine the main stages of tumor pathogenesis, and study the mechanisms of drug resistance (15–18).. The study of gene expression profiles in certain types and subtypes of tumors makes it possible to identify additional markers associated with the clinical course, the risk of invasion and metastasis, as well as to supplement and refine the existing classification or propose a new one based on the molecular characteristics of the tumor (19–22).

The beginnings of gene study date back to Sanger et al. who introduced his chain termination method for sequencing DNA in 1977 (23), that quickly gained great acceptance and popularity around the world, becoming in fact the first generation of the DNA sequencing technology (24, 25).

Next-generation sequencing(NGS) is a method of sequencing multiple DNA or RNA products in parallel. This technique is also known by other names (eg, short-read sequencing, deep sequencing, second-generation sequencing). In contrast to Sanger sequencing, the speed of sequencing and the amount of DNA sequence data generated by NGS are exponentially higher, and the cost of production is significantly lower (26). The most complete molecular assessment lymphoma genetics was obtained by using whole genome sequencing of all coding sequences (exome) by high-throughput next-generation parallel sequencing (WES). WES studies were performed for each of the major immunomorphological subtypes of lymphomas: DLBCL, Burkitt lymphoma, follicular lymphoma, mantle, splenic marginal zone lymphoma, and peripheral T-cell lymphomas (27).

The contemporary diagnosis of NHLs is based on morphological and immunophenotypic studies, as well as chromosomal and molecular analyses, which are indicated as diagnostic procedures to establish high-precision diagnoses (28). The current recommendations, however, do not provide clear stipulations for the conditions of sequencing techniques used for NHL diagnosis and prognosis. There is no uniform strategy at this time, and aspects such as gene selection, sequencing platform, read depth, and variant analysis may vary among laboratories. Therefore, standardization of the panels is needed especially taking into account the fact that the NGS panels of the lymphoid lineage are becoming more accessible for clinical practice.

The purpose of this article is to provide a comprehensive overview of the gene panels that are identified in different NHL types by the use of NGS techniques.

2 B-cell lymphomas

B-cell lymphomas represent the predominant type of NHL diagnosed globally. About 85-90% of NHL cases are derived from B cells, whereas the remaining lymphomas originate from T cells or NK cells (6, 29). This epidemiological circumstance likely explains the greater inclination for studying the genomic and transcriptomic features of these neoplasms by various research groups. In the following sections, we will describe the specifics of gene expression profiles in some of the most common types of NHL. Table 1 includes a summary of the most common GEP associated with different types of B-cell lymphomas.

2.1 Diffuse large B cell lymphoma

Diffuse large B cell lymphoma (DLBCL) is the most frequent type of non-Hodgkin lymphoma in the world, accounting for 30–40% of all occurrences depending on the geographical region (44).

Traditionally, DLBCL cases were classified according to cell-of-origin (COO), with two different subtypes described: germinal center B-cell like (GCB) and activated B-cell like (ABC), and with about 10–15 percent of cases remaining unclassifiable (45).

TABLE 1 Genetic profile of B cell lymphomas.

Type of lymphoma	Genetic profile	References
Diffuse large B cell lymphoma, GCB subtype	*BCL2/BCL6, EZH2,GNA13, IRF8, MYC, SGK1, STAT3, TNFR14*	(17) (30, 31) (6) (32)
Diffuse large B cell lymphoma, ABC subtype	*CD79b, EP300, KMT2D, MYD88d, PIM1, PRDM1*	(17, 30, 31) (6) (32)
Follicular lymphoma	*DTX1, EP300, EZH2, ARID1A, CREBBP, CARD11, FOXO1, HIST1H1E, MEF2B, NOTCH2, UBE2A*	(33, 34) (32) (35)
Marginal zone lymphoma	*BTK, NOTCH2, BCL10, BIRC3, CARD11, KLF2, PLCG2, PTPRD*	(32, 36)
Mantle zone lymphoma	*BTK, NOTCH1/2, MALT1, ATM, BCL10, BIRC3, CDKN2A, IKBKB, MAP3K14, NSD2, PLCG2, SMARCA4, TP53, TRAF2*	(37) (38, 39) (32) (40)
Small lymphocytic lymphoma/ Chronic lymphocytic leukemia	*ATM, BIRC3, BTK, NOTCH1, PLCG2, POT1, SF3B1, TP53d*	(32, 41) (42)
Primary mediastinal large B-cell lymphoma	*STAT6, XPO1, B2M, NFKBIE, PTPN1,TNFAIP3*	(6, 32) (43)
Burkitt lymphoma	*ID3, TCF3, CCND3, TP53, CDKN2A, MYC, DDX3X, PTEN, PIK3R1, ARID1A, SMARCA4, GNA13, ROCK1*	(6, 32)

Patients who have the GCB subtype have a better prognosis than those who have the ABC subtype. Although COO can help predict the outcome, the GCB and ABC subtypes are still very heterogeneous raising the question of a more accurate prognostic stratification (6, 44, 46).

In 2017, Reddy et al. conducted a study that used whole-exome and transcriptome sequencing of tumors from 1,001 newly diagnosed DLBCL patients to determine genetic drivers of the disease and establish probable links to clinical outcomes (47).

As a result, the authors identified 150 genes that are directly involved in the pathogenesis of DLBCL. These genes can be classified into four main categories:

1) genes involved in signaling pathways (for example, *MTOR, PIK3R1, PIM2, BTK*);
2) genes associated with transcription and translation in the cell (for example, *SF3B1, XPO1, HIST1H1E*);
3) genes responsible for the stages of B cell differentiation (for example, *EBF1, IRF4, PAX5, POU2F2, YY1*);
4) genes responsible for cell growth and proliferation (for example, *MYC, CHD8, BCL2*).

Also, *MYD88* was chosen as a critical mutation in the ABC subtype, whereas *XPO1* was chosen as an essential mutation in GCB DLBCL. The publication is limited by the lack of explanation in case of DNA mutation-based disease clustering, focusing only on RNA-based or translocation-based classification with DNA mutations (47).

Chapuy et al. in 2018, proposed a DNA-based classification of DLBCL. In this study WES was performed on 304 patients samples. C1–C5 were the names given to these clusters, permitting the classification of ABC and GCB-DLBCL cases into two different groups with favorable and adverse outcomes. ABC subtypes were divided into two groups: a lower risk group with a putative marginal zone origin (C1) characterized by *NOTCH2* mutations/*BCL6* translocation, and one with a higher risk (C5) with chromosome 18q gain with *BCL2* and *MALT1* gene overexpression and *CD79B* and *MYD88* mutations. The C2 subgroup was associated with biallelic loss or mutation of TP53 and widespread somatic copy number alterations. Additionally, C2 tumors frequently showed copy loss of 9p21.13/CDKN2A and 13q14.2/RB1. Two other subtypes of the GCB were identified (C3 and C4). C4, which was associated with low risk disease and revealed mutations impacting the *BCR/PI3K, JAK/STAT*, and *BRAF* pathways. Conversely, mutations impacting *BCL2* translocation, *PTEN*, and epigenetic mediators such as *KMT2D*, *CREBBP*, and *EZH2* were all linked to the poorer prognosis of the *C3* subgroup (17).

Schmitz et al. used whole-exome and transcriptome sequencing, DNA copy number analysis, and deep targeted amplicon sequencing to examine data from 574 DLBCL patients. As a result, four different subtypes of DLBCL were identified: MCD (based on the presence of *MYD88L265P* and *CD79B* mutations), BN2 (based on *BCL6* fusions and *NOTCH2* mutations), N1 (based on the presence of *NOTCH1* mutations), and EZB (based on the presence of *EZH2* mutations and *BCL2* translocations) (30). There are numerous parallels between Chapuy's and Schmitz's subgroups, including the following: C1 resembles the BN2 group, C3 overalps EZB, and C5 is similar to MCD.

A follow-up to the findings of Schmitz et al. was the research done by Wright et al. examining the initially unclassified cases. The researchers identified two other subtypes, one with high levels of aneuploidy and mutation of *TP53*, and the second *ST2* (*SGK1* and *TET2* mutations). These corresponded closely to the Chapuy subgroups, C2 and C4. Thus, each of the five Chapuy clusters could now be mapped to one of the Schmitz genetic subgroups. LymphGen is the name given to this classification at the moment (31). The correlation between these three molecular classifications is shown in Figure 1.

Recently, studies incorporating clinical, biochemical, and genetic data into multimodal machine learning models have yielded to the elaboration of a gene expression profiling tool that is offering encouraging results in terms of more accurate DLBCL prognostication (48).

2.2 Follicular lymphoma

Follicular lymphoma (FL) represents the second most common non-Hodgkin lymphoma and the most prevalent indolent lymphoma. The chromosomal translocation t(14;18)(q32:q21), in which the immunoglobulin heavy chain (*IGH*) enhancer region at

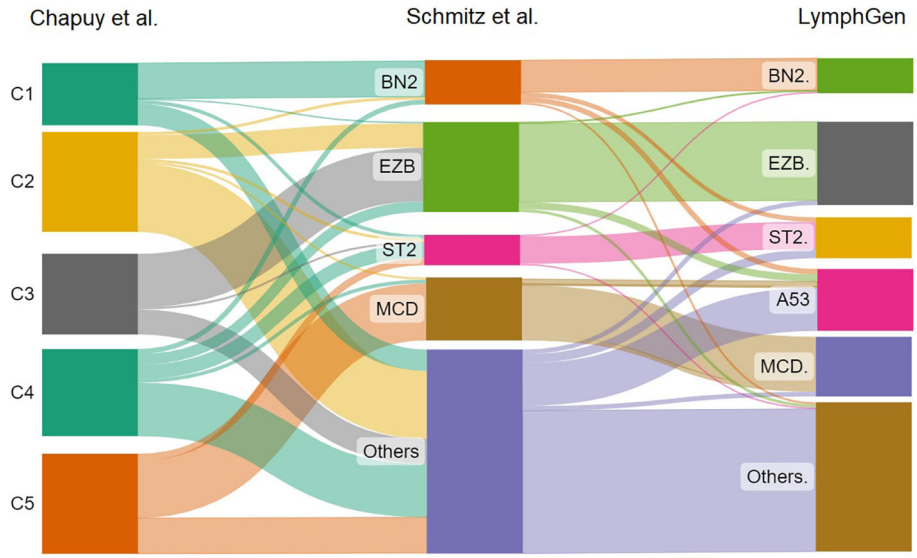

FIGURE 1
The sankey plot shows the relative proportion of cases from the Chapuy et al. classification that correlates with the Schmitz et al. molecular groups and LymphGen classification.

14q32 and the B-cell lymphoma 2 (*BCL2*) gene at 18q21 are juxtaposed, is the hallmark of FL, which is identified in about 90% of cases (49). NGS research has been useful not only in creating a list of genomic events that occur in addition to t(14;18), but also in identifying new potential genetic drivers. The high frequency of mutations affecting epigenetic control is the second distinguishing feature of FL.

Deregulation of such processes (e.g. aberrant DNA hypermethylation) has been recognized as a central feature of hematologic malignancies, and FL in particular, observed in 80% of cases (50). The histone methyltransferases *KMT2D* (90%) and *EZH2* (25%) as well as the histone acetyltransferases *CREBBP* (30–60%) and *EP300* (9%) are among the most commonly mutated genes (51). A clinical-genomic score was created using seven genes including those mentioned above, to predict Failure-Free Survival (FFS) and Overall Survival (OS) (33).

In the era of FL treatment with conventional chemotherapy, the scientists found that mutations in *EP300, FOXO1, CREBBP*, and *CARD11* (providing poor prognosis) and *MEF2B, ARID1A*, and *EZH2* (providing good prognosis) in association with clinical parameters of the FLIPI score, improved PFS and OS prediction. Furthermore, the m7-FLIPI was able to reclassify almost half of the high-risk FLIPI patients into a low-risk m7-FLIPI group, mainly through the discovery of *EZH2* mutations (33, 34). An another research on the m7-FLIPI score across different populations with FL suggests that this molecular score has no impact on patients with FL, treated in the first line, with chemotherapy-free regimens (52, 53). In addition, another study has shown that four mutant genes in FL samples (*NOTCH2, DTX1, UBE2A*, and *HIST1H1E*) were linked to shorter transformation time to DLBCL (35).

In a recent study performed by Gao et al., for the first time, they studied the genomic and transcriptomic characteristics that could predict progression of disease within 24 months (POD24). As a result of this study, they identified genomic markers that are able to predict POD24 in patients with FL. So, HIST1H1D, known as a driver mutation, was significantly correlated with POD24. Furthermore, gains of 6q22.2 (HIST1H1D) and 18q21.33 (BCL2) and loss of 1p36.13 (NBPF1) predicted POD24 independent of FLIPI (54).

2.3 Mantle cell lymphoma

Mantle Cell lymphoma (MCL) is an incurable type of aggressive lymphoma with a median survival of approximately 5 years (38, 55). The revised World Health Organization classification from 2016 identified two molecular routes of MCL dividing them in cases of Nodal MCL and Leukemic non-nodal MCL (10-20% of cases, more indolent) (6, 39).

More than 30 years have passed since the first report of the well-known hallmark genetic alteration t (11,14) (q13; q32)/*CCND1*::*IGH*, which is seen in 95 percent of MCL cases. The result of juxtaposition of heavy-chain immunoglobulin (*IGH*) enhancer region (on 14q32) next to *CCND1* (on 11q13), results in its overexpression of Cyclin D1 (56, 57).

MCL was divided into two categories by the WHO classification: classical MCL and indolent leukemic non-nodal MCL. Indolent leukemic non-nodal MCL is characterized by mutated IGHV and primarily *SOX11* negativity, as well as peripheral blood, bone marrow, and occasionally splenic involvement but no major nodal involvement. Classical MCL is characterised by unmutated or minimally mutated IGHV and mostly *SOX11* positivity (6, 58).

In recent years, genomic techniques have revealed mutations with prognostic implication for MCL. A recent meta-analysis summarized the most common mutations discovered using

molecular methods in MCL patients. Among the most common mutant genes were: *ATM* (43.5%) followed by *TP53* (26.8%), *CDKN2A* (23.9%), and *CCND1* (20.2%). Aberrations in *IGH* (38.4%) and *MYC* (20.8%) were also discovered, mostly by cytogenetic techniques. Other prevalent baseline mutations included *NSD2* (15%), *KMT2A* (8.9%), *S1PR1* (8.6%), and *CARD11* (8.5%). The authors propose that a panel of these genes shall be added to NGS panels (55). *CDNK2A* deletion, *ATM, NOTCH1/2, NSD2* mutations were highlighted as markers of poor prognosis. Other mutations were described to have potential diagnostic, therapeutic and predictive role, such as those in *BIRC3, BTK, PLCG2, SMARCA4* and *MAP3K14* (40, 59, 60).

Agarwal et al. discovered genetic patterns that separates responders and nonresponders in a prospective study performed on patients with MCL. ATM mutations were found in the majority of patients who had a complete response, while chromosome 9p21.1-p24.3 loss and/or mutations in SWI-SNF chromatin-remodeling complex components were found in all patients with primary resistance and two-thirds of patients with relapsed disease (61).

TP53 mutation is another significant indicator of MCL prognosis. Patients with *TP53* mutation were related to the blastoid morphology of MCL, elevated Ki-67, high-risk MIPI, and MIPI-c. When compared to *TP53*-unmutated cases, *TP53* mutations lead to inferior results in terms of response following both induction and autologous stem cell transplantation, as well as shorter PFS (62, 63).

Recently, Yi et al. (37) conducted a WES study on 152 samples of MCL patients, classifying MCL molecularly into 4 distinct clusters (C1-C4). C1 had a 5-year OS of 100% and it was associated with mutant immunoglobulin heavy variable (*IGHV*), *CCND1* mutation, amp(11q13), and active B cell receptor (BCR) signaling. C2 was linked with del(11q)/*ATM* mutations, activation of *NF-kB* and DNA repair pathways, and it was associated with a 5-year OS of 56.7%. C3 was characterized by mutations in *SP140, NOTCH1*, and *NSD2*, as well as downregulation of *BCR* signaling and *MYC* targets, and had a 5-year OS of 47%. C4 included patients with del(17p)/*TP53* mutations, del(13q), and del(9p), as well as active *MYC* pathway and hyperproliferation signatures, and it was associated with a poor prognosis (5-year OS of only 14.2%) (37).

3 NK/T-cell lymphomas

Malignant T/NK lymphomas(TNKL) are a distinct group of non-Hodgkin's lymphomas that account for an estimated 10-15% of the total NHL, with a higher incidence in certain geographic areas (Asia, South America) (64, 65). TNKL, like other malignant proliferative disorders, exhibit genetic instability and chromosomal abnormalities, which combined induce malignant transformation. Therefore, the use of NGS and GEP represent a chance to discover new patterns that can have real prognostic and theranostic impact on TNKL. Despite this, for various reasons, compared to B cell lymphomas, there are fewer reports of the use of NGS, WES, WGS in the case of TNKL. Next, we will attempt to compile the existing data which has genuine prognostic or therapeutic implications. Similarly, data on GEP within distinct TNKLs will be reported in Table 2 separately.

TABLE 2 Genetic profile of T cell lymphomas.

Type of T/NK non-Hodgkin lymphoma	Genetic profile	References
Angioimmunoblastic T-cell lymphoma	*RHOA, TET2, IDH2, DNMT3A, CD28*	(66) (67, 68)
Adult T-cell leukemia/lymphoma	*PLCG1, PRKCB, CARD11, VAV1, IRF4, FYN, CCR4, CCR7, GATA3, HNRNPA2B1, GPR183, CSNK2A1, CSNK2B*	(69–71)
Extranodal natural killer/T-cell lymphoma, nasal type	*TP53, DDX3X, MGA, STAT3, STAT5B, MLL2, ARID1A, EP300, ASXL3, BCOR, MSN, JAK3, KMT2D*	(72–74)
Intestinal T-cell lymphoma	*STAT5B, SETD2, JAK1, JAK3, STAT3, SOCS1, KRAS, TP53*	(75) (76)
Mycosis fungoides/ Sezary Syndrome	*TCR, MYC, TOX, TP53, NCOR1, PTEN, FAS, DNMT3A, USP28, CAAP1, TMEM244, EHD1, MTMR2, RNF123, TOX, BAIAP2, CPN2, GPR128, CAPN12, FIGLA*	(77–79)
Subcutaneous panniculitis-like T-cell lymphoma	*mTOR/AKT/PI3K, HAVCR2*	(80)
Peripheral T- cell lymphoma, NOS	*TP53, CDKN2A, WWOX, ANKRD11, pY-STAT3*	(81)
Breast Implant-Associated Anaplastic Large Cell Lymphoma	*JAK1, STAT3*	(82, 83)

3.1 Angioimunoblastic T-cell lymphoma

AITL is a distinct clinicopathologic, and genetic subtype of peripheral T-cell lymphoma (PTCL). AITL is the second most prevalent PTCL subtype worldwide, accounting for 15% to 20% of all PTCL cases, and the most common subtype in the Western world, accounting for more than 30% of all PTCL cases (18, 84, 85).

In 2007, De Leval et al. identified the cell of origin being the T follicular helper cell (TFH) based on the use of gene expression profile studies (86). *CD28* (9.4–11.3%), *DNMT3A* (20–30%), *IDH2* (20–45%), *TET2* (47–83%), and *RHOA* mutations (50–70%) are the most common genetic alterations detected in AITL.

The *RHOA G17V* is the result of a valine substitution for glycine at aminoacid 17, which causes the protein to lose its ability to bind GTP. Furthermore, patients with *RHOA* mutations are thought to have enhanced microvascular density and to exhibit a high number of follicular helper T-cell markers (87). In contrast to other mutations such as *TET2* and *DNMT3A*, which can occur in both tumor and nontumor cells of AITL patients, *RHOA* mutations appear to be limited to tumor cells, indicating that they play an important role in AITL pathogenesis (67, 88).

TET2 encodes a 2-oxoglutarate/Fe2+–dependent oxygenase that participates in the epigenetic control of gene expression by catalyzing the oxidation of DNA 5-methylcytosine to 5-hydroxymethylcytosine. *TET2* was first described as a tumor suppressor in myeloid neoplasms, but afterward, a high loss of function in *TET2* was identified in PTCL and especially AITL (89–91). *TET2* mutations are also found in hematopoietic cells in a borderline disease called clonal hematopoiesis of indeterminate potential (CHIP), and are associated with the risk of clonal malignancy over time. The fact that not all people with CHIP associated with a *TET2* mutation can develop malignant haemopathy indicates that it is necessary to acquire secondary mutations for the malignant transformation to take place (92, 93). Loss-of-function mutations in *DNMT3A*, a DNA methyltransferase, are common in AITL and frequently co-occur with *TET2* mutations (68). Cooperation between *DNMT3A* and *TET2* mutations has been found to result in malignant transformation in mice models (94).

In the mitochondria, the isocitrate dehydrogenase 2 (*IDH2*) gene normally encodes enzymes that convert isocitrate to alpha-ketoglutarate (2-oxoglutarate, aKG). The neomorphic enzymatic activity of the mutant enzymes catalyzes the conversion of alpha ketoglutarate to 2-hydroxyglutarate (2-HG), an oncometabolite that inhibits the function of the *TET* family of enzymes (68). AITL is the only type of PTCL in which recurrent *IDH2* mutations appear. Mutations in position *R172* of *IDH2* are specific for AITL and typically co-occur with *TET2* mutations (68).

TET2, DNMT3A, and *IDH2* mutations occur early in hematopoietic stem cell development, contributing to increased clonal hematopoiesis and greater hematopoietic stem cell self-renewal, but they do not impact T cell differentiation and are therefore considered non-lineage impact mutations, according to a recent review by Yu et al. (91) Late in the T-cell lineage differentiation, mutations in *RHOA, VAV1, VAV::STAP2, CD28, CTLA::CD28, ITK::SYK, PLCy1*, and *TNFRSF21* induce malignant T-cell transformation (91). Considering hypermethylation as the fundamental pathogenetic mechanism of AITL, the use of hypomethylating drugs appears to be a reasonable therapeutic option, and is currently in the clinical trials phase (95, 96).

3.2 Mycosis fungoides/Sézary syndrome

The nosological entities known as Mycosis fungoides (MF) and Sézary Syndrome (SS) account for about 75% of all Cutaneous T-cell lymphomas (97). SS is a generalized form of the condition that manifests itself clinically with erythrodermic lesions along with lymph node and blood involvement at onset. MF is a disorder with limited expansion in the skin area, being associated with a good prognosis (77, 98).

The difference in COO can explain the clinical distinctions between MF and SS. MF and SS develop from different subtypes of CD4 + memory T cells; The source cell in the case of MF are T resident memory (Trm) cells exhibiting *CCR4 +/CLA +/L-selectin-/CCR7– (TRM)*, which have a higher tropism to the skin and epithelial barriers, while in the case of SS the COO are T-cell central memory cells (Tcm) that express *CCR4 +/Lselectin +/CCR7 +*, and these cells have the ability to migrate between skin, lymph nodes and blood (99).

Recent NGS research in MF/SS has found a high rate of C>T transitions (40–74%), a mutational signature linked to ultraviolet B (UVB) exposure that is uncommon to be seen in other hematological neoplasms (100–102).

Litvinov et al. described 17 genes (*CCL18, CCL26, FYB, T3JAM, MMP12, LEF1, LCK, ITK, GNLY, IL2RA, IL-26, IL-22, CCR4, GTSF1, SYCP1, STAT5A, TOX*) that identified those patients who are at risk of progression and differentiated MF/SS from benign dermatological diseases (103).

The accuracy of diagnosing SS using distinct gene panels has been demonstrated by *Nebozhyn et al.* and *Michel et al.* in two separate papers. *Nebozhyn et al.* used a panel of five genes (STAT4, GATA3, PLS3, CD1D, and TRAIL) that could correctly separate patient samples from controls with 90% accuracy. On the other hand, *Michel et al.* used a signature based on four genes (PLS3, Twist1, CD158k/KIR3DL2, and NKp46) with the ability to separate SS samples from control samples in 100% of cases. They noted that only the Twist1 gene has a diagnostic sensitivity of SS of 91% (104, 105).

The largest retrospective WES evaluation of CTCL to date utilized publicly available sequencing data from nine studies, comprising 220 patients with CTCL, which included 186 SS patients and 25 MF patients (106). This study identified fifty-five putative driver genes and implicated seventeen gene mutations previously not described as being involved in CTCL. These novel mutations target pathways that are involved chromatin remodeling (*BCOR, KDM6A, SMARCB1, TRRAP*), immune surveillance (*CD58, RFXAP*), MAPK signaling (*MAP2K1, NF1*), NF-κB signaling (*PRKCB, CSNK1A1*), PI-3-kinase signaling (*PIK3R1, VAV1*), RHOA/cytoskeleton remodeling (*ARHGEF3*), RNA splicing (*U2AF1*), T-cell receptor signaling (*PTPRN2, RLTPR*), and T-cell differentiation (*RARA*) (106). The *JAK/STAT* pathway, which includes *JAK1, JAK3, STAT3*, and *STAT5B*, is frequently affected by gain-of-function mutations and amplifications in CTCL resulting in the hyperactivation of this signaling pathway (106). Nevertheless, genomic studies in MF/SS do not allow to have a complete picture as in B cell lymphomas on the prognostic stratification of cases or the establishment of molecular classification, this will most likely be the moment of interest for further investigations.

4 The use of liquid biopsy in non-Hodgkin lymphomas

Currently, the diagnosis of non-Hodgkin's lymphoma is based on excisional biopsy of the tumoral tissue. Tissue biopsies, however, are invasive methods of diagnosis with a several disadvantages, such as the risks of tissue biopsy (bleeding, infection, functional disability, etc.), the difficulty of obtaining biopsy samples, and do not allow the dynamic heterogeneity of the case to be assessed (107).

The concept of liquid biopsy which is a non-invasive technique, and can be used to explore the entire mutational landscape of the

lymphoma. Liquid biopsy allows for an evaluation of lymphoma at the stage of diagnosis, and prognostic stratification.

Both healthy cells and malignant cells release nucleic acids (DNA, mRNA, and miRNA) into body fluids like the cerebrospinal fluid, peripheral blood, and urine. The term "cell-free DNA" (cfDNA) refers to non-cell-bound DNA fragments discovered in the circulatory system. cfDNA often contains both normal DNA and circulating tumor DNA (ctDNA). The lysis of circulating tumor cells (CTCs), apoptosis, necrosis, or the release of DNA from tumor cells into the bloodstream are possible origins for the tumor-specific part of cfDNA. Because cfDNA can emerge from both malignant and non-malignant cells, assays for the detection of ctDNA are more specific for tumor identification in the case of non-Hodgkin's lymphomas (108, 109).

Close monitoring of NHL cases by using ctDNA quantification of liquid biopsies can identify the genetic heterogeneities that appear between the primary tumor and the primary areas of metastasis, as well as between various locations of metastases. This information can then be used to find biomarkers indicative of spreading mechanisms and lymphomatous transformation. Multiple studies including a recent meta-analysis, have shown higher levels of cfDNA in cancer patients compared with healthy controls. Different subsets of lymphoma can be distinguished at the time of diagnosis with the help of NGS-based analysis of ctDNA. Furthermore, ctDNA load strongly reflects tumor burden, as it appears to correlate significantly with lactate dehydrogenase (LDH) and the International Prognostic Index (IPI), as observed in DLBCL, NKTCL and other types of lymphomas (110–113). In DLBCL, interim ctDNA monitoring during therapy directly evaluates tumor kinetics response and foretells early treatment failure. The determination of interim levels of ctDNA has a greater sensitivity than existing imaging methods, creating a so-called "window of opportunity" during which, the earlier initiation of salvage therapy prior to clinical relapse to be diagnosed, has the potential to improve outcomes (114).

In the case of DLBCL, initial levels of ctDNA are significantly associated with the International Prognostic Index (IPI), total metabolic tumor volume (TMTV), lactate dehydrogenase (LDH) concentrations, and the Ann Arbor stage. Pretreatment ctDNA concentrations have been demonstrated to be highly accurate predictors of clinical outcomes in univariate and multivariate analysis in those trials, and hence gain prognostic importance (43, 115, 116). ctDNA in DLBCL can also be used for the real-time assessment of treatment response, increases in ctDNA levels and changes in KMT2D mutation status have been found to be useful indicators of disease progression (117). The depth of response is an important predictor of outcomes in the post-treatment surveillance of NHL subtypes. Relapsed NHL likely originates from MRD below the current level of detection, and a recent systematic review demonstrated that between 7% and 20% of DLBCL patients in remission by PET scans will ultimately relapse (118). A recent MRD study on DLBCL patients treated with CAR-T cell therapy has shown better sensitivity and predictive value for progression to treatment than the PET scan (119). This study, among others, suggests that liquid biopsy and NGS would create an excellent platform for assessing the efficacy of treatments (116).

In the case of extranodal natural killer/T cell lymphoma (ENKTCL), a recent study explored the use of ctDNA methylation markers for diagnosing, continuously monitoring, and predicting the prognosis. This research has proposed a score formed by 7 ctDNA markers, namely HLX-AS1, MIR12123, CHST12, DLK1, LINC02115, MIR3973, and NCAM, which achieves over 90% accuracy in distinguishing ENKTCL from nasopharyngeal carcinoma, nasopharyngitis, and normal conditions (120).

However, despite the encouraging data of NHL evaluation by liquid biopsies, few validation studies have been published at the moment (121), with the vast majority of the data presented requiring validation in further research.

5 NGS use for a personalized approach and future perspective of use

NHL remains a condition treated primarily whit chemoimmunotherapy. The standard of care for years has been frontline R-CHOP, despite multiple attempts to investigate more aggressive regimens like R-DA-EPOCH or incorporate new therapies like obinutuzumab, bortezomib, or ibrutinib. Frontline R-CHOP cures around 60% of DLBCL cases. Nowadays, DA-EPOCH-R is utilized as the first-line therapy for double/triple hit lymphomas, primary mediastinal B cell lymphoma, and HIV-associated DLBCL.

The discovery by Wilson et al. that the co-occurrence of mutations in *MYD88* and *CD79B* can predict response to ibrutinib is an illustration of possible clinical utility of genomic profile data in DLBCL, that may have a real impact in the practice (122).

In MCL, the data obtained through genome sequencing allowed the identification of a group of patients in whom there are inactivating mutations in the *SWI-SNF* chromatin-remodeling complex that lead to *BCL-XL* upregulation and subsequent resistance to the therapeutic combination with ibrutinib and venetoclax (61).

Many T-cell lymphomas harbor mutations in epigenetic regulatory genes, such as *TET2*, *DNMT3A*, and *IDH2*, but they are most frequently seen in AITL. Therefore, the use of drugs from the class of HDAC inhibitors or demethylating agents may have a potential beneficial role.

Recently Huang et al. have proposed the DrugComboExplorer, a computational systems biology tool that concurrently integrates pharmacogenomics profiles of 5585 drugs and bioactive compounds from the NIH LINCS program (Library of Integrated Network-based Cellular Signatures) and genomic profiles for specific cancer types (i.e., signaling pathways, interactome, and pharmacological data). This tool does large-scale medication

combination prediction and integrates multi-omics data from cancer patients including non-Hodgkin lymphomas (123).

In conclusion, the knowledge provided by the genomic mapping of non-Hodgkin's lymphomas in near future will allow the targeting of molecular pathways that cause treatment refractoriness or, on the contrary, the inhibition of which is vital in stopping uncontrolled tumor proliferation. Personalized medicine will not only select a single mutation that it will inhibit through the action of a drug, but by selecting molecular targets that have a synergistic costimulatory or inhibitory effect thus self-potentiating. The increased interest in this field confirms that the integration of genomic and transcriptomic data will allow a better understanding of the therapy of malignant lymphomas and of tumor resistance.

Author contributions

VT: Validation, Writing – original draft. AM: Validation, Writing – review & editing. CS: Validation, Writing – original draft. MR: Validation, Writing – original draft. SB: Validation, Writing – original draft. MF: Validation, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Thandra KC, Barsouk A, Saginala K, Padala SA, Barsouk A, Rawla P. Epidemiology of non-hodgkin's lymphoma. *Med Sci* (2021) 9:5. doi: 10.3390/MEDSCI9010005

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71:209–49. doi: 10.3322/CAAC.21660

3. Rappaport H. Tumors of the hematopoietic system. In: *Atlas of tumor pathology sect. 3. Fasicle S*. Washington: DC Armed Forces Institute of pathology (1966). p. 97–161.

4. Lennert K, Stein H, Kaiserling E. Cytological and functional criteria for the classification of Malignant lymphomata. *Br J Cancer* (1975) 31:29–43.

5. Lukes RJ, Collins RD. Immunologic characterization of human Malignant lymphomas. *Cancer* (1974) 34:1488–503. doi: 10.1002/1097-0142(197410)34:8+<1488::AID-CNCR2820340822>3.0.CO;2-C

6. Swerdlow SH, Campo E, Pileri SA, Lee Harris N, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood* (2016) 127:2375–90. doi: 10.1182/BLOOD-2016-01-643569

7. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IB de O, Berti E, et al. The 5th edition of the world health organization classification of haematolymphoid tumours: lymphoid neoplasms. *Leuk* (2022) 36(7):1720–48. doi: 10.1038/s41375-022-01620-2

8. Campo E, Jaffe ES, Cook JR, Quintanilla-Martinez L, Swerdlow SH, Anderson KC, et al. The international consensus classification of mature lymphoid neoplasms: a report from the clinical advisory committee. *Blood* (2022) 140:1229–53. doi: 10.1182/blood.2022015851

9. Binder AF, Brody JD. Non-hodgkin lymphoma. *Oncology* (2020) 342–53. doi: 10.1002/9781119189596.ch30

10. Beham-Schmid C. Aggressive lymphoma 2016: revision of the WHO classification. *Memo - Mag Eur Med Oncol* (2017) 10:248–54. doi: 10.1007/S12254-017-0367-8/FIGURES/5

11. Tomacinschii V, Robu M, Buruiana S, Finciuc V, Grecu A, Dudnic C, et al. Impact of targeted treatment in non-Hodgkin's lymphoma with primary lymph node involvement. *Mold Med J* (2021) 64:56–61. doi: 10.52418/MOLDOVAN-MED-J.64-5.21.11

12. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. *Nature* (2009) 458:719–24. doi: 10.1038/NATURE07943

13. Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, et al. COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res* (2017) 45:D777. doi: 10.1093/NAR/GKW1121

14. Ding Z, Wang N, Ji N, Chen ZS. Proteomics technologies for cancer liquid biopsies. *Mol Cancer* (2022) 21:1–11. doi: 10.1186/S12943-022-01526-8/FIGURES/2

15. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med* (2016) 374:2209–21. doi: 10.1056/NEJMOA1516192

16. Nadeu F, Delgado J, Royo C, Baumann T, Stankovic T, Pinyol M, et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. *Blood* (2016) 127:2122–30. doi: 10.1182/BLOOD-2015-07-659144

17. Chapuy B, Stewart C, Dunford AJ, Kim J, Kamburov A, Redd RA, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat Med* (2018) 24:679–90. doi: 10.1038/S41591-018-0016-8

18. Vose JM, Neumann M, Harris ME. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. *J Clin Oncol* (2008) 26:4124–30. doi: 10.1200/JCO.2008.16.4558

19. Visco C, Li Y, Xu-Monette ZY, Miranda RN, Green TM, Li Y, et al. Comprehensive gene expression profiling and immunohistochemical studies support application of immunophenotypic algorithm for molecular subtype classification in diffuse large B-cell lymphoma: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. *Leukemia* (2012) 26:2103–13. doi: 10.1038/LEU.2012.83

20. Hoppe MM, Jaynes P, Shuangyi F, Peng Y, Sridhar S, Hoang PM, et al. Patterns of oncogene coexpression at single-cell resolution influence survival in lymphoma. *Cancer Discovery* (2023) 13:1144–63. doi: 10.1158/2159-8290.CD-22-0998

21. Ramis-Zaldivar JE, Gonzalez-Farré B, Balagué O, Celis V, Nadeu F, Salmerón-Villalobos J, et al. Distinct molecular profile of IRF4-rearranged large B-cell lymphoma. *Blood* (2020) 135:274–86. doi: 10.1182/BLOOD.2019002699

22. Sukswai N, Lyapichev K, Khoury JD, Medeiros LJ. Diffuse large B-cell lymphoma variants: an update. *Pathology* (2020) 52:53–67. doi: 10.1016/J.PATHOL.2019.08.013

23. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U.S.A.* (1977) 74:5463–7. doi: 10.1073/PNAS.74.12.5463

24. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. *Nature* (2001) 409:860–921. doi: 10.1038/35057062

25. Abdellah Z, Ahmadi A, Ahmed S, Aimable M, Ainscough R, Almeida J, et al. Finishing the euchromatic sequence of the human genome. *Nat* (2004) 431:931–45. doi: 10.1038/nature03001

26. Linnarsson S. Recent advances in DNA sequencing methods – general principles of sample preparation. *Exp Cell Res* (2010) 316:1339–43. doi: 10.1016/J.YEXCR.2010.02.036

27. Intlekofer AM, Younes A. Precision therapy for lymphoma–current state and future directions. *Nat Rev Clin Oncol* (2014) 11:585–96. doi: 10.1038/NRCLINONC.2014.137

28. Zelenetz AD, Gordon LI, Chang JE, Christian B, Abramson JS, Advani RH, et al. NCCN guidelines® Insights: B-cell lymphomas, version 5.2021: featured updates to the NCCN guidelines. *J Natl Compr Cancer Netw* (2021) 19:1218–30. doi: 10.6004/JNCCN.2021.0054

29. Armitage JO, Gascoyne RD, Lunning MA, Cavalli F. Non-hodgkin lymphoma. *Lancet* (2017) 390:298–310. doi: 10.1016/S0140-6736(16)32407-2

30. Schmitz R, Wright GW, Huang DW, Johnson CA, Phelan JD, Wang JQ, et al. Genetics and pathogenesis of diffuse large B-cell lymphoma. *N Engl J Med* (2018) 378:1396–407. doi: 10.1056/NEJMOA1801445/SUPPL_FILE/NEJMOA1801445_DISCLOSURES.PDF

31. Wright GW, Huang DW, Phelan JD, Coulibaly ZA, Roulland S, Young RM, et al. A probabilistic classification tool for genetic subtypes of diffuse large B cell lymphoma with therapeutic implications. *Cancer Cell* (2020) 37:551–568.e14. doi: 10.1016/J.CCELL.2020.03.015

32. Sujobert P, Le Bris Y, De Leval L, Gros A, Merlio JP, Pastoret C, et al. The need for a consensus next-generation sequencing panel for mature lymphoid Malignancies. *HemaSphere* (2019) 3. doi: 10.1097/HS9.0000000000000169

33. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger AM, Szczepanowski M, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: A retrospective analysis of a prospective clinical trial and validation in a population-based registry. *Lancet Oncol* (2015) 16:1111–22. doi: 10.1016/S1470-2045(15)00169-2

34. Jurinovic V, Kridel R, Staiger AM, Szczepanowski M, Horn H, Dreyling MH, et al. Clinicogenetic risk models predict early progression of follicular lymphoma after first-line immunochemotherapy. *Blood* (2016) 128:1112–20. doi: 10.1182/BLOOD-2016-05-717355

35. González-Rincón J, Méndez M, Gómez S, García JF, Martín P, Bellas C, et al. Unraveling transformation of follicular lymphoma to diffuse large B-cell lymphoma. *PloS One* (2019) 14:e0212813. doi: 10.1371/JOURNAL.PONE.0212813

36. Nakamura S, Ponzoni M. Marginal zone B-cell lymphoma: lessons from Western and Eastern diagnostic approaches. *Pathology* (2020) 52:15–29. doi: 10.1016/J.PATHOL.2019.08.012

37. Yi S, Yan Y, Jin M, Bhattacharya S, Wang Y, Wu Y, et al. Genomic and transcriptomic profiling reveals distinct molecular subsets associated with outcomes in mantle cell lymphoma. *J Clin Invest* (2022) 132. doi: 10.1172/JCI153283

38. Maddocks K. Update on mantle cell lymphoma. *Blood* (2018) 132:1647–56. doi: 10.1182/BLOOD-2018-03-791392

39. Li S, Xu J, James You M. The pathologic diagnosis of mantle cell lymphoma. *Histol Histopathol* (2021) 36:1037–51. doi: 10.14670/HH-18-351

40. Jain P, Wang ML. Mantle cell lymphoma in 2022—A comprehensive update on molecular pathogenesis, risk stratification, clinical approach, and current and novel treatments. *Am J Hematol* (2022) 97:638–56. doi: 10.1002/AJH.26523

41. Tausch E, Beck P, Schlenk RF, Jebaraj BMC, Dolnik A, Yosifov DY, et al. Prognostic and predictive role of gene mutations in chronic lymphocytic leukemia: results from the pivotal phase III study COMPLEMENT1. *Haematologica* (2020) 105:2440–7. doi: 10.3324/HAEMATOL.2019.229161

42. Cohen JA, Bomben R, Pozzo F, Tissino E, Härzschel A, Hartmann TN, et al. An updated perspective on current prognostic and predictive biomarkers in chronic lymphocytic leukemia in the context of chemoimmunotherapy and novel targeted therapy. *Cancers (Basel)* (2020) 12. doi: 10.3390/CANCERS12040894

43. Scherer F, Kurtz DM, Newman AM, Stehr H, Craig AFM, Esfahani MS, et al. Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. *Sci Transl Med* (2016) 8:364ra155. doi: 10.1126/SCITRANSLMED.AAI8545

44. Li S, Young KH, Medeiros LJ. Diffuse large B-cell lymphoma. *Pathology* (2018) 50:74–87. doi: 10.1016/J.PATHOL.2017.09.006

45. Alizadeh AA, Elsen MB, Davis RE, Ma CL, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* (2000) 403:503–11. doi: 10.1038/35000501

46. Susanibar-Adaniya S, Barta SK. 2021 Update on Diffuse large B cell lymphoma: A review of current data and potential applications on risk stratification and management. *Am J Hematol* (2021) 96:617–29. doi: 10.1002/AJH.26151

47. Reddy A, Zhang J, Davis NS, Moffitt AB, Love CL, Waldrop A, et al. Genetic and functional drivers of diffuse large B cell lymphoma. *Cell* (2017) 171:481–494.e15. doi: 10.1016/J.CELL.2017.09.027/ATTACHMENT/10836D88-A738-4F08-83E5-08ACDD907DEE/MMC5.XLSX

48. Mosquera Orgueira A, Diaz Arias JA, Cid Lopez M, Peleteiro Raindo A, Lopez Garcia A, Abal Garcia R, et al. Prognostic stratification of diffuse large B-cell lymphoma using clinico-genomic models: validation and improvement of the lymForest-25 model. *HemaSphere* (2022) 6. doi: 10.1097/HS9.0000000000000706

49. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, et al. Frequent mutation of histone modifying genes in non-Hodgkin lymphoma. *Nature* (2011) 476:298. doi: 10.1038/NATURE10351

50. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. *Nature* (2011) 471:189–96. doi: 10.1038/NATURE09730

51. Li H, Kaminski MS, Li Y, Yildiz M, Ouillette P, Jones S, et al. Mutations in linker histone genes HIST1H1 B, C, D, and E; OCT2 (POU2F2); IRF8; and ARID1A underlying the pathogenesis of follicular lymphoma. *Blood* (2014) 123:1487–98. doi: 10.1182/BLOOD-2013-05-500264

52. Lockmer S, Ren W, Brodtkorb M, Østenstad B, Wahlin BE, Pan-Hammarström Q, et al. M7-FLIPI is not prognostic in follicular lymphoma patients with first-line rituximab chemo-free therapy. *Br J Haematol* (2020) 188:259–67. doi: 10.1111/BJH.16159

53. Lockmer S, Ren W, Ostenstad B, Brodtkorb M, Wahlin BE, Pan-Hammarstrom Q, et al. M7-FLIPI not valid in follicular lymphoma patients with first-line rituximab chemo-free therapy. *Blood* (2018) 132:4154. doi: 10.1182/BLOOD-2018-99-117949

54. Gao F, Liu H, Meng X, Liu J, Wang J, Yu J, et al. Integrative genomic and transcriptomic analysis reveals genetic alterations associated with the early progression of follicular lymphoma. *Br J Haematol* (2023) 1151–64. doi: 10.1111/bjh.18974

55. Hill HA, Qi X, Jain P, Nomie K, Wang Y, Zhou S, et al. Genetic mutations and features of mantle cell lymphoma: a systematic review and meta-analysis. *Blood Adv* (2020) 4:2927–38. doi: 10.1182/BLOODADVANCES.2019001350

56. Rimokh R, Berger F, Cornillet P, Wahbi K, Rouault J -P, Ffrench M, et al. Break in the BCL1 locus is closely associated with intermediate lymphocytic lymphoma subtype. *Genes Chromosom Cancer* (1990) 2:223–6. doi: 10.1002/GCC.2870020310

57. Leroux D, Le Marc'hadour F, Gressin R, Jacob M -C, Keddari E, Monteil M, et al. Non-Hodgkin's lymphomas with t(11;14)(q13;q32): a subset of mantle zone/intermediate lymphocytic lymphoma? *Br J Haematol* (1991) 77:346–53. doi: 10.1111/J.1365-2141.1991.TB08582.X

58. Jiang P, Desai A, Ye H. Progress in molecular feature of smoldering mantle cell lymphoma. *Exp Hematol Oncol* (2021) 10:41. doi: 10.1186/s40164-021-00232-3

59. Clot G, Jares P, Giné E, Navarro A, Royo C, Pinyol M, et al. A gene signature that distinguishes conventional and leukemic nonnodal mantle cell lymphoma helps predict outcome. *Blood* (2018) 132:413–22. doi: 10.1182/BLOOD-2018-03-838136

60. Sethi S, Epstein-Peterson Z, Kumar A, Ho C. Current knowledge in genetics, molecular diagnostic tools, and treatments for mantle cell lymphomas. *Front Oncol* (2021) 11:739441/BIBTEX. doi: 10.3389/FONC.2021.739441/BIBTEX

61. Agarwal R, Chan YC, Tam CS, Hunter T, Vassiliadis D, Teh CE, et al. Dynamic molecular monitoring reveals that SWI-SNF mutations mediate resistance to ibrutinib plus venetoclax in mantle cell lymphoma. *Nat Med* (2019) 25:119–29. doi: 10.1038/S41591-018-0243-Z

62. Eskelund CW, Dahl C, Hansen JW, Westman M, Kolstad A, Pedersen LB, et al. TP53 mutations identify younger mantle cell lymphoma patients who do not benefit from intensive chemoimmunotherapy. *Blood* (2017) 130:1903–10. doi: 10.1182/BLOOD-2017-04-779736

63. Condoluci A, Rossi D, Zucca E, Cavalli F. Toward a risk-tailored therapeutic policy in mantle cell lymphoma. *Curr Oncol Rep* (2018) 20. doi: 10.1007/S11912-018-0728-4

64. Jiang M, Bennani NN, Feldman AL. Lymphoma classification update: T-cell lymphomas, Hodgkin lymphomas, and histiocytic/dendritic cell neoplasms. *Expert Rev Hematol* (2017) 10:239. doi: 10.1080/17474086.2017.1281122

65. Wang H, Bi-bo Fu, Gale RP, Liang Y. NK-/T-cell lymphomas. *Leukemia* (2021) 35:2460. doi: 10.1038/S41375-021-01313-2

66. Rohr J, Guo S, Huo J, Bouska A, Lachel C, Li Y, et al. Recurrent activating mutations of CD28 in peripheral T-cell lymphomas. *Leukemia* (2016) 30:1062–70. doi: 10.1038/LEU.2015.357

67. Fujisawa M, Sakata-Yanagimoto M, Nishizawa S, Komori D, Gershon P, Kiryu M, et al. Activation of RHOA–VAV1 signaling in angioimmunoblastic T-cell lymphoma. *Leuk* (2018) 32:694–702. doi: 10.1038/leu.2017.273

68. Cortés JR, Palomero T. The curious origins of Angioimmunoblastic T-Cell Lymphoma. *Curr Opin Hematol* (2016) 23:434. doi: 10.1097/MOH.0000000000000261

69. Kataoka K, Iwanaga M, Yasunaga JI, Nagata Y, Kitanaka A, Kameda T, et al. Prognostic relevance of integrated genetic profiling in adult T-cell leukemia/lymphoma. *Blood* (2018) 131:215–25. doi: 10.1182/BLOOD-2017-01-761874

70. Bonn BR, Huge A, Rohde M, Oschlies I, Klapper W, Voss R, et al. Whole exome sequencing hints at a unique mutational profile of paediatric T-cell lymphoblastic lymphoma. *Br J Haematol* (2015) 168:308–13. doi: 10.1111/BJH.13105

71. Laurent C, Nicolae A, Laurent C, Le Bras F, Haioun C, Fataccioli V, et al. Gene alterations in epigenetic modifiers and JAK-STAT signaling are frequent in breast implant-associated ALCL. *Blood* (2020) 135:360–70. doi: 10.1182/BLOOD.2019001904

72. Zhang Y, Li C, Xue W, Zhang M, Li Z. Frequent mutations in natural killer/T cell lymphoma. *Cell Physiol Biochem* (2018) 49:1–16. doi: 10.1159/000492835

73. Montes-Mojarro IA, Chen BJ, Ramirez-Ibarguen AF, Quezada-Fiallos CM, Pérez-Báez WB, Dueñas D, et al. Mutational profile and EBV strains of extranodal NK/T-cell lymphoma, nasal type in Latin America. *Mod Pathol* (2020) 33:781–91. doi: 10.1038/S41379-019-0415-5

74. Kataoka K, Miyoshi H, Sakata S, Dobashi A, Couronné L, Kogure Y, et al. Frequent structural variations involving programmed death ligands in Epstein-Barr virus-associated lymphomas. *Leukemia* (2019) 33:1687–99. doi: 10.1038/S41375-019-0380-5

75. Moffitt AB, Ondrejka SL, McKinney M, Rempel RE, Goodlad JR, Teh CH, et al. Enteropathy-associated T cell lymphoma subtypes are characterized by loss of function of SETD2. *J Exp Med* (2017) 214:1371–86. doi: 10.1084/JEM.20160894

76. Nairismägi ML, Tan J, Lim JQ, Nagarajan S, Ng CCY, Rajasegaran V, et al. JAK-STAT and G-protein-coupled receptor signaling pathways are frequently altered in epitheliotropic intestinal T-cell lymphoma. *Leukemia* (2016) 30:1311–9. doi: 10.1038/LEU.2016.13

77. Kamijo H, Miyagaki T. Mycosis fungoides and sézary syndrome: updates and review of current therapy. *Curr Treat Options Oncol* (2021) 22. doi: 10.1007/S11864-020-00809-W

78. Iżykowska K, Przybylski GK, Gand C, Braun FC, Grabarczyk P, Kuss AW, et al. Genetic rearrangements result in altered gene expression and novel fusion transcripts in Sézary syndrome. *Oncotarget* (2017) 8:39627–39. doi: 10.18632/ONCOTARGET.17383

79. Hamrouni A, Fogh H, Zak Z, Dum N, Gniadecki R. Clonotypic diversity of the T-cell receptor corroborates the immature precursor origin of cutaneous T-cell lymphoma. *Clin Cancer Res* (2019) 25:3104–14. doi: 10.1158/1078-0432.CCR-18-4099

80. Koh J, Jang I, Mun S, Lee C, Jeong Cha H, Oh YH, et al. Genetic profiles of subcutaneous panniculitis-like T-cell lymphoma and clinicopathological impact of HAVCR2 mutations. *Blood Adv* (2021) 5:3919–30. doi: 10.1182/BLOODADVANCES.2021004562

81. Andersson EI, Brück O, Braun T, Mannisto S, Saikko L, Lagström S, et al. STAT3 mutation is associated with STAT3 activation in CD30 + ALK - ALCL. *Cancers (Basel)* (2020) 12. doi: 10.3390/CANCERS12030702

82. Blombery P, Thompson E, Ryland GL, Joyce R, Byrne DJ, Khoo C, et al. Frequent activating STAT3 mutations and novel recurrent genomic abnormalities detected in breast implant-associated anaplastic large cell lymphoma. *Oncotarget* (2018) 9:36126. doi: 10.18632/ONCOTARGET.26308

83. Jaffe ES, Ashar BS, Clemens MW, Feldman AL, Gaulard P, Miranda RN, et al. Best practices guideline for the pathologic diagnosis of breast implant-associated anaplastic large-cell lymphoma. *J Clin Oncol* (2020) 38:1102–11. doi: 10.1200/JCO.19.02778

84. de Leval L, Parrens M, Le Bras F, Jais JP, Fataccioli V, Martin A, et al. Angioimmunoblastic T-cell lymphoma is the most common T-cell lymphoma in two distinct French information data sets. *Haematologica* (2015) 100:e361–4. doi: 10.3324/HAEMATOL.2015.126300

85. Federico M, Rudiger T, Bellei M, Nathwani BN, Luminari S, Coiffier B, et al. Clinicopathologic characteristics of angioimmunoblastic T-cell lymphoma: analysis of the international peripheral T-cell lymphoma project. *J Clin Oncol* (2013) 31:240–6. doi: 10.1200/JCO.2011.37.3647

86. De Leval L, Rickman DS, Thielen C, De Reynies A, Huang YL, Delsol G, et al. The gene expression profile of nodal peripheral T-cell lymphoma demonstrates a molecular link between angioimmunoblastic T-cell lymphoma (AITL) and follicular helper T (TFH) cells. *Blood* (2007) 109:4952–63. doi: 10.1182/BLOOD-2006-10-055145

87. Ondrejka SL, Grzywacz B, Bodo J, Makishima H, Polprasert C, Said JW, et al. Angioimmunoblastic T-cell lymphomas with the RHOA p.Gly17Val mutation have classic clinical and pathologic features. *Am J Surg Pathol* (2016) 40:335–41. doi: 10.1097/PAS.0000000000000555

88. Nguyen TB, Sakata-Yanagimoto M, Asabe Y, Matsubara D, Kano J, Yoshida K, et al. Identification of cell-type-specific mutations in nodal T-cell lymphomas. *Blood Cancer J* (2017) 7:e516–6. doi: 10.1038/bcj.2016.122

89. Abdel-Wahab O, Mullally A, Hedvat C, Garcia-Manero G, Patel J, Wadleigh M, et al. Genetic characterization of TET1, TET2, and TET3 alterations in myeloid Malignancies. *Blood* (2009) 114:144–7. doi: 10.1182/BLOOD-2009-03-210039

90. Palomero T, Couronné L, Khiabanian H, Kim MY, Ambesi-Impiombato A, Perez-Garcia A, et al. Recurrent mutations in epigenetic regulators, RHOAand FYN kinase in peripheral T cell lymphomas. *Nat Genet* (2014) 46:166. doi: 10.1038/NG.2873

91. Yu DD, Zhang J. Update on recurrent mutations in angioimmunoblastic T-cell lymphoma. *Int J Clin Exp Pathol* (2021) 14:1108–18.

92. Steensma DP. Clinical consequences of clonal hematopoiesis of indeterminate potential. *Blood Adv* (2018) 2:3404–10. doi: 10.1182/BLOODADVANCES.2018020222

93. Steensma DP, Ebert BL. Clonal hematopoiesis as a model for premalignant changes during aging. *Exp Hematol* (2020) 83:48–56. doi: 10.1016/J.EXPHEM.2019.12.001

94. Scourzic L, Couronné L, Pedersen MT, Della Valle V, Diop M, Mylonas E, et al. DNMT3AR882H mutant and Tet2 inactivation cooperate in the deregulation of DNA methylation control to induce lymphoid Malignancies in mice. *Leukemia* (2016) 30:1388. doi: 10.1038/LEU.2016.29

95. Lemonnier F, Dupuis J, Sujobert P, Tournillhac O, Cheminant M, Sarkozy C, et al. Treatment with 5-azacytidine induces a sustained response in patients with angioimmunoblastic T-cell lymphoma. *Blood* (2018) 132:2305–9. doi: 10.1182/BLOOD-2018-04-840538

96. Ruan J, Moskowitz AJ, Mehta-Shah N, Sokol L, Chen Z, Rahim R, et al. Multi-center phase II study of oral azacitidine (CC-486) plus CHOP as initial treatment for peripheral T-cell lymphoma (PTCL). *Blood* (2020) 136:33–4. doi: 10.1182/BLOOD-2020-136023

97. Devata S, Wilcox RA. Cutaneous T-cell lymphoma: A review with a focus on targeted agents. *Am J Clin Dermatol* (2016) 17:225–37. doi: 10.1007/S40257-016-0177-5

98. Larocca C, Kupper T. Mycosis fungoides and sézary syndrome: an update. *Hematol Oncol Clin North Am* (2019) 33:103–20. doi: 10.1016/J.HOC.2018.09.001

99. Campbell JJ, Clark RA, Watanabe R, Kupper TS. Sézary syndrome and mycosis fungoides arise from distinct T-cell subsets: a biologic rationale for their distinct clinical behaviors. *Blood* (2010) 116:767. doi: 10.1182/BLOOD-2009-11-251926

100. Elenitoba-Johnson KSJ, Wilcox R. A new molecular paradigm in mycosis fungoides and Sézary syndrome. *Semin Diagn Pathol* (2017) 34:15–21. doi: 10.1053/J.SEMDP.2016.11.002

101. McGirt LY, Jia P, Baerenwald DA, Duszynski RJ, Dahlman KB, Zic JA, et al. Whole-genome sequencing reveals oncogenic mutations in mycosis fungoides. *Blood* (2015) 126:508–19. doi: 10.1182/BLOOD-2014-11-611194

102. Prasad A, Rabionet R, Espinet B, Zapata L, Puiggros A, Melero C, et al. Identification of gene mutations and fusion genes in patients with sézary syndrome. *J Invest Dermatol* (2016) 136:1490–9. doi: 10.1016/j.jid.2016.03.024

103. Litvinov IV, Netchiporouk E, Cordeiro B, Dore MA, Moreau L, Pehr K, et al. The use of transcriptional profiling to improve personalized diagnosis and management of Cutaneous T-Cell Lymphoma (CTCL). *Clin Cancer Res* (2015) 21:2820. doi: 10.1158/1078-0432.CCR-14-3322

104. Nebozhyn M, Loboda A, Kari L, Rook AH, Vonderheid EC, Lessin S, et al. Quantitative PCR on 5 genes reliably identifies CTCL patients with 5% to 99% circulating tumor cells with 90% accuracy. *Blood* (2006) 107:3189. doi: 10.1182/BLOOD-2005-07-2813

105. Michel L, Jean-Louis F, Begue E, Bensussan A, Bagot M. Use of PLS3, Twist, CD158k/KIR3DL2, and NKp46 gene expression combination for reliable Sézary syndrome diagnosis. *Blood* (2013) 121:1477–8. doi: 10.1182/BLOOD-2012-10-460535

106. Park J, Yang J, Wenzel AT, Ramachandran A, Lee WJ, Daniels JC, et al. Genomic analysis of 220 CTCLs identifies a novel recurrent gain-of-function alteration in RLTPR (p.Q575E). *Blood* (2017) 130:1430–40. doi: 10.1182/BLOOD-2017-02-768234

107. Alizadeh AA, Aranda V, Bardelli A, Blanpain C, Bock C, Borowski C, et al. Toward understanding and exploiting tumor heterogeneity. *Nat Med* (2015) 21:846–53. doi: 10.1038/NM.3915

108. Ray SK, Mukherjee S. Cell free DNA as an evolving liquid biopsy biomarker for initial diagnosis and therapeutic nursing in cancer- an evolving aspect in medical biotechnology. *Curr Pharm Biotechnol* (2022) 23:112–22. doi: 10.2174/1389201021666201211102710

109. Hiemcke-Jiwa LS, Leguit RJ, Snijders TJ, Jiwa NM, Kuiper JJW, de Weger RA, et al. Molecular analysis in liquid biopsies for diagnostics of primary central nervous system lymphoma: Review of literature and future opportunities. *Crit Rev Oncol Hematol* (2018) 127:56–65. doi: 10.1016/J.CRITREVONC.2018.05.010

110. Sun P, Chen C, Xia Y, Wang Y, Liu PP, Bi XW, et al. Mutation profiling of Malignant lymphoma by next-generation sequencing of circulating cell-free DNA. *J Cancer* (2019) 10:323–31. doi: 10.7150/JCA.27615

111. Qi F, Cao Z, Chen B, Chai Y, Lin J, Ye J, et al. Liquid biopsy in extranodal NK/T-cell lymphoma: a prospective analysis of cell-free DNA genotyping and monitoring. *Blood Adv* (2021) 5:2505–14. doi: 10.1182/BLOODADVANCES.2020001637

112. Yao L, Xu H, Wo J, Zhao M, Liu Z, Dong T, et al. Prognostic value of circulating tumor DNA in lymphoma: a meta-analysis. *Clin Exp Med* (2021) 22:1–7. doi: 10.1007/S10238-021-00718-8

113. Herrera AF, Tracy S, Croft B, Opat S, Ray J, Lovejoy AF, et al. Risk profiling of patients with relapsed/refractory diffuse large B-cell lymphoma by measuring circulating tumor DNA. *Blood Adv* (2022) 6:1651. doi: 10.1182/BLOODADVANCES.2021006415

114. Melani C, Wilson WH, Roschewski M. Liquid biopsy in non-Hodgkin's lymphoma. *Hematol Oncol* (2019) 37 Suppl 1:70–4. doi: 10.1002/HON.2587

115. Kurtz DM, Scherer F, Jin MC, Soo J, Craig AFM, Esfahani MS, et al. Circulating tumor DNA measurements as early outcome predictors in diffuse large B-cell lymphoma. *J Clin Oncol* (2018) 36:2845. doi: 10.1200/JCO.2018.78.5246

116. Roschewski M, Dunleavy K, Pittaluga S, Moorhead M, Pepin F, Kong K, et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. *Lancet Oncol* (2015) 16:541–9. doi: 10.1016/S1470-2045(15)70106-3

117. Zhang S, Zhang T, Liu H, Zhao J, Zhou H, Su X, et al. Tracking the evolution of untreated high-intermediate/high-risk diffuse large B-cell lymphoma by circulating tumour DNA. *Br J Haematol* (2022) 196:617–28. doi: 10.1111/BJH.17894

118. Arzuaga-Mendez J, Prieto-Fernández E, Lopez-Lopez E, Martin-Guerrero I, García-Ruiz JC, García-Orad A. Cell-free DNA as a biomarker in diffuse large B-cell lymphoma: A systematic review. *Crit Rev Oncol Hematol* (2019) 139:7–15. doi: 10.1016/J.CRITREVONC.2019.04.013

119. Hossain NM, Dahiya S, Le R, Abramian AM, Kong KA, Muffly LS, et al. Circulating tumor DNA assessment in patients with diffuse large B-cell lymphoma following CAR T-cell therapy. *Leuk Lymphoma* (2019) 60:503–6. doi: 10.1080/10428194.2018.1474463

120. Tian XP, Zhang YC, Lin NJ, Wang L, Li ZH, Guo HG, et al. Diagnostic performance and prognostic value of circulating tumor DNA methylation marker in extranodal natural killer/T cell lymphoma. *Cell Rep Med* (2023) 4. doi: 10.1016/J.XCRM.2022.100859

121. Bastos-Oreiro M, Suárez-González J, Andrés-Zayas C, Carrión NC, Moreno S, Carbonell D, et al. Incorporation of next-generation sequencing in clinical practice using solid and liquid biopsy for patients with non-Hodgkin's lymphoma. *Sci Rep* (2021) 11:1–20. doi: 10.1038/s41598-021-02362-4

122. Wilson WH, Young RM, Schmitz R, Yang Y, Pittaluga S, Wright G, et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. *Nat Med* (2015) 21(8):922–6. doi: 10.1038/nm.3884

123. Huang L, Brunell D, Stephan C, Mancuso J, Yu X, He B, et al. Driver network as a biomarker: systematic integration and network modeling of multi-omics data to derive driver signaling pathways for drug combination prediction. *Bioinformatics* (2019) 35:3709–17. doi: 10.1093/BIOINFORMATICS/BTZ109

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 06 December 2023
DOI 10.3389/fonc.2023.1330977

OPEN ACCESS

EDITED BY
Felix Seyfried,
Ulm University Medical Center, Germany

REVIEWED BY
Mohammad Alibakhshikenari,
Universidad Carlos III de Madrid de Madrid, Spain
Pradeep Kumar Das,
VIT University, India

*CORRESPONDENCE
Basel Elsayed
be1905231@qu.edu.qa
Mohamed Yassin
yassinmoha@gmail.com

RECEIVED 31 October 2023
ACCEPTED 27 November 2023
PUBLISHED 06 December 2023

CITATION
Elsayed B, Elhadary M, Elshoeibi RM, Elshoeibi AM, Badr A, Metwally O, ElSherif RA, Salem ME, Khadadah F, Alshurafa A, Mudawi D and Yassin M (2023) Deep learning enhances acute lymphoblastic leukemia diagnosis and classification using bone marrow images. *Front. Oncol.* 13:1330977. doi: 10.3389/fonc.2023.1330977

Deep learning enhances acute lymphoblastic leukemia diagnosis and classification using bone marrow images

Basel Elsayed[1*], Mohamed Elhadary[1], Raghad Mohamed Elshoeibi[2], Amgad Mohamed Elshoeibi[1], Ahmed Badr[1], Omar Metwally[1], Raghad Alaa ElSherif[1], Mohamed Elsayed Salem[3], Fatima Khadadah[4], Awni Alshurafa[5], Deena Mudawi[5] and Mohamed Yassin[1,5*]

[1]College of Medicine, Qatar University, Doha, Qatar, [2]Faculty of Medicine, Mansoura University, Mansoura, Egypt, [3]Faculty of Medicine, Zagazig University, Zagazig, Egypt, [4]Cancer Genetics Lab, Kuwait Cancer Control Centre, Kuwait City, Kuwait, [5]Department of Medical Oncology, National Center for Cancer Care and Research, Doha, Qatar

Acute lymphoblastic leukemia (ALL) poses a significant health challenge, particularly in pediatric cases, requiring precise and rapid diagnostic approaches. This comprehensive review explores the transformative capacity of deep learning (DL) in enhancing ALL diagnosis and classification, focusing on bone marrow image analysis. Examining ten studies conducted between 2013 and 2023 across various countries, including India, China, KSA, and Mexico, the synthesis underscores the adaptability and proficiency of DL methodologies in detecting leukemia. Innovative DL models, notably Convolutional Neural Networks (CNNs) with Cat-Boosting, XG-Boosting, and Transfer Learning techniques, demonstrate notable approaches. Some models achieve outstanding accuracy, with one CNN reaching 100% in cancer cell classification. The incorporation of novel algorithms like Cat-Swarm Optimization and specialized CNN architectures contributes to superior classification accuracy. Performance metrics highlight these achievements, with models consistently outperforming traditional diagnostic methods. For instance, a CNN with Cat-Boosting attains 100% accuracy, while others hover around 99%, showcasing DL models' robustness in ALL diagnosis. Despite acknowledged challenges, such as the need for larger and more diverse datasets, these findings underscore DL's transformative potential in reshaping leukemia diagnostics. The high numerical accuracies accentuate a promising trajectory toward more efficient and accurate ALL diagnosis in clinical settings, prompting ongoing research to address challenges and refine DL models for optimal clinical integration.

KEYWORDS

acute lymphoblastic leukemia, bone marrow images, medical image analysis, deep learning, convolutional neural networks, diagnosis, classification

1 Introduction

Acute lymphoblastic leukemia (ALL) encompasses a range of lymphoid neoplasms that originate from precursor cells of both B-lineage and T-lineage cells (1). These neoplasms may primarily manifest as an extensive leukemic process involving both the bone marrow and peripheral blood, or they can display localized tissue infiltration with limited bone marrow involvement, termed lymphoblastic lymphoma (LBL) (2). Although ALL and LBLs exhibit distinct clinical features, they appear to represent a continuous biological spectrum. The current classification by the World Health Organization categorizes these conditions as B- or T-lymphoblastic leukemia/lymphoma (3). ALL is the most common pediatric malignancy, with pediatric ALL constituting approximately 80% of cases (4, 5). However, when it arises in adults, ALL takes on a particularly different clinical presentation. Notably, in the recent era of novel agents, not all cases of adult ALL have a poor prognosis; in fact, some individuals now experience good prognoses (6). In the United States, the estimated occurrence of ALL is about 1.6 cases per 100,000 individuals (7, 8). Research conducted among children has pinpointed genetic conditions that make a fraction of ALL cases more likely to occur including Down syndrome, Fanconi anemia, Bloom syndrome, and Ataxia Telangiectasia (9–11).

The initial phase of the diagnostic process for ALL, particularly to distinguish it from acute myeloid leukemia (AML) involves examining the bone marrow. This is crucial because ALL, as per its definition, invariably manifests with bone marrow participation (12, 13). Additional specialized tests are used to complement bone marrow evaluation such as peripheral blood smear (PBS) assessment and flowcytometric immunophenotyping (14, 15). However, bone marrow aspiration and biopsy remains the gold standard for ALL diagnostic confirmation, which provides a complete examination of cellular structure and appearance which could help indicate prognosis and evolution of the disease later on (16). While this approach allows for more precise classification and subtyping, it is an invasive process that can be painful, especially in pediatric patients, and getting appropriate samples can be difficult. Peripheral blood smears, on the other hand, require studying blood samples under a microscope to analyze blood cell morphology. Although they provide a rapid and non-invasive method of detecting blasts, their diagnostic depth may not be as extensive as bone marrow analysis.

Artificial intelligence (AI) and machine learning (ML) breakthroughs have sparked a revolution in medical image analysis and hematological diseases as previously explored by our group (17–21). Deep learning (DL) is a subset of ML that uses artificial neural networks to learn from data. Convolutional neural networks (CNNs) are one type of DL algorithm that has been particularly successful in image classification tasks (22). CNNs are designed to recognize patterns in images by using a series of convolutional layers that extract features from the input image. These features are then passed through a series of fully connected layers that classify the image based on the extracted features (22). CNNs have demonstrated exceptional ability in evaluating and interpreting medical images, including microscopic bone marrow images (23, 24).

In addition to the strides made in deep learning-based approaches, it is essential to acknowledge recent non-DL-based works that have contributed to the field of hematological disorder detection (25). Despite their contributions, these non-deep learning methods often face limitations in handling the complexity and variability present in hematological images. They may struggle to adapt to diverse morphologies and may require extensive manual tuning for optimal performance. DL methods, with their ability to automatically learn hierarchical features and patterns, offer a promising alternative that can potentially overcome some of these limitations, providing a more adaptive and robust solution for hematological disorder detection.

Although DL models in ALL diagnosis are widely studied, the focus has primarily been on peripheral blood smear (PBS) samples, neglecting the crucial bone marrow aspirates and biopsies, which are the gold standard for leukemia diagnosis. Recent reviews have also missed the majority of studies involving digital image analysis of microscopic bone marrow images (26, 27). Therefore, the goal of this review is to investigate the uses of DL in redefining ALL diagnosis and categorization using bone marrow images, possibly leading to the development of automated systems that assist healthcare personnel in making precise and timely ALL diagnoses. Performance metrics of several DL models and architectures in the detection and/or classification of ALL will be discussed. Furthermore, we will discuss the possible limitations and benefits of applying these models.

2 Materials and methods

2.1 Search strategy

We developed our search strategy on the 11th of June 2023 in the PubMed/MEDLINE database. To ensure a broad search strategy, we used many terms, such as ‘acute lymphoblastic leukemia’, ‘acute lymphocytic leukemia’, ‘acute lymphoid leukemia’, ‘ALL’, ‘artificial intelligence’, ‘machine learning’, ‘deep learning’, and ‘neural network’. The search was not restricted by language or time frame. The developed search strategy was transferred to Scopus, Embase, and Web of Science databases using the Polyglot translator (28). The studies were then transferred to EndNote X9, where duplicates were detected and omitted.

2.2 Eligibility criteria

The review will encompass studies that meet specific inclusion criteria: (1) utilization of human ALL samples, (2) publication in English, (3) employment of DL techniques for diagnosing/classifying ALL, (4) utilization of bone marrow samples, and (5) reporting of performance metrics. Studies not meeting these criteria will be excluded, ensuring a focused and relevant analysis.

2.3 Study selection and screening

After applying our search strategy to the mentioned databases, studies were transferred to EndNote X9, where duplicates were identified and removed. Remaining articles were uploaded to the Rayyan platform for additional screening (29). In Rayyan, titles and abstracts were screened for preliminary eligibility by two reviewers, and any discrepancies were settled by consensus. The whole texts of the papers that had been determined to be eligible were then acquired and independently double-screened for inclusion or exclusion using the mentioned criteria, with discrepancies resolved through screening by a third member if needed.

2.4 Data extraction

The data extraction process involved the extraction of pertinent information from included studies, comprising the last name of the primary author and the publication year, country of origin, dataset utilized, the targeted outcome under investigation, the applied validation methodologies, the employed models, and their corresponding performance metrics including accuracy, precision, sensitivity (recall), specificity, and F1 score. Furthermore, the strengths and limitations associated with each model were noted. Two investigators examined and obtained data from the eligible study independently. When they were unable to reach an agreement, they held a meeting with all team members. Only if it was agreed upon in the team meeting was the study included in the final review.

2.5 Aims

This review aims to provide an extensive examination of contemporary DL algorithms employed for the diagnosis and classification of ALL, with a specific emphasis on bone marrow samples. The principal objective entails a comprehensive evaluation of the performance of the diverse DL models featured in each study. Concurrently, a secondary aim involves the analysis of the relative merits and constraints of individual models in comparison to others.

3 Results

3.1 Study selection

The PRISMA flow diagram, shown in Figure 1, depicts the process of selecting studies for this review. Initially, our database search yielded 496 results, with an additional article found through manual extraction. After removing 282 duplicates with EndNote and Rayyan, we evaluated the remaining 215 items based on their titles and abstracts. Through this screening process, we excluded 195 articles not eligible for further screening and were left with 20 for full-text screening. We retrieved and examined the complete texts of the 20 studies and based on a variety of reasons listed in Figure 1, we eliminated 10 more articles. Ultimately, 10 studies were included in our review.

3.2 Study characteristics and data collection

Table 1 presents the attributes and data gathered from the studies included in this analysis. It evaluates the effectiveness of deep learning models implemented for the diagnosis and categorization of ALL through bone marrow imagery. For a more comprehensive understanding, the specific metrics for accuracy (ACC), precision (PRE), sensitivity (SEN), specificity (SPE), and F1-score are provided for each model. In summary, the studies covered were published between 2013 and 2023, predominantly originating from India (n = 5), China (n = 3), KSA (n = 1), and Mexico (n = 1). Among these, five studies utilized the SN-AM dataset, comprising microscopic bone marrow aspirate images from patients diagnosed with B-cell ALL and Multiple Myeloma (MM) (40).

Notably, Yang et al. employed the SN-AM dataset in conjunction with the ALL-IDB1 database of peripheral blood smear images for external validation (38). Their model's training and testing employed bone marrow samples from patients representing diverse leukemia families and subtypes. Zhou et al. introduced a novel "AI-cell platform" database for white blood cell (WBC) classification using bone marrow images, externally validating their model on authentic clinical samples of ALL and acute myeloid leukemia (AML) (39). The remaining four studies retrospectively sourced bone marrow aspirate images from hospital records. In terms of validation methods, only two studies conducted both external and internal validation, specifically utilizing a Train-Test Split approach. The remaining eight studies solely relied on internal validation via Train-Test Split (6) and k-fold cross-validation (2). Most of the studies employed CNNs as their classifier model, incorporating various layers, optimizations, and supplementary algorithms. The architecture of CNNs, as adapted from Kavitha et al, is depicted in Figure 2. A singular study deviated by utilizing radial basis function neural networks with a fuzzy logic algorithm in place of CNNs. The prevalence of CNNs, transfer learning, gradient boosting algorithms, and other elements typically associated with supervised learning tasks underscores these models' intent for tasks involving labeled training data. Most models exhibited remarkable performance in their designated tasks. Notably, the models achieved high accuracy, with some reaching 100%, demonstrating the robustness of DL in ALL diagnosis.

Table 2 provides an overview of the strengths and limitations of each DL model discussed in the review. Each model's outcomes, including feature selection, boosting algorithms, and optimized hyperparameters, are highlighted as strengths. However, limitations such as the lack of external validation, dependency on image quality, and computational complexity are also discussed. These strengths and limitations are crucial in evaluating the practical applicability and potential challenges associated with each DL model. For instance, the discussion on the lack of external validation emphasizes the need for further validation on

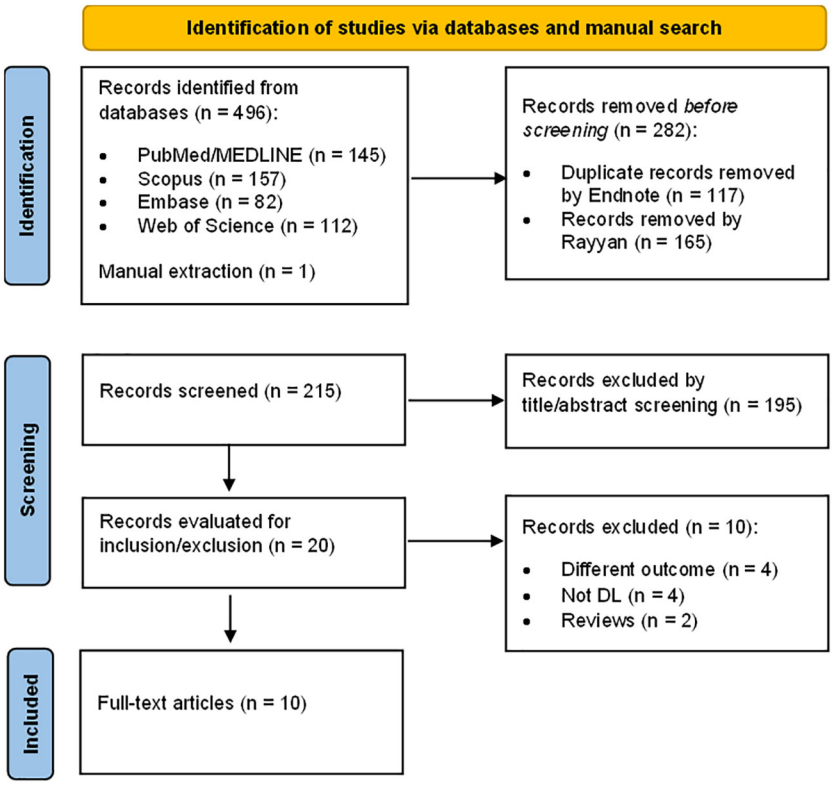

FIGURE 1
Schematic representation of the literature review process.

TABLE 1 Performance of DL models in ALL diagnosis and classification using bone marrow images.

Authors (Year)	Country	Dataset & Sample Size	Validation (IV/EV)	Best Model(s)	ACC (%)	PRE (%)	SEN (%)	SPE (%)	F1 (%)
Devi et al. (2023) (30)	India	SN-AM dataset (B-ALL [90] and MM [100])	IV (Train-Test)	CNN (Convolutional Leaky RELU) with Cat-Boosting algorithm	100	100	99.9		100
				CNN (Convolutional Leaky RELU) with XG-Boosting algorithm	97.12	98.5	99		97.2
Duggal et al. (2017) (31)	India	BM samples (ALL [4469], healthy [4469])	IV (5-fold CV)	Texture-CNN with an additional SD-Layer	93.20				93.08
				CNN (AlexNet) with an additional SD-Layer	88.5				88.32
Huang et al. (2020) (32)	China	BM samples (ALL [23], AML [53], CML [10], healthy [18])	IV (Train-Test)	CNN (DenseNet121) with Transfer Learning technique	99				
Ikechukwu et al. (2022) (33)	India	SN-AM dataset (B-ALL [90] and MM [100])	IV (Train-Test)	CNN (i-Net)	99.18	99.30	99.18		99.19
Kavitha et al. (2022) (34)	India	SN-AM dataset (B-ALL [90] and MM [100])	IV (Train-Test)	CNN with Cat-Swarm Optimization	99.6	99.2	99.5	99.3	99.89
Kumar et al. (2020) (35)	India	SN-AM dataset (B-ALL [90] and MM [100])	IV (Train-Test)	Dense CNN (DCNN)	97.25	100	93.97	95.19	96.89

(Continued)

TABLE 1 Continued

Authors (Year)	Country	Dataset & Sample Size	Validation (IV/EV)	Best Model(s)	ACC (%)	PRE (%)	SEN (%)	SPE (%)	F1 (%)
Ordaz-Gutierrez et al. (2013) (36)	Mexico	BM samples (ALL [118], healthy [62])	IV (Train-Test)	Hybrid of Fuzzy Logic and RBFNN	96.7		98.00	91.00	
Rehman et al. (2018) (37)	KSA	BM samples (ALL L1 [100], ALL L2 [100], ALL L3 [30], healthy [100])	IV (10-fold CV)	CNN (AlexNet)	97.78				
Yang et al. (2023) (38)	China	BM samples (ALL [306], AML [500], CML [162], healthy [291])	IV (Train-Test) EV (SN-AM)	Hybrid of CNN and ViT (MobileViTv2) with MultiPathGAN	96 (IV) 99.72 (EV)				
Zhou et al. (2021) (39)	China	AI-cell database[1] (ALL [24], AML [25])	IV (Train-Test)[1] EV (BM samples)	Ensemble of CNNs (ResNext101_32x8d, ResNext50_32x4d, and ResNet50)	89 (EV)		86 (EV)	95 (EV)	

ACC, Accuracy; PRE, Precision; SEN, Sensitivity SPE, Specificity; BM, Bone Marrow; ALL, Acute Lymphoblastic Leukemia; MM, Multiple Myeloma; AML, Acute Myeloid Leukemia; CML, Chronic Myeloid Leukemia IV, Internal Validation; EV, External Validation; CNN, Convolutional Neural Network; SD-Layer, Stain Deconvolutional Layer; ViT, Vision Transformer; RBFNN, Radial basis function neural network.
[1]Performance metrics of internally validated WBC classifier model: Accuracy: 82.93%, Precision: 85.67%, F1 score: 82.93%, AUC: 98.70%.

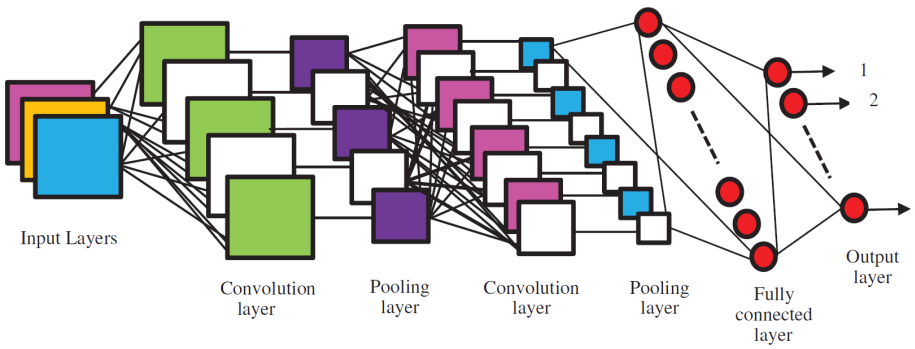

FIGURE 2
Convolutional neural network and its layers, adapted from Kavitha et al.

diverse datasets to ensure the generalizability of the models. Moreover, the acknowledgment of computational complexities and interpretability issues provides insights into areas where improvements and future research could be directed.

3.3 Specialized CNN designs

Section 3.3 is a discussion of studies that focused on developing specialized CNN designs, including the integration of specific enhancements, additional layers, and boosting algorithms to increase classification accuracy. It will be divided into studies using the SN-AM dataset and studies using retrospectively collected hospital bone marrow samples.

3.3.1 B-ALL and MM classification using the SN-AM dataset

B-ALL and MM are hematologic malignancies that arise from various stages of B-cell development. Despite their evident clinical and pathological distinctions, certain resemblances exist in their morphological attributes and molecular characteristics, rendering their differentiation challenging. This subsection focuses on a series of studies that employ the SN-AM dataset, containing bone marrow images from patients with B-ALL and MM, to develop specialized CNN designs for accurate classification. In the four studies, the model was evaluated using internal validation through train-test split, partitioning data into training, validation, and testing sets with no external validation.

The article by Devi et al. addresses the segmentation and classification of white blood cancer cells within bone marrow microscopic images. The research methodology starts with data preprocessing, effectively eliminating dataset anomalies. Following that, dataset diversity and comprehensiveness are augmented through data augmentation techniques. The proposed model then utilizes the Convolutional Leaky RELU with CatBoost and XGBoost (CLR-CXG) algorithm for image segmentation and feature extraction, which are key processes for accurate classification. Binary classification is executed through CNN, accompanied by gradient boosting using CatBoost and XGBoost algorithms individually. The interaction between CNN and boosting

TABLE 2 Strengths and limitations of DL models reported.

Authors (Year)	Outcome	Strengths	Limitations
Devi et al. (2023) (30)	Classification of B-ALL and MM using CNNs with boosting algorithms.	Feature selection Use of boosting algorithms Reduced pre-processing	Lack of external validation Limited dataset Complex segmentation
Duggal et al. (2017) (31)	Differentiating malignant WBCs from normal WBCs using a CNN with a SD-Layer.	Stain deconvolution Minimal additional parameters Generalization potential	Lack of external validation Stain variation challenges Complexity for large datasets
Huang et al. (2020) (32)	Distinguishing between different types of leukemia using a CNN with transfer learning.	Multiple leukemia types Feasibility for small datasets Minimize need for segmentation	Lack of external validation Misclassification of leukemias Limited interpretability
Ikechukwu et al. (2022) (33)	Detection and classification of B-ALL and MM using a CNN with tuned hyperparameters.	Simplified architecture Feature selection capability Real-time applicability	Lack of external validation Limited dataset Limited interpretability
Kavitha et al. (2022) (34)	Detection and classification of B-ALL and MM using a CNN with Cat-Swarm Optimization algorithms.	Outperforms ML models Optimized hyperparameters Real-time applicability	Lack of external validation Limited dataset Computational complexity
Kumar et al. (2020) (35)	Detection and classification of B-ALL and MM using a Dense CNN with fewer parameters.	Outperforms ML models Feature extraction capability Real-world application	Lack of external validation Limited dataset Limited interpretability
Ordaz-Gutierrez et al. (2013) (36)	Diagnosis of ALL using fuzzy logic algorithm and Radial basis function neural network.	Handling of ambiguity Thorough cellular assessment Detection of ALL at early stages	Lack of external validation Dependency on image quality Specific cellular features
Rehman et al. (2018) (37)	Detection and classification of ALL and ALL subtypes (L1, L2. L3) using CNN.	Rapid diagnosis Robust segmentation Assist pathologists	Lack of external validation Limited dataset Dependency on image quality
Yang et al. (2023) (38)	Diagnosis and classification of leukemias using MobileViTv2 classifier and MultiPathGAN.	External validation Lightweight hybrid network Flexibility and adaptability	Sensitivity to data quality Lack of real-world scenarios
Zhou et al. (2021) (39)	Diagnosis of ALL in real clinical scenarios using an ensemble of CNN models.	Real-world & external validation Large dataset Mimics hematologist workflow	Single-center data Prospective validation needed

(Continued)

algorithms mainly occurs in the classification phase. The features extracted by the CNN are used as inputs to the boosting algorithms, which refine the classification decision. This combination allows for a more accurate and efficient classification of blood cancer cells. This CLR-CXG approach aims to minimize bias and amplify accuracy in cancer cell classification, primarily discerning between B-ALL and MM. Internal validation is achieved by partitioning the dataset into train, test, and validate sets. CNNs play a pivotal role in image classification, recognizing important features within images through weight and bias assignment. To address challenges like input-output consistency and GPU expenses, the CLR-CXG model introduces modifications into the CNN architecture. A novel element is the incorporation of the Leaky RELU activation function, elevating the architecture's capabilities. The results impeccably show that CatBoost and XGBoost algorithms enhance accuracy and computational efficiency. The CLRC algorithm achieves an impressive 100% accuracy, precision, and specificity in cancer cell classification, complemented by a sensitivity (recall) of 99.9% and an F1 score of 100. Meanwhile, CLRXG attains 97.12% accuracy, alongside precision, sensitivity (recall), and specificity values of 98.5%, 99%, and 97.2%, correspondingly. Despite these achievements, the article has many limitations including absence of information regarding resource allocation, memory usage, and energy efficiency.

Moreover, the study by Ikechukwu et al. introduces a novel deep CNN model named "i-Net" for classification of ALL using microscopic images. The proposed approach utilizes data from the SN-AM and ALL-IDB datasets, both sourced from the cancer imaging archive (TCIA) repository. Initially, augmentation balanced limited data. Data preprocessing involved grayscale conversion, contrast enhancement, and resizing. For segmentation, they used a UNet model with InceptionV2 architecture, while a custom CNN was designed for image classification. The authors employed two well-known pre-trained deep learning networks, ResNet-50 and VGG-19. However, they adapted the weights and learning parameters instead of using pre-existing ones. An upgraded CNN model, "i-Net," was introduced, adding convolutional layers and fine-tuning hyperparameters for better classification accuracy. To prevent overfitting during training, the authors used data augmentation, dropout regularization, and batch normalization techniques. The proposed "i-Net" achieved 99.18% accuracy on the SN-AM dataset, surpassing ResNet-50 (84.5%) and VGG-19 (93.5%). The model's

generalization was tested, highlighting its potential for clinical decision support systems. Despite limitations due to computational constraints and a smaller dataset, the proposed "i-Net" model outperformed established models, showing promise for clinical use.

Furthermore, Kavitha et al. introduce a groundbreaking methodology for the diagnosis and classification of bone marrow cancers, with a particular focus on ALL and MM. The proposed model employs optimized deep CNNs utilizing a novel CAT (Cat Swarm Optimization) algorithm for hyperparameter tuning (41). The process involves three essential phases: data preparation, data augmentation, and classification. The data preparation phase involves capturing microscopic images from bone marrow aspirate slides, which are stained using the Jenner-Giemsa method. These raw images are then pre-processed to create a dataset that is utilized for both training and testing purposes. Data augmentation techniques are employed to alleviate overfitting concerns and augment the model's ability to generalize. The architecture of the CNN incorporates convolutional layers for feature extraction, pooling layers for dimension reduction, and fully connected layers for accurate classification. The introduction of the CAT algorithm further enhances the model's overall performance by drawing inspiration from the behaviours of cats, combining seeking and tracing modes to effectively optimize the network's parameters. The evaluation of the proposed approach is conducted using the SN-AM dataset. The results showcase remarkable achievements, with an outstanding accuracy of 99.6% attained in accurately predicting ALL. This performance surpasses that of pre-trained deep learning models, such as AlexNets, VGG-16 Nets, and U-Nets. The proposed model's superiority is further substantiated through comprehensive comparisons with other machine learning methodologies, including support vector machines, random forest, and naïve bayes.

Lastly, the study conducted by Kumar et al. introduces a robust mechanism for classifying B-ALL and MM using CNNs. The study leverages deep learning techniques to automate the classification process, eliminating errors associated with manual assessment. The model is trained on cell images, undergoing preprocessing and feature extraction. It employs a dense convolutional neural network (DCNN) framework for classification, depicted in Figure 3, and achieved an impressive overall accuracy of 97.2%. Notably, the model demonstrates exceptional precision, sensitivity, specificity, and F1 score, with a precision of 100%, sensitivity of 93.97%, specificity of 95.19%, and an F1 score of 96.89%. The CNN architecture comprises convolution, max-pooling, and fully connected layers. Data augmentation techniques enhance generalization, while feature selection relies on the Chi-square test. Training utilizes an Adam optimizer with a sigmoid cross-entropy loss function and a learning rate of 0.01. Comparisons with machine learning methods and transferred learning models like VGG-16 were conducted. Random Forests achieved an accuracy of 96.83% on the dataset. However, the proposed CNN model significantly outperforms these approaches, boasting higher precision, sensitivity, specificity, and F1 score. Its capacity to extract features directly from images, coupled with adaptability across datasets, underscores its advantages. Although acknowledging limitations stemming from dataset size, the study underscores the potential of the proposed model as a reliable tool for diagnosing bone marrow blood cancers.

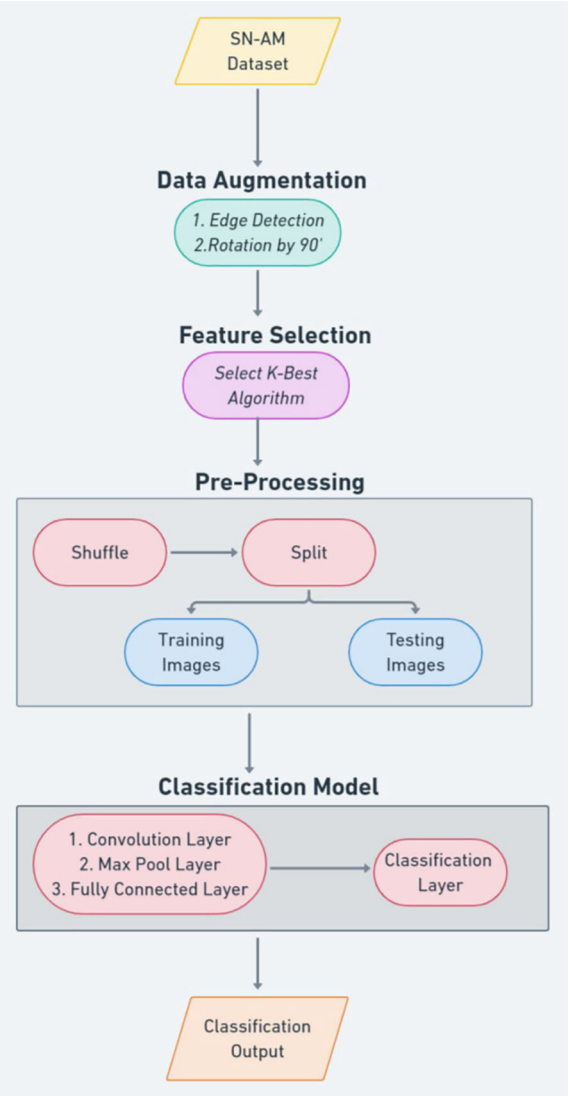

FIGURE 3
Kumar et al.'s proposed convolutional neural network-based methodology.

3.3.2 ALL diagnosis using retrospectively collected hospital bone marrow samples

This subsection focuses on another set of studies that address the diagnosis of ALL through the analysis of retrospectively collected bone marrow samples from hospital patients. These studies emphasize the importance of accurate and efficient diagnosis for different leukemia subtypes.

The article by Duggal et al. presents an innovative convergence of deep learning techniques and stain deconvolution in the domain of medical image analysis. While CNNs have proven successful in medical imaging, the authors highlight a crucial limitation: CNNs primarily function in the RGB color space, potentially missing the nuanced tissue-stain interactions crucial for precise diagnostics. To

address this, the study introduces the stain deconvolution layer (SD-Layer). Positioned at the forefront of CNN architectures, this layer operates in the optical density (OD) color space. Beer-Lambert's law is employed to convert RGB microscopic images into the OD space, revealing pixel stain quantities that hold key diagnostic information. The SD-Layer operates on two fronts: converting RGB to OD space and using backpropagation to derive optimal stain basis vectors for diverse cell types. OD images are then deconvolved with these vectors, providing tissue-specific stain absorption quantities as input for downstream CNN layers. The study focuses on differentiating malignant WBCs from normal ones in cancer detection, particularly ALL. Texture-CNN and CNN (AlexNet) are evaluated using the SD-Layer in two modes: frozen (fixed stain vectors) and trainable (refined vectors). Impressively, the SD-Layer, initialized with stain basis vectors from SVD of the reference image, notably enhances classification accuracy for both architectures. This enhancement is attributed not to model capacity but to the biologically meaningful image representation the SD-Layer offers. With a well-structured dataset of around 9000 cell nuclei, balanced between normal and malignant cells and stained with Jenner-Giemsa, the study's robustness is underscored. Rigorous training and augmentation techniques yield high performances on 5-fold cross-validation accuracy in distinguishing malignant from normal WBCs. The Texture-CNN achieves 93.20% accuracy and 93.08% F1 score with an additional SD-Layer, while CNN (AlexNet) achieves 88.5% accuracy and 88.32% F1 score with an additional SD-Layer. SD-Layer bridges RGB limitations, leveraging the OD space to capture crucial diagnostic insights. As medical imaging evolves, this study paves the way for harnessing stain quantities to enhance classification accuracy and diagnostic efficacy across diverse scenarios.

Moreover, Rehman et al., proposes a computer-aided system that combines image processing and deep learning to improve ALL diagnosis accuracy, depicted in Figure 4. The study focuses on classifying ALL into its subtypes and distinguishing reactive bone marrow (normal) using stained bone marrow images. The authors collect a dataset of bone marrow images from patients with ALL and normal cases. The images are captured using a digital microscope and processed to segment the regions of interest. A novel segmentation technique based on thresholding is introduced, followed by the application of CNNs for classification. The dataset is split into training and testing sets to train the CNN model. The researchers utilize the AlexNet architecture with transfer learning to fine-tune the model to the new data. To assess the effectiveness of their approach, the authors perform experiments and compare the results with other classification methods such as naïve Bayesian, K-nearest neighbor, and support vector machine. The proposed method achieves an impressive accuracy of 97.78% on the test dataset. The classification accuracy is plotted against the number of iterations, demonstrating that higher accuracy can be achieved with more epochs and a lower learning rate. The training time is also noted, with the proposed architecture taking approximately 163.63 seconds for 20 epochs. The authors highlight the significance of their work, as it provides an automated solution for accurate ALL diagnosis and classification. By employing DL techniques, the proposed system improves the accuracy of classification, which could significantly assist hematologists and pathologists in their diagnostic processes. Despite the promising results, this study does have limitations. The dataset size might impact the generalizability of the model, and external validation on larger datasets is necessary.

Lastly, Huang et al.'s study addresses leukemia classification and diagnosis through bone marrow cell morphology, employing CNNs

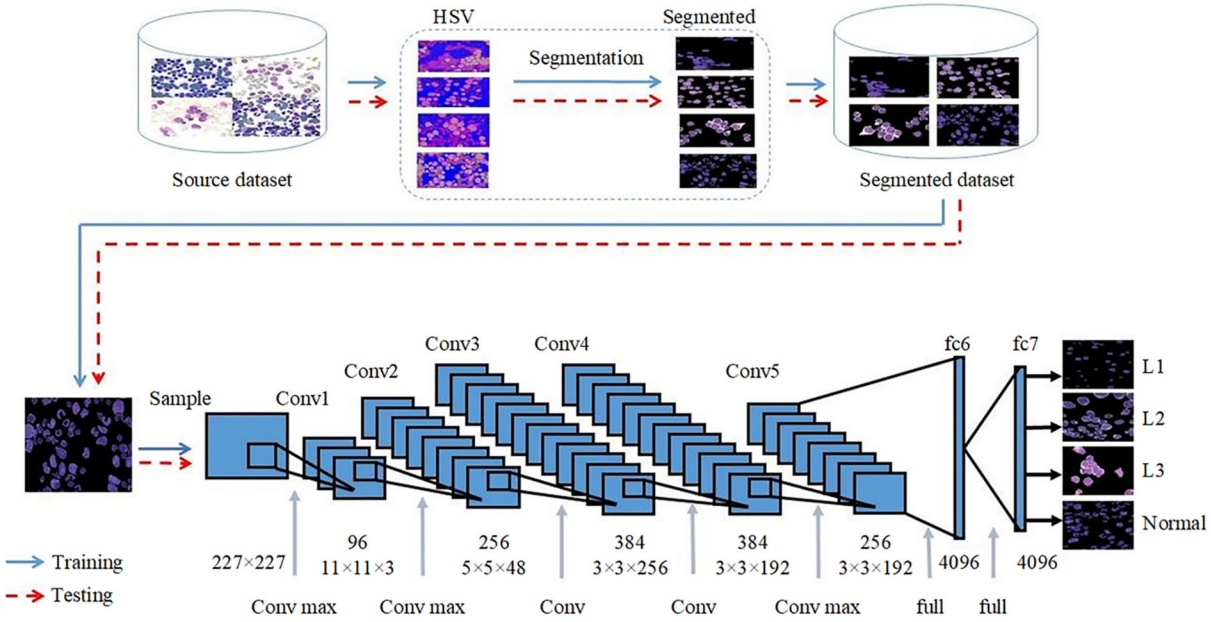

FIGURE 4
Rehman et al.'s proposed convolutional neural network-based methodology.

alongside transfer learning. Traditional manual microscopy for leukemia diagnosis is subjective and error-prone, motivating an automated, precise approach. Their proposed method utilizes CNNs for identifying AML, ALL, and chronic myelocytic leukemia (CML). The researchers obtained microscopy images from healthy subjects and leukemia patients, implementing preprocessing techniques like perfect reflection and adaptive filtering to enhance quality and reduce background noise. They employed three CNN architectures for classification models on both raw and preprocessed datasets: Inception-V3, ResNet50, and DenseNet121. Transfer learning was leveraged to optimize model performance by extracting features or fine-tuning pre-trained models. In line with internal validation practices, the dataset is divided into a training set (991 samples) and a prediction set (331 samples) using a 3:1 ratio. The training set is utilized to train the models, while the prediction set serves as unseen data for testing the model's generalization capability. DenseNet121 excelled among the CNN architectures, consistently achieving superior performance. Transfer learning notably expedited model convergence, significantly boosting accuracy. The study's outcomes indicate that the DenseNet121 model on the preprocessed dataset garnered the highest accuracy at 74.8%. After transfer learning, its accuracy surged to 95.3%, a notable 20.5% improvement. The model exhibited accuracy rates of 90% for normal samples, 99% for ALL, 97% for CML, and 95% for AML, demonstrating efficacy in classifying various leukemia types. Nonetheless, the model faced challenges distinguishing immature granulocytes and lymphocytes, affecting AML classification accuracy. Its adaptability to rare leukemia types remains to be explored. Huang et al.'s study contributes a rapid, accurate, and objective method for leukemia diagnosis by merging CNNs with transfer learning. The combination overcomes the limitations of manual methods, catering to efficient, precise medical imaging despite small sample sizes. Though it confronts challenges, like distinguishing specific cell types, the study offers a promising path towards enhancing leukemia diagnosis and classification.

3.4 Ensemble and hybrid designs

In this section, we explore studies that utilize ensemble techniques and hybrid approaches, combining multiple models to enhance diagnosis accuracy. The subsequent subsections present the findings from these studies and provide insights into their contributions to the field.

3.4.1 ALL Diagnosis using a hybrid of fuzzy logic and radial basis function neural network

This subsection examines the work by Ordaz-Gutierrez et al., which introduces an algorithm for diagnosing ALL using a combination of robust fuzzy logic and radial basis function neural networks (RBFNN). The primary aim of this research is to develop a reliable method for diagnosing ALL, particularly in developing countries like Mexico, where laboratory resources and equipment might be limited. The algorithm leverages bone marrow aspirates to extract specific features related to the disease. The process begins with acquiring microscopic cell images, which are then converted to grayscale to eliminate unnecessary color information and reduce processing time. Histogram equalization enhances image contrast. The segmentation stage involves utilizing the Sobel edge detection and mathematical morphology algorithms to isolate cells from the images. Suitable mathematical expressions are utilized to analyze cell size, circularity, and nuclei-to-cytoplasm ratio, crucial for ALL diagnosis. The heart of the method lies in applying fuzzy logic, chosen for incorporating human knowledge and mathematical modeling. The algorithm determines if a cell has ALL based on computed features. Fuzzy membership values combine using algebraic expressions to generate a diagnosis variable that classifies cells. To enhance the algorithm, a radial basis function (RBF) neural network is introduced, improving classification accuracy. Trained on a dataset, the algorithm achieves high sensitivity (98.00%) and specificity (91.00%). The results of the proposed method are promising, outperforming comparative methods, showing superiority in detection rates. The potential for real-time diagnosis is highlighted due to efficient feature extraction and RBF's computational speed.

3.4.2 Diagnosis of leukemias using a hybrid of CNN and vision transformer

Here, we delve into the article by Yang et al. which presents a deep learning-based approach for diagnosing leukemias using bone marrow aspirates. The study collected 2033 microscopic images of bone marrow samples, encompassing images for 6 disease types and 1 healthy control, from two Chinese medical websites. These images were divided into training, validation, and test datasets. To address variations in staining styles, a novel method called "stain domain augmentation" was introduced using the MultiPathGAN model. This technique normalized stain styles and expanded the dataset. A lightweight hybrid model named MobileViTv2, combining strengths of CNNs and vision transformers (ViTs), was developed for disease classification. MobileViTv2 achieved an average accuracy of 94.28% on the test set, with the highest accuracy values (98%, 96%, and 96%) obtained for MM, ALL, and lymphoma, respectively. Patient-level prediction accuracy averaged 96.72%. The model outperformed both CNNs and ViTs despite using only 9.8 million parameters. Furthermore, MobileViTv2 was compared to other deep learning models, demonstrating its superiority. The model's effectiveness was also externally validated on public datasets (ALL-IDB1 and SN-AM), achieving high accuracy values of 99.75% and 99.72%, respectively. This indicates its robust generalization ability. While the model shows promise, there are some limitations. The dataset size is relatively small, and efforts to collect more images from various scanning devices could enhance its performance. Additionally, the model's application is primarily focused on diagnosing broad disease categories, not specific subtypes. Future research could explore finer disease subtype classification and optimization of the model.

3.4.3 Leukemia diagnosis using ensemble CNN models in real clinical scenarios

This subsection explores Zhou et al.'s study, which develops a deep learning-based system for leukemia diagnosis and evaluates it using real clinical scenarios. The subsection discusses the unique aspects of the system, its effectiveness in diagnosing different types of leukemia, and its practical utility in clinical settings. The researchers collected 1,732 bone marrow images, containing 27,184 cells, from children with leukemia in a dataset named "AI-cell platform". This dataset was used to train a CNN architecture for the differential count of WBCs. Unlike prior approaches that preprocess images, this study used raw images without pre-processing. The developed system mimicked the process of hematologists by detecting and excluding uncountable and crushed cells, classifying remaining cells, and making diagnoses utilizing different configurations of ResNet and ResNeXt architectures for WBC detection and classification, as depicted in Figure 5. The ensemble of CNNs, comprising ResNeXt101_32x8d, ResNeXt50_32x4d, and ResNet50, emerged as the top-performing configuration. On internal validation using Train-Test Split, the ensemble model demonstrated very high performances for classifying WBCs (82.93% accuracy, 86.07% precision, and 82.02% F1 score). On external validation using real-world clinical samples of bone marrow, the system showed notable performance in diagnosing ALL (89% accuracy, 86% sensitivity, and 95% specificity). The validation results reveal significant insights into the ensemble's performances and underscore its robustness and its potential for effectively diagnosing leukemia subtypes. Additionally, it accurately detected bone marrow metastasis of lymphoma and neuroblastoma (average accuracy of 82.93%). The system's development was unique in using raw clinical images and replicating the hematologists' workflow. The CNN differentiated crush cells, commonly excluded during manual counts, and demonstrated high accuracy across diverse WBC types. Furthermore, the system achieved successful ALL diagnosis in clinical practice, providing evidence of its practical utility. Comparison with existing studies revealed the uniqueness of this research in its broader variety of cell types, achieving high accuracy across complex clinical scenarios. Prior studies often focused on single-cell classification or employed pre-processed images, hindering real-world applications. While this study excelled in leukemia diagnosis, the limited dataset size for certain cell types posed challenges. However, the study's innovative use of ensemble models mitigated this issue, enhancing overall accuracy.

4 Discussion

Recent advancements in medical image analysis have yielded remarkable progress in the automated detection and classification of acute leukemia, a critical hematological malignancy. Deep learning techniques have emerged as pivotal tools, demonstrating the potential to revolutionize diagnostic accuracy and efficiency. Key studies have explored acute leukemia detection and classification intricacies, addressing essential elements such as datasets, validation methodologies, and performance metrics.

4.1 Previous literature

The existing literature on AI-based acute lymphoblastic leukemia (ALL) classification, as discussed in systematic reviews by Das et al. (42) and Mustaqim et al. (43), reveals notable limitations that our review seeks to address. While these reviews have explored recent advancements in AI-based ALL classification, they primarily emphasize studies and datasets focused on peripheral blood samples. Although peripheral blood samples provide valuable insights, the gold standard for leukemia diagnosis has long been bone marrow samples, given their ability to offer a more comprehensive understanding of the disease's characteristics. Bone marrow samples are particularly crucial for accurately distinguishing different leukemia subtypes. By including studies that utilize bone marrow samples in the context of AI-based ALL classification, our review fills this crucial gap in the literature, contributing to a more holistic understanding of advancements in leukemia diagnosis and emphasizing the significance of bone marrow analysis in achieving accurate and reliable results. Additionally, Alsalem et al.'s (44) comprehensive review on

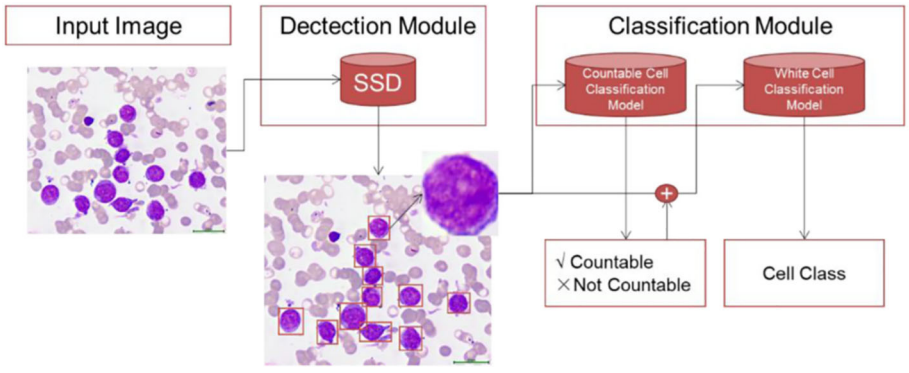

FIGURE 5
Zhou et al.'s proposed framework for white blood cell classification.

automated acute leukemia detection and classification complexities, while valuable, predominantly focuses on studies applying artificial intelligence to peripheral blood smear (PBS) samples (45–48). Similarly, Deshpande et al. (49) adopt an AI-centric approach, enhancing diagnostic accuracy through microscopic blood cell analysis. While these approaches contribute significantly to the field, they underscore the need for a more inclusive examination of bone marrow samples, as we address in our review. Furthermore, the literature showcases the effectiveness of deep learning models in distinguishing acute leukemia subtypes, such as the work by Anilkumar et al. (50) on automated B cell and T cell acute lymphoblastic leukemia differentiation using blood smear samples and Boldú et al.'s (51) introduction of ALNet for effective diagnosis of acute leukemia lineages using peripheral blood cell images. Moreover, Ouyang et al.'s (52) proposal of a convolutional neural network-based acute promyelocytic leukemia diagnosis highlights the versatility of deep learning across various subtypes. Laosai & Chamnongthai's innovative approach using CD markers of blood cells for automated acute leukemia classification (15) adds another dimension to the literature. Notably, the significance of well-annotated datasets, such as ALL-IDB, SN-AM, C-NMC, and SDCT-AuxNet, in standardizing algorithm evaluation is acknowledged in the literature (53, 54). Finally, the literature emphasizes the importance of integrating real-world clinical scenarios and transfer learning to improve model robustness (55, 56), aspects that our review aims to further elucidate and emphasize in the context of bone marrow samples.

4.2 Specialized CNN designs

Several studies have been dedicated to the development of specialized CNN architectures, aiming to enhance the accuracy of classification. Devi et al. introduced the CLR-CXG model, synergizing convolutional leaky rectified linear units (ReLU) with CatBoost and XGBoost boosting algorithms for cancer cell classification. This integration showcased promising results, underlining the potential of hybrid models. The "i-Net" model by Ikechukwu et al. effectively combined pre-trained deep learning networks, segmentation techniques, and data augmentation to achieve exceptional accuracy in segmenting and classifying acute lymphoblastic leukemia (ALL). Kavitha et al. contributed an optimized deep CNN architecture for diagnosing bone marrow cancers, leveraging the Cat Swarm optimization (CAT) algorithm for hyperparameter tuning. By focusing on precise segmentation and feature extraction using deep CNNs, this study demonstrated the power of specialized designs in achieving robust classification. Additionally, Kumar et al. emphasized the automatic detection of white blood cell cancers, specifically ALL and MM using CNNs. Their study highlighted the capacity of CNNs to discern pertinent features within images, effectively enhancing classification accuracy.

4.3 Hybrid and ensemble designs

Hybrid and ensemble methodologies also emerged as valuable avenues for leukemia diagnosis. Ordaz-Gutierrez et al. introduced a practical hybrid approach, uniting the RBFNN with the fuzzy logic algorithm for ALL diagnosis. This study's emphasis on resource-constrained settings underscores the importance of accessible and effective models. Yang et al. ventured into hybrid modeling by integrating CNNs with ViTs to diagnose hematologic malignancies through bone marrow images. By incorporating stain domain augmentation and hybrid modeling, this study showcased the potential of blending diverse deep learning techniques. Zhou et al. devised a deep learning-based system for leukemia diagnosis, employing an ensemble of multiple CNN models. The exceptional performance observed in classifying different white blood cell types and accurately diagnosing ALL in clinical scenarios exemplified the potential of ensemble techniques for practical medical applications.

4.4 Practical implications and limitations

The discussed studies offer a promising path for improving ALL diagnosis and classification through specialized CNN designs, hybrid models, and ensemble techniques. However, recognizing associated limitations is essential. One of the key limitations of the reviewed studies is the relatively small dataset sizes. While these studies demonstrate the potential of DL in ALL diagnosis, the limited data may raise concerns about the generalizability of the results. It is noteworthy that only two out of the ten reviewed studies employed external validation. This is a significant limitation, as relying solely on internal validation can lead to inflated performance metrics. To address these limitations, further efforts are required. This includes exploring larger datasets, refining segmentation techniques, and assessing clinical feasibility. It is imperative to develop a comprehensive evaluation framework that incorporates external validation and real-world clinical testing to enhance the robustness and generalizability of AI models for ALL diagnosis and classification. Moreover, considering the complexity of leukemia diagnosis, incorporating more complex samples, such as those with 10-15% blast in normal marrow, is vital for a thorough assessment of deep learning's potential in distinguishing normal and malignant blasts.

4.5 Future considerations

Future research endeavors should consider the incorporation of molecular and genomic data into the analysis pipeline. Combining these data sources with image-based analyses can potentially provide a more holistic and accurate assessment of ALL cases. Furthermore, the development of AI models that can effectively integrate and interpret both image and molecular/genomic data represents an exciting avenue for future research in the field. By confronting limitations and pursuing the identified research agenda, the field can move into a new era of accurate, efficient, and accessible methods for diagnosing and classifying leukemia using bone marrow images. This advancement holds the potential to revolutionize clinical practices, enabling timely interventions and personalized treatment strategies. Moreover, establishing standardized protocols for external validation and benchmarking

across different datasets and institutions will be instrumental in establishing the reliability and generalizability of deep learning models. Ultimately, as these future directions unfold, they will contribute to the ongoing refinement and deployment of AI-powered tools in the realm of ALL diagnosis and classification, improving patient outcomes and advancing the field of medical image analysis.

5 Conclusion

In conclusion, this review highlights the potential of deep learning models in enhancing acute lymphoblastic leukaemia diagnosis and classification. The proposed methodologies could revolutionize leukaemia diagnostics, providing accurate tools for early detection and treatment. Specialized CNN architectures, hybrid models, and ensemble techniques demonstrate the adaptability of deep learning in medical image analysis. However, limitations like small datasets and lack of external validation must be acknowledged. The reported high model performance metrics might be overestimated without robust validation. Future research should focus on refining and validating models, utilizing larger datasets, and conducting clinical feasibility studies. Collaborative efforts could integrate AI tools for precise leukaemia diagnosis, advancing patient care and reshaping medical imaging diagnostics.

Author contributions

BE: Conceptualization, Methodology, Project administration, Writing – original draft, Writing – review & editing. ME: Methodology, Writing – original draft, Writing – review & editing. RME: Methodology, Writing – original draft, Writing – review & editing. AE: Methodology, Writing – original draft, Writing – review & editing. AB: Validation, Writing – original draft, Writing – review & editing. OM: Validation, Writing – original draft, Writing – review & editing. RAE: Validation, Writing – original draft, Writing – review & editing. MS: Validation, Writing – original draft, Writing – review & editing. FK: Writing – original draft, Writing – review & editing. AA: Writing – original draft, Writing – review & editing. DM: Writing – original draft, Writing – review & editing. MY: Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The open access publication of this article was made possible due to a generous fund from QU Health, Qatar University.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Onciu M. Acute lymphoblastic leukemia. *Hematol Oncol Clin North Am* (2009) 23 (4):655–74. doi: 10.1016/j.hoc.2009.04.009

2. Jabbour E, O'Brien S, Konopleva M, Kantarjian H. New insights into the pathophysiology and therapy of adult acute lymphoblastic leukemia. *Cancer* (2015) 121(15):2517–28. doi: 10.1002/cncr.29383

3. Duffield AS, Mullighan CG, Borowitz MJ. International consensus classification of acute lymphoblastic leukemia/lymphoma. *Virchows Arch* (2023) 482(1):11–26. doi: 10.1007/s00428-022-03448-8

4. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. *CA Cancer J Clin* (2014) 64(2):83–103. doi: 10.3322/caac.21219

5. Bhojwani D, Yang JJ, Pui CH. Biology of childhood acute lymphoblastic leukemia. *Pediatr Clin North Am* (2015) 62(1):47–60. doi: 10.1016/j.pcl.2014.09.004

6. Larson RA. Managing cns disease in adults with acute lymphoblastic leukemia. *Leuk Lymphoma* (2018) 59(1):3–13. doi: 10.1080/10428194.2017.1326597

7. Jemal A, Ward EM, Johnson CJ, Cronin KA, Ma J, Ryerson B, et al. Annual report to the nation on the status of cancer, 1975-2014, featuring survival. *J Natl Cancer Inst* (2017) 109(9). doi: 10.1093/jnci/djx030

8. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: A comprehensive review and 2017 update. *Blood Cancer J* (2017) 7(6):e577. doi: 10.1038/bcj.2017.53

9. Shah A, John BM, Sondhi V. Acute lymphoblastic leukemia with treatment–naive fanconi anemia. *Indian Pediatr* (2013) 50(5):508–10.

10. German J. Bloom's syndrome. Xx. The first 100 cancers. *Cancer Genet Cytogenet* (1997) 93(1):100–6. doi: 10.1016/s0165-4608(96)00336-6

11. Bielorai B, Fisher T, Waldman D, Lerenthal Y, Nissenkorn A, Tohami T, et al. Acute lymphoblastic leukemia in early childhood as the presenting sign of ataxia-telangiectasia variant. *Pediatr Hematol Oncol* (2013) 30(6):574–82. doi: 10.3109/08880018.2013.777949

12. Lai R, Hirsch-Ginsberg CF, Bueso-Ramos C. Pathologic diagnosis of acute lymphocytic leukemia. *Hematol Oncol Clin North Am* (2000) 14(6):1209–35. doi: 10.1016/s0889-8588(05)70183-0

13. Chiaretti S, Zini G, Bassan R. Diagnosis and subclassification of acute lymphoblastic leukemia. *Mediterr J Hematol Infect Dis* (2014) 6(1):e2014073. doi: 10.4084/MJHID.2014.073

14. Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, et al. Proposals for the immunological classification of acute leukemias. European group for the immunological characterization of leukemias (Egil). *Leukemia* (1995) 9(10):1783–6.

15. Dese K, Raj H, Ayana G, Yemane T, Adissu W, Krishnamoorthy J, et al. Accurate machine-learning-based classification of leukemia from blood smear images. *Clin Lymphoma Myeloma Leuk* (2021) 21(11):e903–e14. doi: 10.1016/j.clml.2021.06.025

16. Thomas X, Le QH, Danaila C, Lheritier V, Ffrench M. Bone marrow biopsy in adult acute lymphoblastic leukemia: morphological characteristics and contribution to the study of prognostic factors. *Leuk Res* (2002) 26(10):909–18. doi: 10.1016/s0145-2126(02)00034-6

17. Elsabagh AA, Elhadary M, Elsayed B, Elshoeibi AM, Ferih K, Kaddoura R, et al. Artificial intelligence in sickle disease. *Blood Rev* (2023) 61:101102. doi: 10.1016/j.blre.2023.101102

18. Ferih K, Elsayed B, Elshoeibi AM, Elsabagh AA, Elhadary M, Soliman A, et al. Applications of artificial intelligence in thalassemia: A comprehensive review. *Diagnostics (Basel)* (2023) 13(9):1551. doi: 10.3390/diagnostics13091551

19. Elhadary M, Elsabagh AA, Ferih K, Elsayed B, Elshoeibi AM, Kaddoura R, et al. Applications of machine learning in chronic myeloid leukemia. *Diagnostics (Basel)* (2023) 13(7):1330. doi: 10.3390/diagnostics13071330

20. Elsayed B, Elshoeibi AM, Elhadary M, Ferih K, Elsabagh AA, Rahhal A, et al. Applications of artificial intelligence in philadelphia-negative myeloproliferative neoplasms. *Diagnostics (Basel)* (2023) 13(6):1123. doi: 10.3390/diagnostics13061123

21. Elshoeibi AM, Ferih K, Elsabagh AA, Elsayed B, Elhadary M, Marashi M, et al. Applications of artificial intelligence in thrombocytopenia. *Diagnostics (Basel)* (2023) 13(6):1060. doi: 10.3390/diagnostics13061060

22. Alzubaidi L, Zhang J, Humaidi AJ, Al-Dujaili A, Duan Y, Al-Shamma O, et al. Review of deep learning: concepts, cnn architectures, challenges, applications, future directions. *J Big Data* (2021) 8(1):53. doi: 10.1186/s40537-021-00444-8

23. Chan HP, Samala RK, Hadjiiski LM, Zhou C. Deep learning in medical image analysis. *Adv Exp Med Biol* (2020) 1213:3–21. doi: 10.1007/978-3-030-33128-3_1

24. Litjens G, Kooi T, Bejnordi BE, Setio AAA, Ciompi F, Ghafoorian M, et al. A survey on deep learning in medical image analysis. *Med Image Anal* (2017) 42:60–88. doi: 10.1016/j.media.2017.07.005

25. Das PK, Meher S, Panda R, Abraham A. An efficient blood-cell segmentation for the detection of hematological disorders. *IEEE Trans Cybern* (2022) 52(10):10615–26. doi: 10.1109/TCYB.2021.3062152

26. Raina R, Gondhi NK, Chaahat, Singh D, Kaur M, Lee H-N. A systematic review on acute leukemia detection using deep learning techniques. *Arch Comput Methods Eng* (2023) 30(1):251–70. doi: 10.1007/s11831-022-09796-7

27. Salah HT, Muhsen IN, Salama ME, Owaidah T, Hashmi SK. Machine learning applications in the diagnosis of leukemia: current trends and future directions. *Int J Lab Hematol* (2019) 41(6):717–25. doi: 10.1111/ijlh.13089

28. Clark JM, Sanders S, Carter M, Honeyman D, Cleo G, Auld Y, et al. Improving the translation of search strategies using the polyglot search translator: A randomized controlled trial. *J Med Libr Assoc* (2020) 108(2):195–207. doi: 10.5195/jmla.2020.834

29. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev* (2016) 5(1):210. doi: 10.1186/s13643-016-0384-4

30. Devi TG, Patil N, Rai S, Sarah CP. Segmentation and classification of white blood cancer cells from bone marrow microscopic images using duplet-convolutional neural network design. *Multimedia Tools Appl* (2023) 82(23):1–23. doi: 10.1007/s11042-023-14899-9

31. Duggal R, Gupta A, Gupta R, Mallick P. (2017). Sd-layer: stain deconvolutional layer for cnns in medical microscopic imaging, In: *Medical image computing and computer assisted intervention – MICCAI 2017*. (Cham, Switzerland: Springer) 10435:435–43. doi: 10.1007/978-3-319-66179-7_50

32. Huang F, Guang P, Li F, Liu X, Zhang W, Huang W. Aml, all, and cml classification and diagnosis based on bone marrow cell morphology combined with convolutional neural network: A stard compliant diagnosis research. *Med (Baltimore)* (2020) 99(45):e23154. doi: 10.1097/md.0000000000023154

33. Ikechukwu AV, Murali S. I-net: A deep cnn model for white blood cancer segmentation and classification. *Int J Advanced Technol Eng Explor* (2022) 9(95):17. doi: 10.19101/IJATEE.2021.875564

34. Kavitha R, Viswanathan N. Cat-inspired deep convolutional neural network for bone marrow cancer cells detection. *Intelligent Automation Soft Computing* (2022) 33 (2):16. doi: 10.32604/iasc.2022.022816

35. Kumar D, Jain N, Khurana A, Mittal S, Satapathy SC, Senkerik R, et al. Automatic detection of white blood cancer from bone marrow microscopic images using convolutional neural networks. *IEEE Access* (2020) 8:142521–31. doi: 10.1109/access.2020.3012292

36. Ordaz-Gutierrez S, Gallegos-Funes FJ, Rosales-Silva AJ, Carvajal-Gamez BE, Mujica-Vargas D. Diagnosis of acute lymphoblastic leukaemia using fuzzy logic and neural networks. *Imaging Sci J* (2013) 61(1):8. doi: 10.1179/1743131X11Y.0000000057

37. Rehman A, Abbas N, Saba T, Rahman SIU, Mehmood Z, Kolivand H. Classification of acute lymphoblastic leukemia using deep learning. *Microsc Res Tech* (2018) 81(11):1310–7. doi: 10.1002/jemt.23139

38. Yang G, Qin Z, Mu J, Mao H, Han M. Efficient diagnosis of hematologic Malignancies using bone marrow microscopic images: A method based on multipathgan and mobilevitv2. *Comput Methods Programs BioMed* (2023) 237:107583. doi: 10.1016/j.cmpb.2023.107583

39. Zhou M, Wu KF, Yu LS, Xu MD, Yang JJ, Shen Q, et al. Development and evaluation of a leukemia diagnosis system using deep learning in real clinical scenarios. *Front Pediatr* (2021) 9:693676. doi: 10.3389/fped.2021.693676

40. Clark K, Vendt B, Smith K, Freymann J, Kirby J, Koppel P, et al. The cancer imaging archive (Tcia): maintaining and operating a public information repository. *J Digit Imaging* (2013) 26(6):1045–57. doi: 10.1007/s10278-013-9622-7

41. Chu S-C, Tsai P-w, Pan J-S. Cat swarm optimization, in: *PRICAI 2006: trends in artificial intelligence*. Berlin, Heidelberg: Springer (2006). p. 854–8.

42. Das PK DVA, Meher S, Panda R, Abraham A. A systematic review on recent advancements in deep and machine learning based detection and classification of acute lymphoblastic leukemia. *IEEE Access* (2022) 10:81741–63. doi: 10.1109/ACCESS.2022.3196037

43. Mustaqim T, Fatichah C, Suciati N. Deep learning for the detection of acute lymphoblastic leukemia subtypes on microscopic images: A systematic literature review. *IEEE Access* (2023) 11:16108–27. doi: 10.1109/ACCESS.2023.3245128

44. Alsalem MA, Zaidan AA, Zaidan BB, Hashim M, Madhloom HT, Azeez ND, et al. A review of the automated detection and classification of acute leukaemia: coherent taxonomy, datasets, validation and performance measurements, motivation, open challenges and recommendations. *Comput Methods Programs BioMed* (2018) 158:93–112. doi: 10.1016/j.cmpb.2018.02.005

45. Abunadi I, Senan EM. Multi-method diagnosis of blood microscopic sample for early detection of acute lymphoblastic leukemia based on deep learning and hybrid techniques. *Sensors (Basel)* (2022) 22(4):1629. doi: 10.3390/s22041629

46. Sampathila N, Chadaga K, Goswami N, Chadaga RP, Pandya M, Prabhu S, et al. Customized deep learning classifier for detection of acute lymphoblastic leukemia using blood smear images. *Healthcare (Basel)* (2022) 10(10):1812. doi: 10.3390/healthcare10101812

47. Atteia G, Alhussan AA, Samee NA. Bo-allcnn: bayesian-based optimized cnn for acute lymphoblastic leukemia detection in microscopic blood smear images. *Sensors (Basel)* (2022) 22(15):5520. doi: 10.3390/s22155520

48. Rastogi P, Khanna K, Singh V. Leufeatx: deep learning-based feature extractor for the diagnosis of acute leukemia from microscopic images of peripheral blood smear. *Comput Biol Med* (2022) 142:105236. doi: 10.1016/j.compbiomed.2022.105236

49. Deshpande NM, Gite S, Aluvalu R. A review of microscopic analysis of blood cells for disease detection with ai perspective. *PeerJ Comput Sci* (2021) 7:e460. doi: 10.7717/peerj-cs.460

50. Anilkumar KK, Manoj VJ, Sagi TM. Automated detection of leukemia by pretrained deep neural networks and transfer learning: A comparison. *Med Eng Phys* (2021) 98:8–19. doi: 10.1016/j.medengphy.2021.10.006

51. Boldu L, Merino A, Acevedo A, Molina A, Rodellar J. A deep learning model (Alnet) for the diagnosis of acute leukaemia lineage using peripheral blood cell images. *Comput Methods Programs BioMed* (2021) 202:105999. doi: 10.1016/j.cmpb.2021.105999

52. Ouyang N, Wang W, Ma L, Wang Y, Chen Q, Yang S, et al. Diagnosing acute promyelocytic leukemia by using convolutional neural network. *Clin Chim Acta* (2021) 512:1–6. doi: 10.1016/j.cca.2020.10.039

53. Gupta R, Gehlot S, Gupta A. C-nmc: B-lineage acute lymphoblastic leukaemia: A blood cancer dataset. *Med Eng Phys* (2022) 103:103793. doi: 10.1016/j.medengphy.2022.103793

54. Gehlot S, Gupta A, Gupta R. Sdct-auxnet(Theta): dct augmented stain deconvolutional cnn with auxiliary classifier for cancer diagnosis. *Med Image Anal* (2020) 61:101661. doi: 10.1016/j.media.2020.101661

55. Shafique S, Tehsin S. Acute lymphoblastic leukemia detection and classification of its subtypes using pretrained deep convolutional neural networks. *Technol Cancer Res Treat* (2018) 17:1533033818802789. doi: 10.1177/1533033818802789

56. Hamza MA, Albraikan AA, Alzahrani JS, Dhahbi S, Al-Turaiki I, Al Duhayyim M, et al. Optimal deep transfer learning-based human-centric biomedical diagnosis for acute lymphoblastic leukemia detection. *Comput Intell Neurosci* (2022) 2022:7954111. doi: 10.1155/2022/7954111

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 06 December 2023
DOI 10.3389/fonc.2023.1308869

OPEN ACCESS

EDITED BY
Gabor Mikala,
Central Hospital of Southern Pest, Hungary

REVIEWED BY
Ferenc Uher,
Central Hospital of Southern Pest, Hungary
Francesca Vinchi,
New York Blood Center, United States

*CORRESPONDENCE
Weiying Feng
fengweiying1997@126.com

RECEIVED 07 October 2023
ACCEPTED 24 November 2023
PUBLISHED 06 December 2023

CITATION
Chen Z, Zheng S, Han J, Fu L, Fu J, Zhang Z, Hong P and Feng W (2023) Molecular mechanisms of ferroptosis and its roles in leukemia. *Front. Oncol.* 13:1308869. doi: 10.3389/fonc.2023.1308869

Molecular mechanisms of ferroptosis and its roles in leukemia

Zhe Chen, Suying Zheng, Jiongping Han, Leihua Fu, Jiaping Fu, Zhijian Zhang, Pan Hong and Weiying Feng*

Department of Hematology, Shaoxing People's Hospital (Shaoxing Hospital, Zhejiang University School of Medicine), Shaoxing, China

Cell death is a complex process required to maintain homeostasis and occurs when cells are damage or reach end of life. As research progresses, it is apparent that necrosis and apoptosis do not fully explain the whole phenomenon of cell death. Therefore, new death modalities such as autophagic cell death, and ferroptosis have been proposed. In recent years, ferroptosis, a new type of non-apoptotic cell death characterized by iron-dependent lipid peroxidation and reactive oxygen species (ROS) accumulation, has been receiving increasing attention. Ferroptosis can be involved in the pathological processes of many disorders, such as ischemia-reperfusion injury, nervous system diseases, and blood diseases. However, the specific mechanisms by which ferroptosis participates in the occurrence and development of leukemia still need to be more fully and deeply studied. In this review, we present the research progress on the mechanism of ferroptosis and its role in leukemia, to provide new theoretical basis and strategies for the diagnosis and treatment of clinical hematological diseases.

KEYWORDS

programmed cell death, ferroptosis, leukemia, reactive oxygen species, immunotherapy, iron metabolism

1 Introduction

The term "ferroptosis" was coined in 2012, when screens for small-molecule compounds capable of inhibiting the growth of RAS-mutant cancer cells were performed. In the 1950s, Harry Eagle et al. found that cysteine-deficient cells had a different pattern of cell death than those caused by other amino acid deficiencies. In the 1970s, a cysteine-dependent liver cell death involving glutathione (GSH) depletion was reported. At the same time, Shiro et al. found that alpha-tocopherol, an inhibitor of lipid peroxidation, saved cell death from GSH and cysteine deficiency. Ursini et al. isolated an enzyme named glutathione peroxidase 4 (GPX4) in 1982, which can inhibit iron-catalyzed lipid peroxidation. GPX4 protects cell death related to lipid peroxidation and oxidative stress. Dolma et al. discovered in 2003 that a small molecule compound named erastin

could target the inhibition of RAS expressing tumor cells. Erastin induced death cell showed no apoptotic features and could not be inhibited by apoptosis inhibitors, suggesting new non-apoptotic cell death form. In 2012, Dixon et al. coined the term "ferroptosis" as erastin induced cell death. Ferroptosis refers to an iron-dependent form of regulatory cell death caused by lipid peroxide overload on the cell membrane. This is a new kind of cell death, which is different from the traditional forms of autophagy, apoptosis, necrosis, and other cell death. Morphologically, mitochondrial volume decreases, density increases, mitochondrial crest disappears, and lipid reactive oxygen species (ROS) increases in the cytoplasm (1). The fatal accumulation of lipid peroxides is a fundamental feature of ferroptosis and involves the confrontation between ferroptosis production and ferroptosis defense systems in cells. Ferroptosis occurs when its promotion of cellular activity significantly exceeds the antioxidant buffer provided by the ferroptosis defense system (2–4).

Ferroptosis is affected by a range of different genes including multiple cancer-related signaling pathways which have been shown to participate in ferroptosis. For example, p53 and BRCA1-related protein 1 (BAP1) induce ferroptosis in tumor cells through multiple signaling pathways, which act as a natural barrier to cancer development (5, 6). Oncogene-mediated or oncogene-signal-mediated ferroptosis avoidance contributes to tumor occurrence, progression, metastasis, and treatment resistance regulation (7, 8). Conversely, the unique metabolism of cancer cells, their high load of ROS, and their specific mutations make some of these cells inherently susceptible to ferroptosis, thus exposing therapeutic targets for certain cancer types (9–11). With the continuous development of research, ferroptosis has been confirmed to be closely related to the occurrence of tumors, respiratory system, cardiovascular system, nervous system, ischemia reperfusion injury, and other diseases. Recent studies have shown that ferroptosis also plays an important role in the development and progression of hematological diseases, especially leukemia. The present study mainly describes the role of ferroptosis in leukemia, research progress and provides new targets and new ideas for the diagnosis and treatment of leukemia.

2 Mechanism of ferroptosis

Ferroptosis is caused by the accumulation of lipid peroxidation, leading to the destruction of membrane structures. The prerequisite for ferroptosis is polyunsaturated fatty acids -containing phospholipids (PUFA-PLs) synthesis with peroxidation. Sensitivity to ferroptosis is regulated by several factors, including GSH and REDOX regulatory systems, such as System Xc-, GPX4 regulation, CoQ10-NAD (P) pathway, glutamine metabolic pathway, and NRF2 regulation (Figure 1). In this section, there are mainly describes Ferroptosis prerequisites, Ferroptosis defense mechanisms, and Upregulation of ferroptosis defenses.

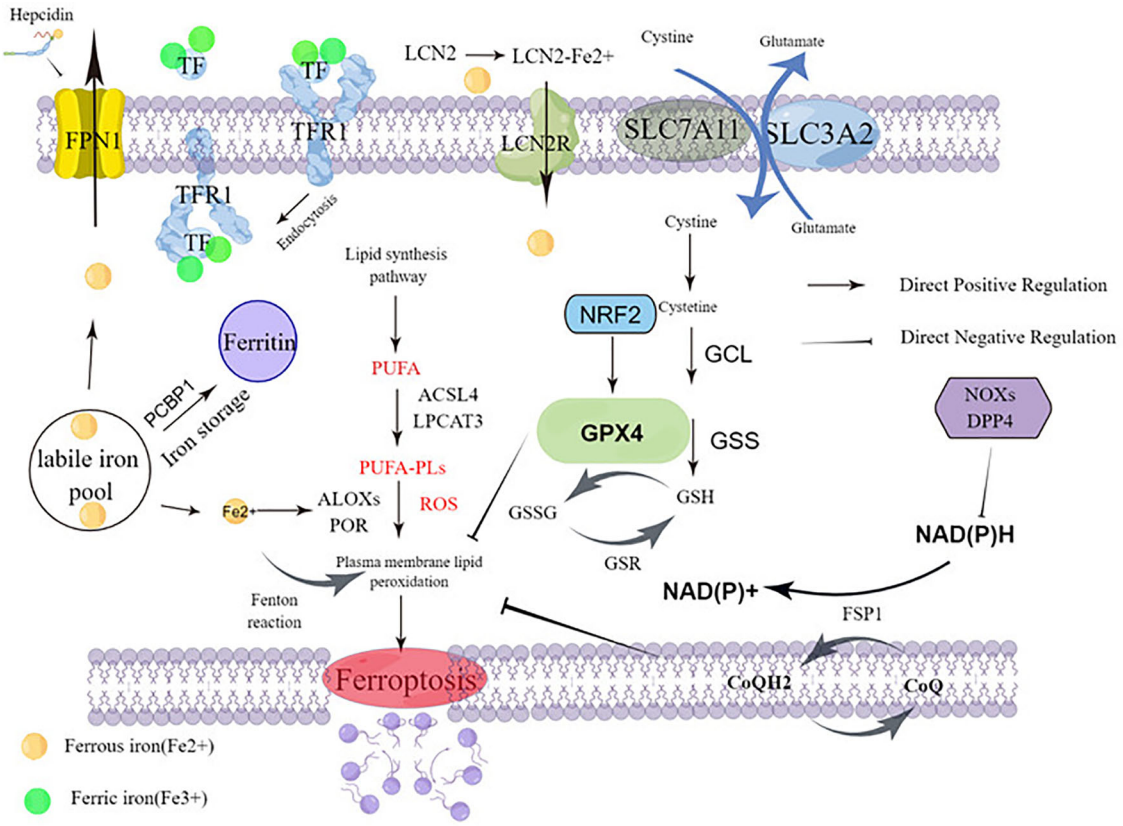

FIGURE 1
Molecular mechanisms of ferroptosis.

2.1 Ferroptosis prerequisites

The crux of ferroptosis execution is PUFA-PLs synthesis with peroxidation. As outlined in this section, PUFA-PL synthesis and peroxidation, Iron metabolism, and Mitochondrial metabolism constitute the main prerequisites driving ferroptosis.

PUFA-PL synthesis and peroxidation: The key to triggering ferroptosis is the catalytic oxidation of phospholipids containing PUFA into polyunsaturated fatty acids, which leads to the fatal accumulation of lipid peroxides on the cell membrane and subsequent membrane rupture, resulting in ferroptosis. It is the main prerequisite of ferroptosis. Acyl-coenzyme A (CoA) synthetase long chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3) are critical mediators of PUFA-PL synthesis (12, 13). ACSL4 and LPCAT3 play an important role in the biosynthesis and remodeling of phosphatidylethanolamine (PE), which can activate PUFAs and affect transmembrane properties (14). ACSL4 catalyzes the linking of free PUFAs, which include arachidonic and adrenal acid to CoA forming PUFA-CoAs such as arachidonic acid-CoA or adrenal acid -CoA. These products are then subsequently re-esterified by LPCAT3 and incorporated into PLs to form PUFA-PLs which include arachidonic acid-phosphatidylethanolamine or adrenic acid-phosphatidylethanolamine (Figure 1).

Acetyl-coa carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to produce malonyl CoA, which is required for synthesis of some PUFAs (15, 16). Human cytochrome P450 redox reductase (POR) mediated or arachidonic lipoxygenase (ALOXs) mediated enzymatic reactions have also been shown to promote lipid peroxidation (17, 18) (Figure 1). POR's ability to promote lipid peroxidation appears to be indirect, through the production of H2O2 (18). The ALOX gene plays an important role in driving ferroptosis. The mammalian ALOX family, consisting of six members (ALOXE3, ALOX5, ALOX12, ALOX12B, ALOX15, and ALOX15B), which play a context-dependent role in driving ferroptosis. For example, spermidine/spermidine n1-acetyltransferase 1 (SAT1), a target gene for tumor protein p53 (TP53), mediates the expression of ALOX15 (but not ALOX5 and ALOX12) and is involved in TP53-mediated ferroptosis (3). Interestingly, other studies have cast doubt on the role of ALOX genes in ferroptosis (19). ALOX12 does not depend on GPX4 and ALOX15 binds to phosphatidylethanolamine binding protein 1 (PEBP1), mediating RSL3-induced ferroptosis in bronchial epithelial cells, renal epithelial cells, and neuronal cells (20).

Iron metabolism: Iron is a key nutrient involved in ATP production via the mitochondrial chain complex, DNA synthesis in the process of ribonucleic acid reductase, oxygen transport, antioxidant defense (peroxidase and catalase), oxygen sensitive factors such as hypoxia-inducer factor-HiIF - and proline hydroxylase, and many other enzymes. Nutrient iron exists mainly as iron ions, which can be reduced by iron reductase. Systemic iron homeostasis is maintained by a balance of iron uptake, recycling, and loss. Iron mainly comes from food intake and the elimination of aging red blood cells, existing as Fe2+ and Fe3+. Ferrous ions are internalized into intestinal cells by active transport mechanisms in the gastrointestinal tract. Iron can also be internalized into the blood through the basolateral membrane through ferroportin 1 (FPN1; the only known iron exporter), iron through the binding of ferriferous carriers to lipidin-2 (LCN2), and subsequent endocytosis returned into the cell (21, 22). However, Fe3+ combines with transferrin (TF) on the cell membrane to form TF-Fe3+, which is finally combined with transferrin receptor 1 (TFR1) and is swallowed *in vivo*. Excess iron is stored in the liver primarily through ferritin (FTH and FTL). High iron levels cause the liver system to secrete hepcidin, the most relevant regulator of iron metabolism in the system. Hepcidin is a protein of iron transport from cells which binds to ferritin transporters on iron-storage cells such as intestinal epithelial cells and macrophages. This leads to internalization and degradation of the hepcidin-transporter complex, which effectively shuts down nutrient iron uptake and iron release from internal iron stores. Expression of hepcidin is controlled by a regulatory feedback mechanism of active erythropoiesis: erythropoiet-derived erythroferone (ERFE), growth differentiation factor 11 (GDF11), growth differentiation factor 15 (GDF15), and twisted gastrin protein homology 1 (TWSG1) have been shown to affect liver hepcidin secretion. Interestingly, leukemia cells require more iron than normal cells. In particular, cancer patients require a large number of red blood cell transfusions due to normal dyserythrogenesis and anemia caused by chemotherapy, and excess iron is common in leukemia patients.

Excess iron and reactive oxygen species (ROS) catalyze production and promote malignant transformation of hematopoietic stem cells through niacinamide adenine dinucleotide phosphate oxidase (NOX) and subsequent glutathione (GSH) consumption (23).

Iron's ability to gain and lose electrons between its oxidized Fe3+ and Fe2+ forms allow it to participate in radical generation reactions. Among these processes is the Fenton reaction, where ferrous iron contributes an electron to hydrogen peroxide to produce hydroxyl radicals that induce ROS production. Abnormal iron accumulation and subsequent excess ROS levels produce oxidative stress, induce DNA, protein, or lipid damage, and even lead to cell death. It is important to note that these oxidation actions of iron can promote the development of tumors and are thought to be necessary for the development of cancer (24). Nuclear receptor coactivator 4 (NCOA4) is the target of the ferritin transporter, which mediates ferritinophagy, a selective autophagy that degrades ferritin by lysosomes. Selective autophagy degrades ferritin. Ferritinophagy increases free iron in cells. Iron pools can be coated by lysosomes via NCOA4, and then degrade and release a large amount of Fe2+, which increases the sensitivity of cells to ferroptosis (25). Inhibition of ferritin macrophages mediated by NCOA4 increases iron storage and limits iron cell apoptosis in ferroptosis (25, 26)(Figure 1).

Mitochondrial metabolism: Overexpression of ferritin mitochondria (FTMT), an iron-storage protein in mitochondria whose primary function is to provide energy for cells through oxidative phosphorylation, is a major site of iron metabolism and ROS production which inhibits erastin-induced ferroptosis in neuroblastoma cells (27). This suggests that FTMT has a widespread anti-iron declining effect. Iron chaperone PCBP1 delivers Fe2+ to ferritin, thereby limiting ferroptosis in hepatocytes (28). The role of mitochondria in the biosynthetic

pathway of cell metabolism also contributes to ferroptosis. Ferroptosis requires tricarboxylic acid (TCA) cycling (29) and various non-fusion reactions in mitochondria. These reactions may drive ferroptosis by promoting ROS, ATP, and/or PUFA-PL production (30, 31).

2.2 Ferroptosis defense mechanisms

The imbalance between injury and defense signals eventually leads to cell death. Ferroptosis defence mechanisms involve cellular antioxidant systems that directly neutralize lipid peroxides. As discussed below, there are mainly introduction the following two systems.

The GPX4–GSH system: GPX4 belongs to the GPX protein family (32, 33) and is the only GPX member capable of converting PL hydroperoxides into PL alcohols (34, 35). GPX4 is a key regulator of ferroptosis, and inhibition of its activity leads to the accumulation of lipid peroxides in cells, which signal ferroptosis in cells. Down-regulation of GPX4 increased susceptibility to ferroptosis, while up-regulation inhibited ferroptosis (36). GPX4 consists of three subtypes with different subcellular localization, namely cytoplasmic GPX4, mitochondrial GPX4, and nuclear GPX4. These isomers are encoded by the same GPX4 gene and have different transcription start sites, resulting in the n-terminal GPX4 protein of the mitochondrial or nuclear localization sequence. Only cytoplasmic GPX4 has a protective effect against ferroptosis (37). Cytoplasmic GPX4 re-expression significantly inhibited GPX4-deletion induced cell death in mouse embryonic fibroblasts. The expression or activity of GPX4 is controlled by selenium and glutathione (38, 39). Reducing glutathione (GSH), for GPX4 is a thiol-containing tripeptide derived from glycine, glutamic acid, and cysteine, of which cysteine is the rate-limiting precursor. GSH is the most abundant reducing agent in mammalian cells and is a cofactor of many enzymes. For glutathione synthesis, it is mainly formed by the redox of cysteine through the xc-/cystine/glutamate transporter (Figure 1).

Cystine glutamate transporter (system Xc-) is an amino acid reverse transporter widely distributed in the phospholipid bilayer and is an important part of the cellular antioxidant system. System Xc- is composed of solute carrier family 7 member 11 (SLC7A11) solute carrier family 3 member 2 (SLC3A2), an amino acid reverse transporter that can transfer cystine into cells and glutamate 1:1. Most cancer cells acquire intracellular cysteine-mediated cystine uptake (the oxydimer form of cysteine) primarily through the Xc- system, followed by reduction of cystine to cysteine in the cytoplasm (40, 41). Through the exchange of system Xc- (40), cysteine and glutamic acid are transported inside and outside the cell, and then participate in the synthesis of GSH. Inhibition of cysteine absorption can inhibit the activity of system Xc-, which can affect the synthesis of GSH and ultimately inhibit the activity of GPX, leading to the decline of cellular antioxidant capacity, lipid ROS accumulation, and inducing ferroptosis. Overexpression of apoptosis-inducing factor associated 2 (AIFM2) may eliminate GPX4 to inhibit ferroptosis (42) (Figure 1).

Seven members of solute vector family 11 (SLC7A11; Also called xCT) (43) is a transporter subunit in the system Xc-. The expression or activity of SLC7A11 is regulated by many factors, such as TP53 (6), NRF2 (44), BRCA1-related protein 1 (BAP1) (5), and BECN1 (45). Inhibition of SLC7A11 by small molecule compounds (such as erastin) can cause glutathione depletion to trigger ferroptosis (46). After GPX4 inactivation (10), some cancer cell lines remain resistant to ferroptosis, suggesting the presence of additional ferroptosis defense mechanisms (Figure 1).

CoQH2 system: Some recent studies suggest that ferroptosis defense systems can be divided into two main parts, GPX4 system and CoQH2 system. CoQH2 is an endogenous ferroptosis inhibitor, which has antioxidant effect in cell membrane and can reduce the oxidative damage of cell membrane. In addition to GPX4 system, DHODH/FSP1/CoQ pathway is another key inhibitory mechanism for lipid peroxidation and ferroptosis. DHODH is an enzyme involved in pyrimidine synthesis, which can reduce ubiquinone (CoQ) to ubiquinol (CoQH2) in the mitochondrial inner membrane. When GPX4 is dramatically inactivated, the flux through DHODH (3) increases significantly, leading to enhanced CoQH2 production, neutralizing lipid peroxidation, and defense against ferroptosis in mitochondria. The inactivation of mitochondrial GPX4 and DHODH releases powerful mitochondrial lipid peroxidation and triggers intense ferroptosis. Cytoplasmic GPX4 was also found to be significantly localized in the mitochondrial membrane gap (37) (Figure 1).

As a major suppressor of ferroptosis, FSP1 was originally described as a p53 response gene and therefore was originally called p53 response gene 3 (PRG3). FSP1, also known as AIFM2, is localized in the plasma membrane (as well as other subcellular compartments), and its plasma membrane localization appears to be necessary and sufficient to function FSP1's role in inhibiting ferroptosis (47, 48). Doll et al. and Bersuker et al. found that FSP1 inhibits lipid peroxidation and ferroptosis by reducing CoQ (or its partially oxidized product hemihydroquinone) to CoQ2. This may directly reduce lipid radicals to terminate lipid autoxidation, or indirectly via regenerating oxidized α-tocopheryl radical (Vitamin E), a powerful natural antioxidant (47, 48). FSP1 acts as a NADPH-dependent CoQ redox enzyme, and can catalyze CoQ10 regeneration depending on NADPH, thereby improving the ability of free radical capture to protect cells, and also has a protective effect against ferroptosis caused by GPX4 deletion. This protective effect of CoQ reveals why some cells and tissues, such as highly metabolically active liver cells, contain large amounts of extracellular CoQ, which is inconsistent with its typical role in the mitochondrial electron transport chain. CoQ is synthesized mainly in the mitochondria (49), but detected in non-mitochondrial membranes, including the plasma membrane (50). Unanswered questions remain about the potential role of other CoQH2 producing mitochondrial enzymes in the regulation of ferroptosis.

2.3 Upregulation of ferroptosis defenses

Despite the prevailing importance of GPX4 and CoQH2 for limiting ferroptosis, both the signal pathways and the tumor

microenvironment influence the function of ferroptosis in tumorigenesis and tumor therapy.This section focuses on the role of these two pathways that inhibit ferroptosis.

Hippo–YAP signaling in ferroptosis: The Hippo -Yap pathway is involved in a variety of biological functions, including cell proliferation and organ size control (51). Wu et al. demonstrated the role of intercellular interactions and intracellular NF2-YAP signaling in dictating ferroptosis, which can promote the survival of GPX4 knockout cells (9). Because YAP targets include several regulators of ferroptosis, including transferrin receptors ACSL4, TFR1, and possibly other genes, susceptibility to ferroptosis depends on Hippo pathway activity, with increased susceptibility in response to Hippo inhibition and YAP activation (9). Yang et al. found that in renal cell carcinoma (RCC), Transcription Regulator 1 (TAZ) is abundantly expressed and regulates ferroptosis through Epithelial Membrane Protein 1 (EMP1)-NOX4 (52).

Nuclear factor E2 erythroid 2-like-2 (NRF2): The NRF2 transcription pathway can up-regulate the expression of antioxidant genes or cell protective genes in various oxidative stress processes. As a major regulator of antioxidant defense, transcription factor NRF2 (53, 54) controls the transcription of many genes involved in GPX4-GSH-mediated ferroptosis defense. Sun et al. report that NRF2 plays a central role in protecting hepatocellular carcinoma (HCC) cells against ferroptosis, and NRF2 signaling is up-regulated in many human cancer types (55) (Figure 1).

Ferroptosis in the tumor microenvironment: Recent studies have also shown that the tumor microenvironment (TME), which is a multicellular environment, includes the extracellular matrix, immune cells, blood vessels, tumor cells, and other cells. In particular, immune cells determine whether ferroptosis in tumor cells will occur. CD8+ cytotoxic T cells are the main agents of anti-tumor immunity in the TME (56), secreting interferon-γ (IFN-γ) and subsequently inhibiting cystine uptake by cancer cells via down-regulation of SLC7A11 expression, thereby increasing lipid peroxidation and ferroptosis in tumors. Ferroptotic cancer cells can release several immunostimulatory signals, such as high mobility group box 1 (HMGB1) (57), calreticulin (58), ATP (59), and phosphatidylethanolamine (60). These factors can promote dendritic cell maturation, increasing the efficiency of macrophages in the phagocytosis of ferroptotic cancer cells, and further enhance the infiltration of CD8+ T cells into tumors.

Immunotherapy, combined with induction of ferroptosis, is a promising therapeutic approach. Drijvers et al. (61) found that Acyl-CoA synthetase ACSL4 mediates GPX4 inhibitor-induced sensitivity changes and ferroptosis in activated CD8+ T cells. CD8+ T cells can inhibit tumor cells by inducing iron decay and pyrosis (62, 63).

3 The role of ferroptosis in leukemia

Leukemia comprises a group of heterogeneous hematopoietic stem/progenitor cell malignancies characterized by abnormal proliferation of primitive cells in the bone marrow that interfere with normal blood cell production. Its occurrence involves multiple gene changes including the transferrin receptor 1 gene, the hemochromatosis (HFE) gene, and several genes related to iron metabolism.

At present, chemotherapy, immunotherapy, and hematopoietic stem cell transplantation (HSCT) are still the main treatments for leukemia. Despite advances in treatment, the results remain disheartening. Relapse or refractory disease and resistance to chemotherapy are the main reasons for treatment failure. Overcoming drug resistance is a major challenge in cancer treatment. Combination therapy prevents drug resistance by combining drugs with different targets, modes of action, and distribution of side effects in the body to reduce toxicity (64).

As a newly discovered programmed cell death mode, ferroptosis is regulated by multiple pathways such as lipid metabolism, mitochondrial metabolism and iron metabolism. Through this new death mode, it provides a new idea for improving the prognosis of leukemia patients. However, leukemia cells seem to be able to escape oxidative stress and reduce ferroptosis through some mechanisms.

3.1 Acute myeloid leukemia

Acute myeloid leukemia (AML) is a clonal hematopoietic disease caused by a variety of genetic and epigenetic impairments, characterized by impaired differentiation and uncontrolled proliferation, with varying prognoses (65). The incidence of AML increases with age, with a mortality rate of over 90% (66) at diagnosis after age 65. Ferroptosis provides a new idea for the treatment of AML, and a variety of drugs have been shown to induce ferroptosis.

Sorafenib has been approved as a tyrosine kinase inhibitor for the treatment of liver, kidney, and thyroid cancers for more than 15 years, and has recently been shown to be effective in AML patients with FLT3-ITD mutations (67). Concurrently, it also inhibits system Xc- and thus induces ferroptosis (46). Imatinib Mesylate (IMA) (68)down-regulates the expression of NRF2 and up-regulates the expression of p53 and TFR. These results provided compelling evidence that ferroptosis participates in IMA-induced cardiotoxicity. Ferroptosis could be regarded as a target to protect against cardiotoxicity in IMA-exposed patients.

APR-246 (69) is a novel drug for the treatment of TP53-mutant AML. Its main mechanism of action is to promote the binding of p53 mutants to DNA targets to reactivate the transcriptional activity of p53 and exert tumor inhibitory effects. APR-246 (70) increases oxidative stress by depleting GSH and inhibiting thioredoxin reductase, leading to the accumulation of ROS and further promoting tumor cell death. Birsen et al. (71) found that the observed early p53-independent cell death induced by APR-246 is ferroptosis.

NEAT1 (72) is bound to cytoplasmic disheveled 2 (DVL2) and tripartite motif containing 56 (TRIM56), which promotes the degradation of DVL2 and inhibits Wnt signaling, inhibiting the self-renewal of AML stem cells. Zhang et al. (73) found that ferroptosis inducers erastin and RSL3 increased NEAT1

expression by promoting the binding of p53 to the NEAT1 promoter. Induced NEAT1 promoted the expression of MIOX by competitively binding to miR-362-3p. MIOX increased ROS production and decreased the intracellular levels of NADPH and GSH, resulting in enhanced erastin- and RSL3-induced ferroptosis.

Aldo-keto reductase family 1 member C2 (AKR1C2), suppressor of cytokine signaling1 (SOCS1) (74), dipetidyl peptidase-4 (DPP4) (75), and human immunodeficiency virus type I enhancer-binding protein zinc finger 3(HIVEP3) (76) can be used as predictive adverse prognosis. Long non-coding RNAs (lncrnas) (77) associated with ferroptosis have also been shown to accurately predict the prognosis of AML and optimize treatment strategies for AML.

Acetaldehyde dehydrogenase 3a2 (Aldh3a2) (78) is l-gmp dependent and not seen in n-gmp. It protects AML cells from oxidative cell death, and Aldh3a2 inhibition improves leukemia outcomes *in vivo* without compromising normal hematopoiesis. Aldh3a2 inhibition combined with ferroptosis inducer or standard AML induction chemotherapy deserves further consideration as a cancer treatment.

High mobility base Box 1 (HMGB1) (79) is a transcription factor involved in the process of chromatin remodeling, DNA recombination, and repair. HMGB1 is found in the cytoplasm and, via translocation, is expressed on the cell surface membrane or diffuse in the extracellular space. This can be caused by various cellular stressors, causing HMGB1 to migrate from the nucleus to the cytoplasm in response to erastin in HL-60/NRASQ61L cell lines and acts as a positive regulator of ferroptosis, possibly enhancing resistance to anticancer therapy.

At present, a variety of drugs have been confirmed to promote or inhibit ferroptosis in AML cells, but there is still a lack of large-scale studies, and further research is still needed to support the development. There are still many challenges in the clinical application of ferroptosis in the treatment of leukemia.

3.2 Acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is a common malignant disease of the blood system, which manifests as abnormal clonal proliferation of naive or immature T and B lymphocytes. These cells will infiltrate bone marrow, blood, or other tissues and organs, causing abnormal hematopoietic function of bone marrow and immune dysfunction. Vincristine (VCR) is often used as a treatment for ALL (80). Studies have found that VCR promotes ferroptosis by enhancing the expression of lncRNA LINC00618 and inhibiting the transcription of SLC7A11, suggesting that ferroptosis is involved in the mechanism of action of VCR.

RSL3 is an inducer of ferroptosis that binds and inactivates GPX4, mediating ferroptosis regulated by GPX4 (81). Probst et al. treated ALL cell lines with RSL3 causing lipid peroxidation, ROS production, and cell death (82). Hydnocarpin D (HD) can trigger ferroptosis through the accumulation of lipid ROS and the reduction of GSH and GPX4, while the inhibition of autophagy prevents the ferroptosis (83). PAQR3 (progestin and adipoQ receptor family member 3) is involved in the occurrence of many tumors as a tumor suppressor and can inhibit the proliferation of human leukemia cells and induce cell apoptosis (84). Jin et al. found that PAQR3 inhibits cell proliferation and aggravates ferroptosis in ALL by regulating the stability of NRF2. Hong et al. (85, 86) demonstrated the critical role of ferroptosis in Philadelphia chromosome negative (Ph-neg) B-ALL patients, with sorafenib potentially improving survival in high-risk Ph-neg B-ALL patients.

Artesunate (ART), a semi-synthetic water-soluble derivative of Artemisia annua L., is a natural product extracted from artemisia annua L. Apoptosis induced by ART corresponded to the activation of caspase-8/9/3. The expression of Bcl-xL, Bcl-2, myeloid leukemia-1, survivin, X-linked apoptosis inhibitor protein, and apoptosis inhibitor 1/2 were decreased, with increased expression of Bak. ART increased the activation of intracellular ROS and DNA damage marker gamma-H2Ax. In the ATLL mouse model, intraperitoneal injection of ART reduced tumor burden (87).

Poricoic acid A (PAA) (88) strongly reduced the cell viability of T-ALL cell lines. Mitochondrial dysfunction was also elevated by PAA, along with enhanced cellular reactive oxygen species (ROS) production. PAA treatments provoked ferroptosis in T-ALL cells with reduced glutathione (GSH) levels and elevated malonaldehyde (MDA) contents. As a new mode of regulatory cell death, amplification of ferroptosis effect may be a new idea for drug development and disease treatment.

3.3 Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is a disease with different genetic characteristics and treatment responses. CLL is characterized by the cloning and proliferation of mature CD5-positive B cells in the blood, bone marrow, lymph nodes, and spleen, resulting in immune system decline, organ dysfunction, and slow progressive systemic failure and depletion.

Ferroptosis is less well studied in the CLL field. SLC7A11 is the main functional subunit of system Xc- to transport cystine into cells to synthesize GSH. Inhibition of SLC7A11 expression can induce ferroptosis. The expression of SLC7A11 is low in CLL compared to the high expression level of SLC7A11 in other systemic solid tumors. This will lead to an increase in intracellular ROS, and as CLL cells are more prone to oxidative stress, they may be sensitive to ferroptosis inducers.

Ferroptosis is an autophagy-dependent form of cell death. BECN1 affects the occurrence and progression of autophagy, and its repeated allelic deletion and expression variation have been reported in tumors (89). Gong et al. (90) proposed a novel FPS model for prognostic prediction of CLL and established nine ferroptosis genes associated with CLL prognosis.

3.4 Chronic myelogenous leukemia

Chronic myelogenous leukemia (CML) is a hematopoietic malignancy caused by reciprocal translocation of Philadelphia chromosomes 9 and 22. Ferroptosis has been less studied in the field of CML. Cysteine metabolism plays a key role in cancer cell

survival in the study of CML related fields. Cysteine deficiency has been reported to inhibit tumor growth and induce ferroptosis in pancreatic cancer cells. It has also been reported that cysteine depletion can induce ferroptosis in CML cells *in vitro*, and thioredoxin reductase 1 (TXNRD1) (91), which is related to cell redox metabolism, is a key factor regulating ferroptosis.

4 Conclusion and outlook

Ferroptosis, as a newly discovered form of programmed cell death, has a broad prospect in tumor therapy. We have systematically and comprehensively illustrated the relationship between ferroptosis and leukemia, and found that ferroptosis plays an important role in disease progression. PUFA-PL synthesis and peroxidation, intracellular ROS levels, and homeostasis of various metabolic pathways can affect cell sensitivity to ferroptosis, thus inducing ferroptosis in blood cells. Iron accumulation and lipid peroxidation may be considered as intermediate events, but they are not the final executors of ferroptosis. Leukemia cells seem to escape oxidative stress through certain mechanisms, such as the upregulation of ferroptosis defenses and ferroptosis defense mechanisms, which reduce the occurrence of ferroptosis. However, the study of ferroptosis in hematological diseases is still in the early stage, and its specific mechanism needs to be further studied. At present, most studies affect the activity of antioxidants such as GPX4 through exogenous ferroptosis inducers, causing the accumulation of ROS and thus promoting ferroptosis. There are few large studies on ferroptosis inducers in the treatment of leukemia. The pathogenesis of leukemia is complex, and often involves multiple pathways and targets. The previous multi-drug combination chemotherapy with cytotoxic drugs could easily cause serious adverse consequences such as bone marrow suppression and immune destruction. Novel target inhibitors related to ferroptosis, or their combination with existing cytotoxic agents, may further enhance the efficacy of existing single agents, delay drug resistance and improve prognosis. Although the treatment of leukemia with ferroptosis inducers is not mature at present, but it still has high research value.

In summary, we are currently in the middle of an important phase in the development of ferroptosis research. The occurrence and development of ferroptosis, its transcriptional regulation mechanism, and the development of effective regulatory targeted drugs are of utmost importance, providing a new direction for clinical diagnosis and treatment of blood diseases. New treatments based on ferroptosis will be developed and put into clinical use soon, guided by specific biomarkers and a precise assessment of a patient's background.

Author contributions

ZC: Writing – review & editing. SZ: Data curation, Writing – original draft. JH: Data curation, Writing – original draft. LF: Writing – original draft. JF: Funding acquisition, Writing – review & editing. ZZ: Methodology, Writing – review & editing. PH: Writing – original draft. WF: Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Sponsored by Zhejiang Provincial Natural Science Foundation of China (LBY23H080002), the Zhejiang Provincial Program for the Cultivation of High-level Innovative Health Talents.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, et al. Ferroptosis: A regulated cell death nexus linking metabolism, redox biology, and disease. *Cell* (2017) 171(2):273–85. doi: 10.1016/j.cell.2017.09.021

2. Ingold I, Berndt C, Schmitt S, Doll S, Poschmann G, Buday K, et al. Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis. *Cell* (2018) 172(3):409–22.e21. doi: 10.1016/j.cell.2017.11.048

3. Mao C, Liu X, Zhang Y, Lei G, Yan Y, Lee H, et al. DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. *Nature* (2021) 593(7860):586–90. doi: 10.1038/s41586-021-03539-7

4. Soula M, Weber RA, Zilka O, Alwaseem H, La K, Yen F, et al. Metabolic determinants of cancer cell sensitivity to canonical ferroptosis inducers. *Nat Chem Biol* (2020) 16(12):1351–60. doi: 10.1038/s41589-020-0613-y

5. Zhang Y, Shi J, Liu X, Feng L, Gong Z, Koppula P, et al. BAP1 links metabolic regulation of ferroptosis to tumour suppression. *Nat Cell Biol* (2018) 20(10):1181–92. doi: 10.1038/s41556-018-0178-0

6. Jiang L, Kon N, Li T, Wang SJ, Su T, Hibshoosh H, et al. Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* (2015) 520(7545):57–62. doi: 10.1038/nature14344

7. Ubellacker JM, Tasdogan A, Ramesh V, Shen B, Mitchell EC, Martin-Sandoval MS, et al. Lymph protects metastasizing melanoma cells from ferroptosis. *Nature* (2020) 585(7823):113–8. doi: 10.1038/s41586-020-2623-z

8. Zhang R, Liu Y, You J, et al. Tanshinone IIA inhibits ischemia-reperfusion-induced inflammation, ferroptosis and apoptosis through activation of the PI3K/Akt/mTOR pathway. *Hum Exp Toxicol* (2023) 42:9603271231180864. doi: 10.1177/09603271231180864

9. Wu J, Minikes AM, Gao M, Bian H, Li Y, Stockwell BR, et al. Intercellular interaction dictates cancer cell ferroptosis via NF2-YAP signalling. *Nature* (2019) 572 (7769):402–6. doi: 10.1038/s41586-019-1426-6

10. Qi D, Peng M. Ferroptosis-mediated immune responses in cancer. *Front Immunol* (2023) 14(1188365). doi: 10.3389/fimmu.2023.1188365

11. Zou Y, Henry WS, Ricq EL, Graham ET, Phadnis VV, Maretich P, et al. Plasticity of ether lipids promotes ferroptosis susceptibility and evasion. *Nature* (2020) 585 (7826):603–8. doi: 10.1038/s41586-020-2732-8

12. Kagan VE, Tyurina YY, Vlasova II, Kapralov AA, Amoscato AA, Anthonymuthu TS, et al. Redox epiphospholipidome in programmed cell death signaling: catalytic mechanisms and regulation. *Front Endocrinol* (2020) 11(628079). doi: 10.3389/fendo.2020.628079

13. Xue Q, Kang R, Klionsky DJ, Tang D, Liu J, Chen X. Copper metabolism in cell death and autophagy. *Autophagy* (2023) 19(8):2175–95. doi: 10.1080/15548627.2023.2200554

14. Bouchaoui H, Mahoney-Sanchez L, Garçon G, Berdeaux O, Alleman LY, Devos D, et al. ACSL4 and the lipoxygenases 15/15B are pivotal for ferroptosis induced by iron and PUFA dyshomeostasis in dopaminergic neurons. *Free Radical Biol Med* (2023) 195:145–157. doi: 10.1016/j.freeradbiomed.2022.12.086

15. Qi Y, Zhang X, Wu Z, Tian M, Chen F, Guan W, et al. Ferroptosis regulation by nutrient signalling. *Nutr Res Rev* (2022) 35(2):282–94. doi: 10.1017/S0954422421000226

16. Li C, Dong X, Du W, Shi X, Chen K, Zhang W, et al. LKB1-AMPK axis negatively regulates ferroptosis by inhibiting fatty acid synthesis. *Signal Transduct Target Ther* (2020) 5(1):187. doi: 10.1038/s41392-020-00297-2

17. Cui S, Simmons G Jr., Vale G, Deng Y, Kim J, Kim H, et al. FAF1 blocks ferroptosis by inhibiting peroxidation of polyunsaturated fatty acids. *Proc Natl Acad Sci U States America* (2022) 119(17):e2107189119. doi: 10.1073/pnas.2107189119

18. Yan B, Ai Y, Sun Q, Ma Y, Cao Y, Wang J, et al. Membrane damage during ferroptosis is caused by oxidation of phospholipids catalyzed by the oxidoreductases POR and CYB5R1. *Mol Cell* (2021) 81(2):355–69.e10. doi: 10.1016/j.molcel.2020.11.024

19. Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, et al. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat Cell Biol* (2014) 16(12):1180–91. doi: 10.1038/ncb3064

20. Wenzel SE, Tyurina YY, Zhao J, St Croix CM, Dar HH, Mao G, et al. PEBP1 wardens ferroptosis by enabling lipoxygenase generation of lipid death signals. *Cell* (2017) 171(3):628–41.e26. doi: 10.1016/j.cell.2017.09.044

21. Chi Y, Remsik J, Kiseliovas V, Derderian C, Sener U, Alghader M, et al. Cancer cells deploy lipocalin-2 to collect limiting iron in leptomeningeal metastasis. *Sci (New York NY)* (2020) 369(6501):276–82. doi: 10.1126/science.aaz2193

22. Jończy A, Mazgaj R, Smuda E, Żelazowska B, Kopeć Z, Starzyński RR, et al. The role of copper in the regulation of ferroportin expression in macrophages. *Cells* (2021) 10(9):2259. doi: 10.3390/cells10092259

23. Hole PS, Zabkiewicz J, Munje C, Newton Z, Pearn L, White P, et al. Overproduction of NOX-derived ROS in AML promotes proliferation and is associated with defective oxidative stress signaling. *Blood* (2013) 122(19):3322–30. doi: 10.1182/blood-2013-04-491944

24. Battaglia AM, Chirillo R, Aversa I, Sacco A, Costanzo F, Biamonte F. Ferroptosis and cancer: mitochondria meet the "Iron Maiden" Cell death. *Cells* (2020) 9(6):1505. doi: 10.3390/cells9061505

25. Chappell G, Geer M, Gatza E, Braun T, Churay T, Brisson J, et al. Maintenance sorafenib in FLT3-ITD AML following allogeneic HCT favorably impacts relapse and overall survival. *Bone Marrow Transplant* (2019) 54(9):1518–20. doi: 10.1038/s41409-019-0493-5

26. Fang Y, Chen X, Tan Q, Zhou H, Xu J, Gu Q. Inhibiting ferroptosis through disrupting the NCOA4-FTH1 interaction: A new mechanism of action. *ACS Cent Sci* (2021) 7(6):980–9. doi: 10.1021/acscentsci.0c01592

27. Wang YQ, Chang SY, Wu Q, Gou YJ, Jia L, Cui YM, et al. The protective role of mitochondrial ferritin on erastin-induced ferroptosis. *Front Aging Neurosci* (2016) 8. doi: 10.3389/fnagi.2016.00308

28. Protchenko O, Baratz E, Jadhav S, Li F, Shakoury-Elizeh M, Gavrilova O, et al. Iron chaperone poly rC binding protein 1 protects mouse liver from lipid peroxidation and steatosis. *Hepatol (Baltimore Md)* (2021) 73(3):1176–93. doi: 10.1002/hep.31328

29. Friedman JR, Nunnari J. Mitochondrial form and function. *Nature* (2014) 505 (7483):335–43. doi: 10.1038/nature12985

30. Zheng D, Liu J, Piao H, Zhu Z, Wei R, Liu K. ROS-triggered endothelial cell death mechanisms: Focus on pyroptosis, parthanatos, and ferroptosis. *Front Immunol* (2022) 13. doi: 10.3389/fimmu.2022.1039241

31. Gao M, Yi J, Zhu J, Minikes AM, Monian P, Thompson CB, et al. Role of mitochondria in ferroptosis. *Mol Cell* (2019) 73(2):354–63.e3. doi: 10.1016/j.molcel.2018.10.042

32. Wu F, Du Y, Yang J, Shao B, Mi Z, Yao Y, et al. Peroxidase-like active nanomedicine with dual glutathione depletion property to restore oxaliplatin chemosensitivity and promote programmed cell death. *ACS nano* (2022) 16(3):3647–63. doi: 10.1021/acsnano.1c06777

33. BRIGELIUS-Flohé R, Flohé L. Regulatory phenomena in the glutathione peroxidase superfamily. *Antioxid Redox Signaling* (2020) 33(7):498–516. doi: 10.1089/ars.2019.7905

34. Seibt TM, Proneth B, Conrad M. Role of GPX4 in ferroptosis and its pharmacological implication. *Free Radical Biol Med* (2019) 133:144–52. doi: 10.1016/j.freeradbiomed.2018.09.014

35. Ursini F, Maiorino M. Lipid peroxidation and ferroptosis: The role of GSH and GPx4. *Free Radical Biol Med* (2020) 152:175–185. doi: 10.1016/j.freeradbiomed.2020.02.027

36. Wei J, Xie Q, Liu X, Wan C, Wu W, Fang K, et al. Identification the prognostic value of glutathione peroxidases expression levels in acute myeloid leukemia. *Ann Trans Med* (2020) 8(11):678. doi: 10.21037/atm-20-3296

37. Gan B. Mitochondrial regulation of ferroptosis. *J Cell Biol* (2021) 220(9): e202105043. doi: 10.1083/jcb.202105043

38. Jankowski CSR, Rabinowitz JD. Selenium modulates cancer cell response to pharmacologic ascorbate. *Cancer Res* (2022) 82(19):3486–98. doi: 10.1158/0008-5472.CAN-22-0408

39. Xu C, Sun S, Johnson T, Qi R, Zhang S, Zhang J, et al. The glutathione peroxidase Gpx4 prevents lipid peroxidation and ferroptosis to sustain Treg cell activation and suppression of antitumor immunity. *Cell Rep* (2021) 35(11):109235. doi: 10.1016/j.celrep.2021.109235

40. Koppula P, Zhang Y, Zhuang L, Gan B. Amino acid transporter SLC7A11/xCT at the crossroads of regulating redox homeostasis and nutrient dependency of cancer. *Cancer Commun (London England)* (2018) 38(1):12. doi: 10.1186/s40880-018-0288-x

41. Liu X, Olszewski K, Zhang Y, Lim EW, Shi J, Zhang X, et al. Cystine transporter regulation of pentose phosphate pathway dependency and disulfide stress exposes a targetable metabolic vulnerability in cancer. *Nat Cell Biol* (2020) 22(4):476–86. doi: 10.1038/s41556-020-0496-x

42. Wei X, Yi X, Zhu XH, Jiang DS. Posttranslational modifications in ferroptosis. *Oxid Med Cell Longevity* (2020) 2020:8832043. doi: 10.1155/2020/8832043

43. Koppula P, Zhuang L, Gan B. Cystine transporter SLC7A11/xCT in cancer: ferroptosis, nutrient dependency, and cancer therapy. *Protein Cell* (2021) 12(8):599–620. doi: 10.1007/s13238-020-00789-5

44. Dong H, Qiang Z, Chai D, Peng J, Xia Y, Hu R, et al. Nrf2 inhibits ferroptosis and protects against acute lung injury due to intestinal ischemia reperfusion via regulating SLC7A11 and HO-1. *Aging* (2020) 12(13):12943–59. doi: 10.18632/aging.103378

45. Song X, Zhu S, Chen P, Hou W, Wen Q, Liu J, et al. AMPK-mediated BECN1 phosphorylation promotes ferroptosis by directly blocking system X(c)(-) activity. *Curr Biol CB* (2018) 28(15):2388–99.e5. doi: 10.1016/j.cub.2018.05.094

46. Wang L, Liu Y, Du T, Yang H, Lei L, Guo M, et al. ATF3 promotes erastin-induced ferroptosis by suppressing system Xc(). *Cell Death Differ* (2020) 27(2):662–75. doi: 10.1038/s41418-019-0380-z

47. Bersuker K, Hendricks JM, Li Z, Magtanong L, Ford B, Tang PH, et al. The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature* (2019) 575 (7784):688–92. doi: 10.1038/s41586-019-1705-2

48. Doll S, Freitas FP, Shah R, Aldrovandi M, da Silva MC, Ingold I, et al. FSP1 is a glutathione-independent ferroptosis suppressor. *Nature* (2019) 575(7784):693–8. doi: 10.1038/s41586-019-1707-0

49. Guerra RM, Pagliarini DJ. Coenzyme Q biochemistry and biosynthesis. *Trends Biochem Sci* (2023) 48(5):463–76. doi: 10.1016/j.tibs.2022.12.006

50. Santoro MM. The antioxidant role of non-mitochondrial CoQ10: mystery solved! *Cell Metab* (2020) 31(1):13–5. doi: 10.1016/j.cmet.2019.12.007

51. Wang M, Dai M, Wang D, Xiong W, Zeng Z, Guo C. The regulatory networks of the Hippo signaling pathway in cancer development. *J Cancer* (2021) 12(20):6216–30. doi: 10.7150/jca.62402

52. Yang WH, Ding CC, Sun T, Rupprecht G, Lin CC, Hsu D, et al. The hippo pathway effector TAZ regulates ferroptosis in renal cell carcinoma. *Cell Rep* (2019) 28 (10):2501–8.e4. doi: 10.1016/j.celrep.2019.07.107

53. Wei R, Zhao Y, Wang J, Yang X, Li S, Wang Y, et al. Tagitinin C induces ferroptosis through PERK-Nrf2-HO-1 signaling pathway in colorectal cancer cells. *Int J Biol Sci* (2021) 17(11):2703–17. doi: 10.7150/ijbs.59404

54. Hsieh CH, Hsieh HC, Shih FS, Wang PW, Yang LX, Shieh DB, et al. An innovative NRF2 nano-modulator induces lung cancer ferroptosis and elicits an immunostimulatory tumor microenvironment. *Theranostics* (2021) 11(14):7072–91. doi: 10.7150/thno.57803

55. Sun X, Ou Z, Chen R, Niu X, Chen D, Kang R, et al. Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. *Hepatol (Baltimore Md)* (2016) 63(1):173–84. doi: 10.1002/hep.28251

56. Wang W, Green M, Choi JE, Gijón M, Kennedy PD, Johnson JK, et al. CD8(+) T cells regulate tumour ferroptosis during cancer immunotherapy. *Nature* (2019) 569 (7755):270–4. doi: 10.1038/s41586-019-1170-y

57. Chen R, Zou J, Kang R, Tang D. The redox protein HMGB1 in cell death and cancer. *Antioxid Redox Signaling* (2023). doi: 10.1089/ars.2023.0007

58. Yu B, Choi B, Li W, Kim DH. Magnetic field boosted ferroptosis-like cell death and responsive MRI using hybrid vesicles for cancer immunotherapy. *Nat Commun* (2020) 11(1):3637. doi: 10.1038/s41467-020-17380-5

59. Efimova I, Catanzaro E, Van Der Meeren L, Turubanova VD, Hammad H, Mishchenko TA, et al. Vaccination with early ferroptotic cancer cells induces efficient antitumor immunity. *J Immunother Cancer* (2020) 8(2):e001369. doi: 10.1136/jitc-2020-001369

60. Luo X, Gong HB, Gao HY, Wu YP, Sun WY, Li ZQ, et al. Oxygenated phosphatidylethanolamine navigates phagocytosis of ferroptotic cells by interacting with TLR2. *Cell Death Differ* (2021) 28(6):1971–89. doi: 10.1038/s41418-020-00719-2

61. Drijvers JM, Gillis JE, Muijlwijk T, Nguyen TH, Gaudiano EF, Harris IS, et al. Pharmacologic screening identifies metabolic vulnerabilities of CD8(+) T cells. *Cancer Immunol Res* (2021) 9(2):184–99. doi: 10.1158/2326-6066.CIR-20-0384

62. Liao P, Wang W, Wang W, Kryczek I, Li X, Bian Y, et al. CD8(+) T cells and fatty acids orchestrate tumor ferroptosis and immunity via ACSL4. *Cancer Cell* (2022) 40 (4):365–78.e6. doi: 10.1016/j.ccell.2022.02.003

63. Wang Q, Wang Y, Ding J, Wang C, Zhou X, Gao W, et al. A bioorthogonal system reveals antitumour immune function of pyroptosis. *Nature* (2020) 579 (7799):421–6. doi: 10.1038/s41586-020-2079-1

64. Gurnari C, Voso MT, Maciejewski JP, Visconte V. From bench to bedside and beyond: therapeutic scenario in acute myeloid leukemia. *Cancers* (2020) 12(2):357. doi: 10.3390/cancers12020357

65. Krali O, Palle J, Bäcklin CL, Abrahamsson J, Norén-Nyström U, Hasle H, et al. DNA methylation signatures predict cytogenetic subtype and outcome in pediatric acute myeloid leukemia (AML). *Genes (Basel)* (2021) 12(6):895. doi: 10.3390/genes12060895

66. Horikoshi N, Cong J, Kley N, Shenk T. Isolation of differentially expressed cDNAs from p53-dependent apoptotic cells: activation of the human homologue of the Drosophila peroxidasin gene. *Biochem Biophys Res Commun* (1999) 261(3):864–9. doi: 10.1006/bbrc.1999.1123

67. Pollard JA, Alonzo TA, Gerbing R, Brown P, Fox E, Choi J, et al. Sorafenib in combination with standard chemotherapy for children with high allelic ratio FLT3/ITD + Acute myeloid leukemia: A report from the children's oncology group protocol AAML1031. *J Clin Oncol* (2022) 40(18):2023–35. doi: 10.1200/JCO.21.01612

68. Song C, Li D, Zhang J, Zhao X. Role of ferroptosis in promoting cardiotoxicity induced by Imatinib Mesylate via down-regulating Nrf2 pathways *in vitro* and *in vivo*. *Toxicol Appl Pharmacol* (2022) 435:115852. doi: 10.1016/j.taap.2021.115852

69. Cluzeau T, Sebert M, Rahmé R, Cuzzubbo S, Lehmann-Che J, Madelaine I, et al. Eprenetapopt plus azacitidine in TP53-mutated myelodysplastic syndromes and acute myeloid leukemia: A phase II study by the groupe francophone des myélodysplasies (GFM). *J Clin Oncol* (2021) 39(14):1575–83. doi: 10.1200/JCO.20.02342

70. Xin Q, Ji Q, Zhang Y, Ma W, Tian B, Liu Y, et al. Aberrant ROS served as an acquired vulnerability of cisplatin-resistant lung cancer. *Oxid Med Cell Longevity* (2022) 2022:1112987. doi: 10.1155/2022/1112987

71. Birsen R, Larrue C, Decroocq J, Johnson N, Guiraud N, Gotanegre M, et al. APR-246 induces early cell death by ferroptosis in acute myeloid leukemia. *Haematologica* (2022) 107(2):403–16. doi: 10.3324/haematol.2020.259531

72. Yan H, Wang Z, Sun Y, Hu L, Bu P. Cytoplasmic NEAT1 suppresses AML stem cell self-renewal and leukemogenesis through inactivation of Wnt signaling. *Advanced Sci (Weinheim Baden-Wurttemberg Germany)* (2021) 8(22):e2100914. doi: 10.1002/advs.202100914

73. Zhang Y, Luo M, Cui X, O'Connell D, Yang Y. Long noncoding RNA NEAT1 promotes ferroptosis by modulating the miR-362-3p/MIOX axis as a ceRNA. *Cell Death Differ* (2022) 29(9):1850–63. doi: 10.1038/s41418-022-00970-9

74. Zhou F, Chen B. Prognostic significance of ferroptosis-related genes and their methylation in AML. *Hematol (Amsterdam Netherlands)* (2021) 26(1):919–30. doi: 10.1080/16078454.2021.1996055

75. Wei J, Nai GY, Dai Y, Huang XJ, Xiong MY, Yao XY, et al. Dipetidyl peptidase-4 and transferrin receptor serve as prognostic biomarkers for acute myeloid leukemia. *Ann Trans Med* (2021) 9(17):1381. doi: 10.21037/atm-21-3368

76. Zhang X, Zhang X, Liu K, Li W, Wang J, Liu P, et al. HIVEP3 cooperates with ferroptosis gene signatures to confer adverse prognosis in acute myeloid leukemia. *Cancer Med* (2022) 11(24):5050–65. doi: 10.1002/cam4.4806

77. Zheng Z, Wu W, Lin Z, Liu S, Chen Q, Jiang X, et al. Identification of seven novel ferroptosis-related long non-coding RNA signatures as a diagnostic biomarker for acute myeloid leukemia. *BMC Med Genomics* (2021) 14(1):236. doi: 10.1186/s12920-021-01085-9

78. Yusuf RZ, Saez B, Sharda A, van Gastel N, Yu VWC, Baryawno N, et al. Aldehyde dehydrogenase 3a2 protects AML cells from oxidative death and the synthetic lethality of ferroptosis inducers. *Blood* (2020) 136(11):1303–16. doi: 10.1182/blood.2019001808

79. Ye F, Chai W, Xie M, Yang M, Yu Y, Cao L, et al. HMGB1 regulates erastin-induced ferroptosis via RAS-JNK/p38 signaling in HL-60/NRAS(Q61L) cells. *Am J Cancer Res* (2019) 9(4):730–9.

80. Wang Z, Chen X, Liu N, Shi Y, Liu Y, Ouyang L, et al. A nuclear long non-coding RNA LINC00618 accelerates ferroptosis in a manner dependent upon apoptosis. *Mol Ther J Am Soc Gene Ther* (2021) 29(1):263–74. doi: 10.1016/j.ymthe.2020.09.024

81. Li S, He Y, Chen K, Sun J, Zhang L, He Y, et al. RSL3 drives ferroptosis through NF-κB pathway activation and GPX4 depletion in glioblastoma. *Oxid Med Cell Longevity* (2021) 2021:2915019. doi: 10.1155/2021/2915019

82. Probst L, Dächert J, Schenk B, Fulda S. Lipoxygenase inhibitors protect acute lymphoblastic leukemia cells from ferroptotic cell death. *Biochem Pharmacol* (2017) 140:41–52. doi: 10.1016/j.bcp.2017.06.112

83. Lou S, Hong H, Maihesuti L, Gao H, Zhu Z, Xu L, et al. Inhibitory effect of hydnocarpin D on T-cell acute lymphoblastic leukemia via induction of autophagy-dependent ferroptosis. *Exp Biol Med (Maywood NJ)* (2021) 246(13):1541–53. doi: 10.1177/15353702211004870

84. Xu Y, Deng N, Wang X, Chen Y, Li G, Fan H. RKTG overexpression inhibits proliferation and induces apoptosis of human leukemia cells via suppression of the ERK and PI3K/AKT signaling pathways. *Oncol Lett* (2017) 14(1):965–70. doi: 10.3892/ol.2017.6182

85. Jin L, Tong L. PAQR3 inhibits proliferation and aggravates ferroptosis in acute lymphoblastic leukemia through modulation Nrf2 stability. *Immun Inflammation Dis* (2021) 9(3):827–39. doi: 10.1002/iid3.437

86. Hong Y, Zhang L, Tian X, Xiang X, Yu Y, Zeng Z, et al. Identification of immune subtypes of Ph-neg B-ALL with ferroptosis related genes and the potential implementation of Sorafenib. *BMC Cancer* (2021) 21(1):1331. doi: 10.1186/s12885-021-09076-w

87. Ishikawa C, Senba M, Mori N. Evaluation of artesunate for the treatment of adult T-cell leukemia/lymphoma. *Eur J Pharmacol* (2020) 872:172953. doi: 10.1016/j.ejphar.2020.172953

88. Chen L, Fang W, Liu J, Qi X, Zhao L, Wang Y, et al. Poricoic acid A (PAA) inhibits T-cell acute lymphoblastic leukemia through inducing autophagic cell death and ferroptosis. *Biochem Biophys Res Commun* (2022) 608:108–15. doi: 10.1016/j.bbrc.2022.03.105

89. Zhou B, Liu J, Kang R, Klionsky DJ, Kroemer G, Tang D. Ferroptosis is a type of autophagy-dependent cell death. *Semin Cancer Biol* (2020) 66:89–100. doi: 10.1016/j.semcancer.2019.03.002

90. Gong H, Li H, Yang Q, Zhang G, Liu H, Ma Z, et al. A ferroptosis molecular subtype-related signature for predicting prognosis and response to chemotherapy in patients with chronic lymphocytic leukemia. *BioMed Res Int* (2022) 2022:5646275. doi: 10.1155/2022/5646275

91. Liu S, Wu W, Chen Q, Zheng Z, Jiang X, Xue Y, et al. TXNRD1: A key regulator involved in the ferroptosis of CML cells induced by cysteine depletion *in vitro*. *Oxid Med Cell Longevity* (2021) 2021:7674565. doi: 10.1155/2021/7674565

frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 11 December 2023
DOI 10.3389/fonc.2023.1303677

OPEN ACCESS

EDITED BY
Felix Seyfried,
Ulm University Medical Centre, Germany

REVIEWED BY
Valentina S. Caputo,
London South Bank University,
United Kingdom
Rao Prabhala,
Harvad Medical School, United States

*CORRESPONDENCE
Helena Jernberg Wiklund
helena.jernberg_wiklund@igp.uu.se

†These authors have contributed equally to this work and share first authorship

RECEIVED 28 September 2023
ACCEPTED 27 November 2023
PUBLISHED 11 December 2023

CITATION
Nylund P, Garrido-Zabala B, Kalushkova A and Wiklund HJ (2023) The complex nature of lncRNA-mediated chromatin dynamics in multiple myeloma. *Front. Oncol.* 13:1303677. doi: 10.3389/fonc.2023.1303677

The complex nature of lncRNA-mediated chromatin dynamics in multiple myeloma

Patrick Nylund†, Berta Garrido-Zabala†, Antonia Kalushkova and Helena Jernberg Wiklund*

Science for Life Laboratory, Department of Immunology, Genetic and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden

Extensive genome-wide sequencing efforts have unveiled the intricate regulatory potential of long non-protein coding RNAs (lncRNAs) within the domain of haematological malignancies. Notably, lncRNAs have been found to directly modulate chromatin architecture, thereby impacting gene expression and disease progression by interacting with DNA, RNA, and proteins in a tissue- or condition-specific manner. Furthermore, recent studies have highlighted the intricate epigenetic control of lncRNAs in cancer. Consequently, this provides a rationale to explore the possibility of therapeutically targeting lncRNAs themselves or the epigenetic mechanisms that govern their activity. Within the scope of this review, we will assess the current state of knowledge regarding the epigenetic regulation of lncRNAs and how, in turn, lncRNAs contribute to chromatin remodelling in the context of multiple myeloma.

KEYWORDS

lncRNA, chromatin regulation, multiple myeloma, epigenetics, RNA modifications, haematological malignancies

Introduction

Multiple myeloma (MM) is a heterogeneous haematological malignancy characterized by the clonal expansion of malignant plasma cells within the bone marrow (1). It represents the second most prevalent haematological malignancy and it is marked by complex genetic aberrations, including chromosomal translocations, copy number alterations, and somatic mutations, affecting pathways critical to cell cycle regulation, DNA repair, and epigenetic modulation (2–4). Treatment strategies include high-dose chemotherapy regimens, autologous stem cell transplantation, as well as targeted therapies such as proteasome inhibitors and immunomodulatory agents. Despite these therapeutic innovations, disease relapse and drug resistance remain as substantial challenges (5). Thus, treatment of MM is clinically challenging and new therapeutic interventions are required. Prior studies, by us and others, have suggested that the epigenetic machinery plays a crucial role in MM pathogenesis, including aberrant DNA methylation and abnormal histone modification patterns (6–14). Furthermore, more recently, dysregulation of long non-protein coding

RNAs (lncRNAs) has been suggested to contribute to MM pathogenesis, patient outcome and drug resistance (15–17). Additionally, dysregulation of lncRNAs has been shown to contribute to disease progression by influencing critical pathways involved in proliferation, apoptosis, immune response, and drug resistance (18, 19). Unravelling the complex network of lncRNA-mediated molecular mechanisms could therefore unveil novel therapeutic targets and diagnostic markers in MM.

lncRNAs represent the largest group of non-protein coding RNAs, however, to date their functions remain largely unexplored. lncRNAs are transcripts exceeding 200 nucleotides in length, and their transcriptional regulation mirrors that of protein-coding genes, including processes such as histone modifications, chromatin compaction, and chromatin remodelling. The biogenesis of lncRNAs encompasses a spectrum of events, including 5' capping, splicing, variation in exon and intron dimensions, and the addition of polyadenylation (poly(A)+) tails. Notably, features like poly(A)+ tails and 5' capping play fundamental roles in determining the transcript stability of lncRNAs. In contrast to messenger RNAs (mRNAs), lncRNAs transcripts exhibit a diminished steady-state, as they are commonly less evolutionary conserved (20). lncRNAs can be transcribed from multiple genomic locations, including promoters, enhancers, intergenic regions, as well as in bidirectional and antisense directions. Typically residing within the nucleus, lncRNAs tend to manifest pronounced cell and tissue specificity (21). In addition, a substantial fraction i.e., 81% of lncRNAs, exhibit a limited degree of evolutionary conservation, while 3% of lncRNAs manifest ultra-conservation (22).

Functionally, lncRNAs perform a diverse array of functions both within the nucleus and the cytoplasm. These molecules regulate gene expression by engaging in intricate interactions with RNA, DNA and proteins, including chromatin-modifying enzymes. Within the nuclear domain, lncRNAs have been categorized into four fundamental archetypes: signal, decoy, guide, and scaffold lncRNAs (Figure 1). Signal lncRNAs respond to specific stimuli, promoting integration of signals for the transcription of targeted genes (23). Decoy lncRNAs, on the other hand, can bind proteins, such as transcription factors and chromatin modifiers, resulting in transcriptional control by impeding the binding capacity to their targets (24). Guide lncRNAs have the ability to reposition ribonucleoprotein complexes to designated loci, both in *cis* and in *trans*, thereby altering the gene expression patterns. Finally, scaffold-associated lncRNAs engage in temporally and spatially regulated interactions with DNA, different types of RNAs and proteins, thereby bolstering the stability of complexes involved in either transcriptional activation or suppression. Additionally, lncRNAs operate as microRNA (miRNA) sponges, sequestering miRNAs to avert mRNA degradation (25). To date, various lncRNAs have been described to localize with chromatin, where they interact with different chromatin-associated proteins to promote or repress their binding potential to specific DNA locations. These chromatin-associated lncRNAs have been implicated in MM pathogenesis and disease outcome. In addition, lncRNAs do not only act as regulators of the epigenetic landscape but can also themselves be epigenetically regulated by DNA and chromatin modifications as well as by RNA modifications, referred to as the epitranscriptomics. Among these, RNA modifications such

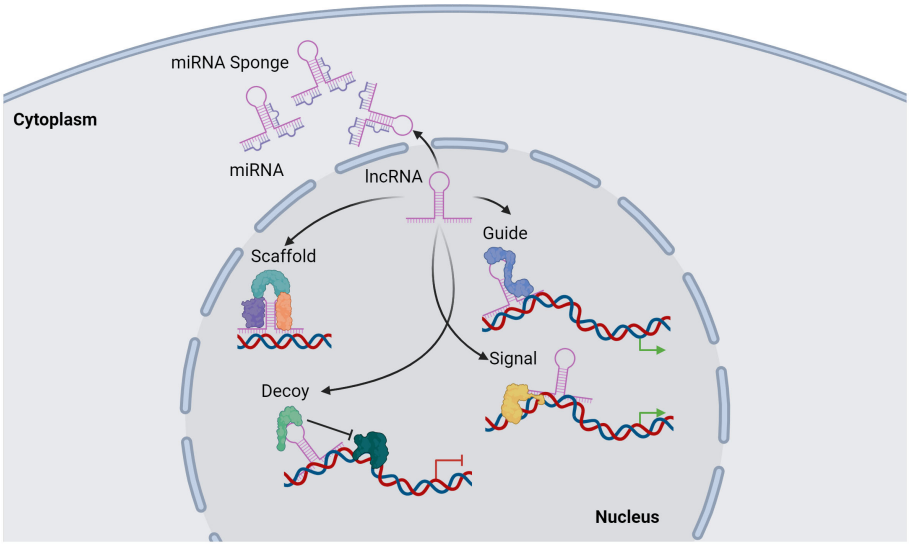

FIGURE 1
Overview of lncRNA functions. lncRNAs can regulate transcription by acting as a scaffold by binding proteins together in a complex structure. A secondary function of a lncRNA is as a guide of proteins or other molecules to target genomic location. lncRNAs can directly bind to genomic regions within the genome to transduce signal activation of DNA-bound molecules. Furthermore, a lncRNA can act as a decoy, preventing different molecules such as proteins to bind to targeted genomic regions. In addition, lncRNAs can regulate miRNAs function by acting as a miRNA sponge, preventing miRNA-mRNA binding, thus inhibiting mRNAs degradation. Image was created with biorender.com.

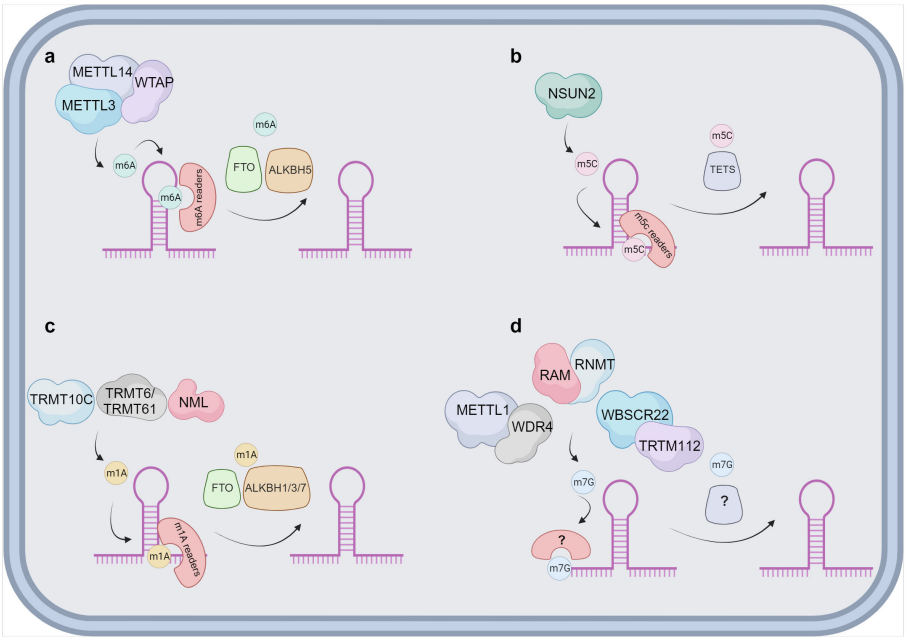

FIGURE 2
Proposed overview of RNA modifications on lncRNA. Schematic overviews are provided for the different lncRNAs modifications. **(A)** The m6A modification is deposited by a protein complex constituted mainly by METTL3, METTL14 and the cofactor WTAP. There are multiple m6A readers identified, namely YTHDF1/2/3, IGF2BP1/2/3, HNRNPs and ZC3H13. The demethylation of m6A is catalysed by FTO and ALKBH5. **(B)** NSUN2 has been reported as the sole writer of the m5C modification in lncRNAs. The m5C can be identified by ALYREF and YBX1 and it is speculated that the removal is catalysed by TETs. **(C)** m1A is suggested to be deposited by TRMT10C, TRMT6/61 and NML on lncRNAs. Multiple readers have been identified in other types of RNAs, as YTDHF1-3 and YTHDC1 and the removal is mainly associated to FTO and ALKBH1/3/7. **(D)** The exact manner of how m7G modification is deposited on lncRNAs is currently unknown. Three different complexes can take the role of a writer, METTL1 and WDR4, RAM and RNMT or WBSCR22 and TRTM112. Currently both readers and erasers on m7G are unknown and represented with ? in the figure. Image was created with biorender.com.

as, N6-methyladenosine (m6A), N1-methyladenosine (m1A), 5-methylcytosine (m5C), and 7-methylguanosine (m7G), play a crucial role in regulating various aspects of lncRNA function, structure, stability, localization, and lncRNA-mediated interactions (26–34) (Figure 2).

We and others have shown the importance of lncRNAs in chromatin remodelling and the impact they have on MM patient outcome (6, 15–17). In fact, aberrant lncRNA expression has been demonstrated to have an oncogenic role in MM pathogenesis and progression (35, 36). In this review, we present an overview of the role of lncRNAs in the context of MM through their epigenetic regulation and functional effects on chromatin remodelling.

The functional impact of lncRNAs in epigenetic regulation

lncRNAs have been suggested to affect multiple layers of cellular function, encompassing processes such as cellular biogenesis of macromolecules, differentiation, gene expression and chromatin remodelling. The recent establishment of a comprehensive genome-wide lncRNA-chromatin interactome has provided insight into the intricate orchestration of chromatin compaction by lncRNAs, subsequently impacting gene expression patterns (37–39). Notably, the functional implications of lncRNA-mediated chromatin remodelling in the context of cancer have gained considerable interest, however a summary within the domain of MM is currently lacking.

Epigenetic regulation of lncRNAs by DNA methylation and histone modifications

The expression of lncRNAs can be regulated by different epigenetic machineries, such as DNA methylation (40). DNA methylation plays an important role in regulating cell-type specific gene expression. The DNA methylation process consists of the deposition of methyl groups to the 5-carbon position of cytosine in a CpG dinucleotide, resulting in gene suppression when located along the promoter or transcription start site and gene transcription when found in the gene body. This process is catalysed by the DNA methyltransferases, DNMT1 and DNMT3A/B and can be reversed by the DNA demethylase enzymes TET1-3 (41). Disrupted DNA methylation has been shown to promote carcinogenesis and disease progression in multiple cancers (3, 42, 43). In fact, it has previously been suggested that promoter DNA hypermethylation is accountable for decreased expression of 35 lncRNAs in hepatocellular carcinoma (40, 44). Furthermore, patients with lower expression of these lncRNAs had increased expression of the DNA methyltransferase genes *DNMT1*, *DNMT3A*, and *DNMT3B*. In contrast, patients with higher

expression of this panel of lncRNAs, exhibited lower expression of the DNA methyltransferases (40). Li et al. reported that the lncRNA *BM742401*, defined as a tumour suppressor in gastric cancer and chronic lymphocytic leukaemia, undergoes silencing in MM cell lines due to promoter hypermethylation. Notably, decreased *BM742401* levels enhanced MM cell migration, while in newly diagnosed MM patients, silencing by elevated DNA methylation levels in the promoter of the lncRNA *BM742401* correlated with poor overall survival. This underscores the significant impact that epigenetic regulation of lncRNAs can exert on disease progression (45). Similarly, DNA methylation-mediated silencing of the lncRNA *KIAA0495* has been reported in MM cell lines, although it was not found to be relevant for the progression of the disease (46) (Table 1).

An additional level of transcriptional regulation is through chromatin compaction. DNA is packed into chromatin fibres wrapped around a histone octamer, ultimately forming a nucleosome. The nucleosome consists of the four histone proteins H2A, H2B, H3 and H4. Each histone protein has in its N-terminal domain a histone tail that can be reversibly subjected to methylation, acetylation, phosphorylation, ubiquitination, sumoylation and histone tail clipping which control chromatin compaction, thus either promoting or inhibiting transcription factor binding, DNA repair, replication and genomic recombination. The majority of studies have concentrated on examining histone modifications related to protein coding genes and non-protein coding genes such as miRNAs (59–62). Consequently, additional research is warranted to elucidate the influence of histone modifications on lncRNAs' regulation and the potential implications in various diseases, including MM.

Interplay between lncRNAs and chromatin modifiers

Although, data is largely lacking how regulation of lncRNAs by the deposition of histone modifications may directly influence their expression, there is now emerging data indicating that lncRNAs may act as recruiters, guides and scaffolds for protein complexes including chromatin modifiers, thus epigenetically influencing the expression of other genes. Prior studies have shown that PRC2-mediated gene silencing is important for MM pathogenesis and disease progression, both *in vivo* and *in vitro* (7, 8, 10, 43). Furthermore, several lncRNAs have been suggested to regulate the enzymatic activity of PRC2 by binding to the catalytic subunit EZH2. Moreover, lncRNAs can modulate PRC2 activity by acting as a complex recruiter to target genomic locations. For instance, the lncRNA *PVT1* was recently described to be overexpressed in primary MM patient samples and associated with poor prognosis, a seemingly independent feature from patients' cytogenetic background (6). Moreover, *PVT1* was shown to interact directly with EZH2, facilitating recruitment of PRC2 to target genomic loci and transcriptional repression of genes associated with pro-apoptotic and tumour suppressor functions (6) (Table 1).

TABLE 1 Function and clinical implications of lncRNAs in multiple myeloma.

lncRNA	Expression	Function	Downstream effects	Prognosis	Reference
BM742401	Downregulated	Unknown	Promote cell migration	Poor OS	(45)
KIAA0495	Downregulated	Unknown	Unknown	Not involved	(46)
PVT1	Upregulated	PRC2 recruiter	Silencing of tumor suppressor genes & pro-apoptotic genes	Poor OS	(6)
ANRIL	Upregulated	Guide for PRC1/2	Resistance to bortezomib	Poor OS	(47)
H19	Upregulated	miRNA sponge & activator of BRD4	Imbalance of osteogenesis/osteolysis	Poor OS	(48)
CRNDE	Upregulated	Unknown	Proliferation through IL6 signalling	Poor OS	(49)
MIAT	Upregulated	Unknown	Resistance to bortezomib	Poor OS	(50)
HOTAIR	Upregulated	Activation of NF-κB & JAK2/STAT3 signalling	Resistance to dexamethasone	Unknown	(51)
RROL	Unknown	Chromatin scaffold	Promote cell growth	Unknown	(52)
AIR	Upregulated	Unknown	Unknown	Unknown	(53)
HOXB-AS1	Upregulated	mRNA stabilizer	Unknown	Unknown	(54)
DARS-AS1	Unknown	Unknown	Promoting the mTOR pathway	Unknown	(55)
MALAT1	Upregulated	Scaffold for protein complexes & miRNA sponge	Increased proliferation & reduction of pro-apoptotic gene expression	Poor OS	(25, 56, 57)
NEAT1	Upregulated	Unknown	Chemotherapeutic resistance	Poor OS	(25, 58)
GAS5	Upregulated	Unknown	Unknown	Poor OS	(25)

Similarly to the function of *PVT1*, the lncRNA *ANRIL*, was described to exert a guiding function for PRC1 and PRC2 DNA binding in MM and was demonstrated to promote resistance to conventional therapies such as bortezomib by guiding PRC2 to promote gene silencing of the tumour suppressor gene *PTEN*. High expression of *ANRIL* has been associated with poor overall survival in MM (47) (Table 1). Furthermore, upregulation of the lncRNA *H19* correlates with worse prognosis and promotes the imbalance of osteogenesis and osteolysis in MM by acting as a miRNA sponge, resulting in upregulation of E2F7, which is a transcriptional activator of EZH2 and thus affecting the suppression of *PTEN* (48). In addition, increased *H19* activity has been shown to activate the chromatin reader protein BRD4 in MM (63). BRD4 is a well-known epigenetic reader of acetylated lysine and assists in the transmission of epigenetic memory during cell division (64, 65). BRD4 has been identified as a therapeutic vulnerability and potential target in MM (66) (Table 1).

The lncRNA *CRNDE* epigenetically regulates the transcription of *DUSP5* and *CDKN1A* in solid tumours by facilitating PRC2 recruitment (67). Overexpression of *CRNDE* has been described to be associated with poor prognosis by regulating proliferative capacity through IL6 signalling in MM, however, no direct interaction between *CRNDE* and PRC2 has been proven (49). Recruitment of the histone H3 lysine 4 methyltransferase MLL has been suggested to occur through the binding to the lncRNA *MIAT*, which can then guide MLL to the promoter region of the collagen degradation enzyme *MMP9*. Inhibition of *MIAT* resulted in the loss of transcriptional activity of *MMP9*, which is suggested to reduce proliferative capacity and cell migration in non-small cell lung cancer (68). In MM, *MIAT is* overexpressed and has been associated with sensitivity to bortezomib treatment (50) (Table 1).

Interestingly, additional lncRNAs have been suggested to play important roles in chromatin regulation. The lncRNA *HOTAIR* has been demonstrated to bind to the PRC2 complex and can further interact with the TF-silencing complex formed by LSD1/CoREST/repressor element 1, promoting gene repression (69). In addition, *HOTAIR* may function as a stabilizing component of PRC2, as well as a scaffold for complex-complex interactions (69). In MM, *HOTAIR* has been described to be upregulated in primary patient samples and to contribute to the oncogenic activation of the JAK2/STAT3 signalling pathway (51) (Table 1). Similarly, the MIR17HG-derived lncRNA, *RROL*, has been demonstrated to act as a chromatin scaffold for protein interactions and to promote MM cell growth (52). lncRNAs such as *AIR* and *HOXB-AS1* have been described to have a guiding function through which they recruit the histone methyltransferases G9a and SET1/MLL to target locations to induce gene repression or activation, respectively (53, 70). Interestingly, *HOXB-AS1* has been described to be upregulated in MM, acting as a stabilizer for mRNA (54) (Table 1). In another aspect of epigenetic regulation, *DARS-AS1* promotes the recruitment of the histone methyltransferases METTL3 and METTL14 to *DARS* mRNA to induce m6A modification and enhance translation in cervical cancer (71). In MM, *DARS-AS1* has been described to regulate HIF-1α in promoting the mTOR pathway (55) (Table 1).

Increased expression of the lncRNAs *GAS5*, *MALAT1* and *NEAT1* in MM patients, is associated with poor outcome and worse overall survival (25) (Table 1). *GAS5* has the ability to act as decoy for different molecules by functioning as a DNA mimic, thus preventing DNA motif binding (72). One of the most abundant and most studied lncRNAs is *MALAT1* which has been implicated in various functions during MM pathogenesis by acting as a scaffold for proteins involved in DNA repair (56) and as a miRNA sponge (57). Interestingly it has also been described to promote gene silencing by PRC2 recruitment in various cancers (73–75). Recent studies in colorectal cancer have suggested that the lncRNA *NEAT1* promotes histone H3 lysine 27 acetylation in genes associated with stemness (76). In addition, *NEAT1* has further been implicated in lung cancer by recruiting DNMT1 to the promoter regions of genes regulating cytotoxic T-cell infiltration. In fact, inhibition of *NEAT1* leads to loss of DNMT1 binding to these promoter regions and thus activating gene expression (77). In MM, overexpression of *NEAT1* has been associated with poor patient outcome. In addition, and further supporting a clinical relevance, inhibition of *NEAT1* promoted increased sensitivity to chemotherapeutic treatment (58) (Table 1).

Epigenetic regulation of lncRNAs by RNA modifications

RNA modifications on lncRNAs may influence their stability, subcellular localization, and interactions with DNA, proteins and other RNA molecules. These modifications can also affect lncRNA regulation and contribute to their reported functional diversity (33). Dysregulation of RNA modifications on lncRNAs has been associated with various diseases, including MM (33, 78–80).

The deposition of the N6-methyladenosine (m6A) mark may give rise to structural changes in lncRNAs, thus modifying lncRNA-protein interactions. Additionally, the m6A modification can modulate gene transcription, influence the subcellular localization of lncRNAs and regulate lncRNAs' stability (81–84). There is an interdependent connection between the m6A modification and lncRNAs. Notably, lncRNAs have the ability to influence the stability and degradation of enzymes involved in m6A, as well as facilitate their integration into protein complexes (85–87). One example of this function is the lncRNA *FEZF1-AS1*, the knockdown of which led to an increased apoptosis by regulating the signalling of IGF2BP1, an m6A reader protein, in MM (88). Furthermore, dysregulation of m6A-related enzymes has been associated with disease progression, enhancing tumour growth and cell proliferation in MM (89–95). Significantly, m6A studies in MM showed a correlation between exosome-induced drug resistance and high levels of m6A on the lncRNAs *LOC606724* and *SNHG*. Wang et al. identified METTL7A as an additional component of the m6A methyltransferase complex and described how its regulation is mediated by EZH2. Depletion of EZH2 simultaneously reduced METLL7A protein methylation levels, thus altering the m6A levels on the lncRNAs *LOC606724* and *SNHG* (96). Studies in prostate cancer show that high levels of m6A on *NEAT1* have been

associated with bone metastasis (79, 97). Although no studies of m6A on *NEAT1* have been performed in MM, high expression of *NEAT1* in patients have been correlated with poor prognosis (98). In addition, *NEAT1* can enhance the preservation of DNA integrity, thus promoting survival of MM cells (99). Moreover, knockdown of *NEAT1* improved dexamethasone drug response in MM cell lines (100).

5-methylcytosine (m5C) has previously been described to exert important functions on DNA and has also been found to occur on RNA (78). The biological impact of RNA m5C primarily affects RNA localization, stability and transcription efficiency (101). Interestingly, NSUN2 has been reported as the sole writer of the m5C mark on lncRNAs (79). In MM, dysregulated deposition of RNA m5C has been correlated with disease progression and immune microenvironment regulation (102). Furthermore, recent studies have elucidated the importance of this modification in various other cancer types, including lung adenocarcinoma, pancreatic cancer, and colon cancer (79, 103–105).

Modifications of lncRNAs also include the deposition of N1-methyladenosine (m1A), which alters RNA secondary and tertiary structure, subsequently affecting its capacity to interact with RNA binding proteins. However, the function of m1A in lncRNAs is not fully elucidated, and the m1A modification has so far only been reported in the lncRNA *MALAT1* (80, 106). Despite the absence of studies focusing on the m1A modification in MM, as previously mentioned, *MALAT1* overexpression is correlated with worse prognosis, and the oncogenic role of *MALAT1* in promoting MM tumorigenesis has been widely studied (35, 56, 107). *MALAT1* dysregulation in MM has been associated with a wide range of processes including cell proliferation, DNA repair mechanisms, metastasis, drug resistance, and angiogenesis pathways (57, 107–109). Nonetheless, if these functions are mediated by chromatin remodelling and regulated via RNA modifications remains to be further investigated.

The N7-methylguanosine (m7G) modification is predominantly found at the 5′cap of mRNA, ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs). However, the impact of m7G on lncRNAs remains uncertain, probably attributed to the absence of 5′cap on less conserved lncRNAs (110, 111). Nevertheless, Yang et al. constructed the first model based on eight m7G-related lncRNAs to predict patient prognosis in colon cancer (112). Similarly, RNA m7G MeRIP-seq uncovered the significance of m7G-enriched lncRNAs in acute myeloid leukemia cells and unravelled a potential role of this modification in modulating gene expression, thereby enhancing drug resistance (111). However, the role of m7G modification in MM remains at present unknown.

Discussion

The pathogenic impact of lncRNAs in MM and other haematological malignancies is unravelling. Recently, there have been large sequencing efforts in various cancers including MM that have suggested a clinical importance of lncRNAs. In MM, lncRNAs have been implicated in clinically relevant elements such as disease development, progression, drug resistance and patient outcome (25). Studies on the epitranscriptomics of lncRNAs through the addition of methyl groups to the lncRNA transcripts have gained increased attention and have furthered added an additional level of complexity to how lncRNAs contribute to cellular processes, such as RNA stability, translational efficiency of mRNAs and protein complex formation. However, the exact nature of these modifications needs to be further investigated in the context of MM. Moreover, not only can the expression of lncRNAs be epigenetically regulated but can in turn regulate chromatin modifying enzymes. Although lncRNA-chromatin interactions are clearly more dynamically investigated in some areas, such as in the recently shown context of PRC2 recruitment, deep functional evaluation of lncRNAs in MM is still lacking. It is apparent that this field is underdeveloped and a complete picture of how lncRNAs impact the pathophysiological processes in MM remains uncertain. While their functions continue to unfold, targeting lncRNAs arises as compelling innovative treatment option in cancer, including MM.

Author contributions

PN: Conceptualization, Project administration, Visualization, Writing – original draft, Writing – review & editing. BG-Z: Conceptualization, Project administration, Visualization, Writing – original draft, Writing – review & editing. AK: Supervision, Writing – review & editing. HW: Funding acquisition, Supervision, Writing – review & editing, Conceptualization, Visualization.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The project was supported by grants from the Swedish Research Council (K2019-64X-20102-13-3/KDB 1335/17) and the Swedish Cancer Society (CAN 2016/458, 200727 PjVSF).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Fairfield H, Falank C, Avery L, Reagan MR. Multiple myeloma in the marrow: pathogenesis and treatments. *Ann N Y Acad Sci* (2016) 1364(1):32–51. doi: 10.1111/nyas.13038

2. Kazandjian D. Multiple myeloma epidemiology and survival: A unique Malignancy. *Semin Oncol* (2016) 43(6):676–81. doi: 10.1053/j.seminoncol.2016.11.004

3. Alzrigat M, Parraga AA, Jernberg-Wiklund H. Epigenetics in multiple myeloma: From mechanisms to therapy. *Semin Cancer Biol* (2018) 51:101–15. doi: 10.1016/j.semcancer.2017.09.007

4. Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. *Nat Rev Cancer* (2012) 12(5):335–48. doi: 10.1038/nrc3257

5. Pawlyn C, Davies FE. Toward personalized treatment in multiple myeloma based on molecular characteristics. *Blood.* (2019) 133(7):660–75. doi: 10.1182/blood-2018-09-825331

6. Nylund P, Garrido-Zabala B, Parraga AA, Vasquez L, Pyl PT, Harinck GM, et al. PVT1 interacts with polycomb repressive complex 2 to suppress genomic regions with pro-apoptotic and tumour suppressor functions in multiple myeloma. *Haematologica* (2023). doi: 10.3324/haematol.2023.282965

7. Kalushkova A, Fryknas M, Lemaire M, Fristedt C, Agarwal P, Eriksson M, et al. Polycomb target genes are silenced in multiple myeloma. *PloS One* (2010) 5(7):e11483. doi: 10.1371/journal.pone.0011483

8. Agarwal P, Alzrigat M, Parraga AA, Enroth S, Singh U, Ungerstedt J, et al. Genome-wide profiling of histone H3 lysine 27 and lysine 4 trimethylation in multiple myeloma reveals the importance of Polycomb gene targeting and highlights EZH2 as a potential therapeutic target. *Oncotarget.* (2016) 7(6):6809–23. doi: 10.18632/oncotarget.6843

9. Alzrigat M, Parraga AA, Agarwal P, Zureigat H, Osterborg A, Nahi H, et al. EZH2 inhibition in multiple myeloma downregulates myeloma associated oncogenes and upregulates microRNAs with potential tumor suppressor functions. *Oncotarget.* (2017) 8(6):10213–24. doi: 10.18632/oncotarget.14378

10. Nylund P, Atienza Parraga A, Haglof J, De Bruyne E, Menu E, Garrido-Zabala B, et al. A distinct metabolic response characterizes sensitivity to EZH2 inhibition in multiple myeloma. *Cell Death Dis* (2021) 12(2):167. doi: 10.1038/s41419-021-03447-8

11. Walker BA, Wardell CP, Chiecchio L, Smith EM, Boyd KD, Neri A, et al. Aberrant global methylation patterns affect the molecular pathogenesis and prognosis of multiple myeloma. *Blood.* (2011) 117(2):553–62. doi: 10.1182/blood-2010-04-279539

12. Agirre X, Castellano G, Pascual M, Heath S, Kulis M, Segura V, et al. Whole-epigenome analysis in multiple myeloma reveals DNA hypermethylation of B cell-specific enhancers. *Genome Res* (2015) 25(4):478–87. doi: 10.1101/gr.180240.114

13. Alaterre E, Ovejero S, Herviou L, de Boussac H, Papadopoulos G, Kulis M, et al. Comprehensive characterization of the epigenetic landscape in multiple Myeloma. *Theranostics* (2022) 12(4):1715–29. doi: 10.7150/thno.54453

14. Ordoñez R, Kulis M, Russiñol N, Chapaprieta V, Carrasco-Leon A, García-Torre B, et al. Chromatin activation as a unifying principle underlying pathogenic mechanisms in multiple myeloma. *Genome Res* (2020) 30(9):1217–27. doi: 10.1101/gr.265520.120

15. Zang X, Wang J, Xia Y, Li J, Chen L, Gu Y, et al. LncRNA MEG3 promotes the sensitivity of bortezomib by inhibiting autophagy in multiple myeloma. *Leuk Res* (2022) 123:106967. doi: 10.1016/j.leukres.2022.106967

16. Pan Y, Zhang Y, Liu W, Huang Y, Shen X, Jing R, et al. LncRNA H19 overexpression induces bortezomib resistance in multiple myeloma by targeting MCL-1 via miR-29b-3p. *Cell Death Dis* (2019) 10(2):106. doi: 10.1038/s41419-018-1219-0

17. Chen T, Sun Z, Cui Y, Ji J, Li Y, Qu X. Identification of long noncoding RNA NEAT1 as a key gene involved in the extramedullary disease of multiple myeloma by bioinformatics analysis. *Hematology.* (2023) 28(1):2164449. doi: 10.1080/16078454.2022.2164449

18. Meng H, Han L, Hong C, Ding J, Huang Q. Aberrant lncRNA expression in multiple myeloma. *Oncol Res* (2018) 26(5):809–16. doi: 10.3727/096504017X15123872205507

19. Carrasco-Leon A, Ezponda T, Meydan C, Valcarcel LV, Ordonez R, Kulis M, et al. Characterization of complete lncRNAs transcriptome reveals the functional and clinical impact of lncRNAs in multiple myeloma. *Leukemia.* (2021) 35(5):1438–50. doi: 10.1038/s41375-021-01147-y

20. Clark MB, Johnston RL, Inostroza-Ponta M, Fox AH, Fortini E, Moscato P, et al. Genome-wide analysis of long noncoding RNA stability. *Genome Res* (2012) 22(5):885–98. doi: 10.1101/gr.131037.111

21. Cabili MN, Dunagin MC, McClanahan PD, Biaesch A, Padovan-Merhar O, Regev A, et al. Localization and abundance analysis of human lncRNAs at single-cell and single-molecule resolution. *Genome Biol* (2015) 16(1):20. doi: 10.1186/s13059-015-0586-4

22. Butova R, Vychytilova-Faltejskova P, Souckova A, Sevcikova S, Hajek R. Long non-coding RNAs in multiple myeloma. *Noncoding RNA* (2019) 5(1). doi: 10.3390/ncrna5010013

23. Shi W, Wang Q, Bian Y, Fan Y, Zhou Y, Feng T, et al. Long noncoding RNA PANDA promotes esophageal squamous carcinoma cell progress by dissociating from NF-YA but interact with SAFA. *Pathol Res Pract* (2019) 215(10):152604. doi: 10.1016/j.prp.2019.152604

24. Chen CL, Tseng YW, Wu JC, Chen GY, Lin KC, Hwang SM, et al. Suppression of hepatocellular carcinoma by baculovirus-mediated expression of long non-coding RNA PTENP1 and MicroRNA regulation. *Biomaterials.* (2015) 44:71–81. doi: 10.1016/j.biomaterials.2014.12.023

25. Carrasco-Leon A, Amundarain A, Gomez-Echarte N, Prosper F, Agirre X. The role of lncRNAs in the pathobiology and clinical behavior of multiple myeloma. *Cancers (Basel)* (2021) 13(8):1976. doi: 10.3390/cancers13081976

26. Helm M, Motorin Y. Detecting RNA modifications in the epitranscriptome: predict and validate. *Nat Rev Genet* (2017) 18(5):275–91. doi: 10.1038/nrg.2016.169

27. Wang X, He C. Dynamic RNA modifications in posttranscriptional regulation. *Mol Cell* (2014) 56(1):5–12. doi: 10.1016/j.molcel.2014.09.001

28. Yang X, Liu M, Li M, Zhang S, Hiju H, Sun J, et al. Epigenetic modulations of noncoding RNA: a novel dimension of Cancer biology. *Mol Cancer* (2020) 19(1):64. doi: 10.1186/s12943-020-01159-9

29. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, et al. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. *Nature.* (2012) 485(7397):201–6. doi: 10.1038/nature11112

30. Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell.* (2012) 149(7):1635–46. doi: 10.1016/j.cell.2012.05.003

31. Yang X, Yang Y, Sun BF, Chen YS, Xu JW, Lai WY, et al. 5-methylcytosine promotes mRNA export - NSUN2 as the methyltransferase and ALYREF as an m(5)C reader. *Cell Res* (2017) 27(5):606–25. doi: 10.1038/cr.2017.55

32. Enroth C, Poulsen LD, Iversen S, Kirpekar F, Albrechtsen A, Vinther J. Detection of internal N7-methylguanosine (m7G) RNA modifications by mutational profiling sequencing. *Nucleic Acids Res* (2019) 47(20):e126. doi: 10.1093/nar/gkz736

33. Haruehanroengra P, Zheng YY, Zhou Y, Huang Y, Sheng J. RNA modifications and cancer. *RNA Biol* (2020) 17(11):1560–75. doi: 10.1080/15476286.2020.1722449

34. Dominissini D, Nachtergaele S, Moshitch-Moshkovitz S, Peer E, Kol N, Ben-Haim MS, et al. The dynamic N(1)-methyladenosine methylome in eukaryotic messenger RNA. *Nature.* (2016) 530(7591):441–6. doi: 10.1038/nature16998

35. Cho SF, Chang YC, Chang CS, Lin SF, Liu YC, Hsiao HH, et al. MALAT1 long non-coding RNA is overexpressed in multiple myeloma and may serve as a marker to predict disease progression. *BMC Cancer* (2014) 14:809. doi: 10.1186/1471-2407-14-809

36. Meng YB, He X, Huang YF, Wu QN, Zhou YC, Hao DJ. Long noncoding RNA CRNDE promotes multiple myeloma cell growth by suppressing miR-451. *Oncol Res* (2017) 25(7):1207–14. doi: 10.3727/096504017X14886679715637

37. Bonetti A, Agostini F, Suzuki AM, Hashimoto K, Pascarella G, Gimenez J, et al. RADICL-seq identifies general and cell type-specific principles of genome-wide RNA-chromatin interactions. *Nat Commun* (2020) 11(1):1018. doi: 10.1038/s41467-020-14337-6

38. Bell JC, Jukam D, Teran NA, Risca VI, Smith OK, Johnson WL, et al. Chromatin-associated RNA sequencing (ChAR-seq) maps genome-wide RNA-to-DNA contacts. *Elife* (2018) 7. doi: 10.7554/eLife.27024

39. Mumbach MR, Granja JM, Flynn RA, Roake CM, Satpathy AT, Rubin AJ, et al. HiChIRP reveals RNA-associated chromosome conformation. *Nat Methods* (2019) 16(6):489–92. doi: 10.1038/s41592-019-0407-x

40. Recalde M, Garate-Rascon M, Herranz JM, Elizalde M, Azkona M, Unfried JP, et al. DNA methylation regulates a set of long non-coding RNAs compromising hepatic identity during hepatocarcinogenesis. *Cancers (Basel)* (2022) 14(9):2048. doi: 10.3390/cancers14092048

41. Smith ZD, Meissner A. DNA methylation: roles in mammalian development. *Nat Rev Genet* (2013) 14(3):204–20. doi: 10.1038/nrg3354

42. Kulis M, Esteller M. DNA methylation and cancer. *Adv Genet* (2010) 70:27–56. doi: 10.1016/B978-0-12-380866-0.60002-2

43. Kalushkova A, Nylund P, Parraga AA, Lennartsson A, Jernberg-Wiklund H. One omics approach does not rule them all: the metabolome and the epigenome join forces in haematological Malignancies. *Epigenomes.* (2021) 5(4):22. doi: 10.3390/epigenomes5040022

44. Unfried JP, Serrano G, Suarez B, Sangro P, Ferretti V, Prior C, et al. Identification of coding and long noncoding RNAs differentially expressed in tumors and preferentially expressed in healthy tissues. *Cancer Res* (2019) 79(20):5167–80. doi: 10.1158/0008-5472.CAN-19-0400

45. Li Z, Kumar S, Jin DY, Calin GA, Chng WJ, Siu KL, et al. Epigenetic silencing of long non-coding RNA BM742401 in multiple myeloma: impact on prognosis and myeloma dissemination. *Cancer Cell Int* (2020) 20:403. doi: 10.1186/s12935-020-01504-4

46. Wong KY, Li Z, Zhang X, Leung GK, Chan GC, Chim CS. Epigenetic silencing of a long non-coding RNA KIAA0495 in multiple myeloma. *Mol Cancer* (2015) 14:175. doi: 10.1186/s12943-015-0444-8

47. Yang LH, Du P, Liu W, An LK, Li J, Zhu WY, et al. LncRNA ANRIL promotes multiple myeloma progression and bortezomib resistance by EZH2-mediated epigenetically silencing of PTEN. *Neoplasma.* (2021) 68(4):788–97. doi: 10.4149/neo_2021_210205N184

48. Guo N, Song Y, Zi F, Zheng J, Cheng J. Abnormal expression pattern of lncRNA H19 participates in multiple myeloma bone disease by unbalancing osteogenesis and osteolysis. *Int Immunopharmacol* (2023) 119:110058. doi: 10.1016/j.intimp.2023.110058

49. David A, Zocchi S, Talbot A, Choisy C, Ohnona A, Lion J, et al. The long non-coding RNA CRNDE regulates growth of multiple myeloma cells via an effect on IL6 signalling. *Leukemia.* (2021) 35(6):1710–21. doi: 10.1038/s41375-020-01034-y

50. Fu Y, Liu X, Zhang F, Jiang S, Liu J, Luo Y. Bortezomib-inducible long non-coding RNA myocardial infarction associated transcript is an oncogene in multiple myeloma that suppresses miR-29b. *Cell Death Dis* (2019) 10(4):319. doi: 10.1038/s41419-019-1551-z

51. Guan R, Wang W, Fu B, Pang Y, Lou Y, Li H. Increased lncRNA HOTAIR expression promotes the chemoresistance of multiple myeloma to dexamethasone by regulating cell viability and apoptosis by mediating the JAK2/STAT3 signaling pathway. *Mol Med Rep* (2019) 20(4):3917–23. doi: 10.3892/mmr.2019.10603

52. Morelli E, Fulciniti M, Samur MK, Ribeiro CF, Wert-Lamas L, Henninger JE, et al. A MIR17HG-derived long noncoding RNA provides an essential chromatin scaffold for protein interaction and myeloma growth. *Blood.* (2023) 141(4):391–405. doi: 10.1182/blood.2022016892

53. Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R, et al. The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. *Science.* (2008) 322(5908):1717–20. doi: 10.1126/science.1163802

54. Chen R, Zhang X, Wang C. LncRNA HOXB-AS1 promotes cell growth in multiple myeloma via FUT4 mRNA stability by ELAVL1. *J Cell Biochem* (2020) 121 (10):4043–51. doi: 10.1002/jcb.29573

55. Tong J, Xu X, Zhang Z, Ma C, Xiang R, Liu J, et al. Hypoxia-induced long non-coding RNA DARS-AS1 regulates RBM39 stability to promote myeloma Malignancy. *Haematologica.* (2020) 105(6):1630–40. doi: 10.3324/haematol.2019.218289

56. Hu Y, Lin J, Fang H, Fang J, Li C, Chen W, et al. Targeting the MALAT1/PARP1/LIG3 complex induces DNA damage and apoptosis in multiple myeloma. *Leukemia.* (2018) 32(10):2250–62. doi: 10.1038/s41375-018-0104-2

57. Sun Y, Jiang T, Jia Y, Zou J, Wang X, Gu W. LncRNA MALAT1/miR-181a-5p affects the proliferation and adhesion of myeloma cells via regulation of Hippo-YAP signaling pathway. *Cell Cycle* (2019) 18(19):2509–23. doi: 10.1080/15384101.2019.1652034

58. Taiana E, Favasuli V, Ronchetti D, Todoerti K, Pelizzoni F, Manzoni M, et al. Long non-coding RNA NEAT1 targeting impairs the DNA repair machinery and triggers anti-tumor activity in multiple myeloma. *Leukemia.* (2020) 34(1):234–44. doi: 10.1038/s41375-019-0542-5

59. Agirre X, Martinez-Climent JA, Odero MD, Prosper F. Epigenetic regulation of miRNA genes in acute leukemia. *Leukemia.* (2012) 26(3):395–403. doi: 10.1038/leu.2011.344

60. Malumbres M. miRNAs and cancer: an epigenetics view. *Mol Aspects Med* (2013) 34(4):863–74. doi: 10.1016/j.mam.2012.06.005

61. Bure IV, Nemtsova MV, Kuznetsova EB. Histone modifications and non-coding RNAs: mutual epigenetic regulation and role in pathogenesis. *Int J Mol Sci* (2022) 23 (10):5801. doi: 10.3390/ijms23105801

62. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res* (2011) 21(3):381–95. doi: 10.1038/cr.2011.22

63. Zheng JF, Guo NH, Zi FM, Cheng J. Long noncoding RNA H19 promotes tumorigenesis of multiple myeloma by activating BRD4 signaling by targeting microRNA 152-3p. *Mol Cell Biol* (2020) 40(3). doi: 10.1128/MCB.00382-19

64. Wu SY, Lee AY, Lai HT, Zhang H, Chiang CM. Phospho switch triggers Brd4 chromatin binding and activator recruitment for gene-specific targeting. *Mol Cell* (2013) 49(5):843–57. doi: 10.1016/j.molcel.2012.12.006

65. Wang R, Li Q, Helfer CM, Jiao J, You J. Bromodomain protein Brd4 associated with acetylated chromatin is important for maintenance of higher-order chromatin structure. *J Biol Chem* (2012) 287(14):10738–52. doi: 10.1074/jbc.M111.323493

66. Stubbs MC, Burn TC, Sparks R, Maduskuie T, Diamond S, Rupar M, et al. The novel bromodomain and extraterminal domain inhibitor INCB054329 induces vulnerabilities in myeloma cells that inform rational combination strategies. *Clin Cancer Res* (2019) 25(1):300–11. doi: 10.1158/1078-0432.CCR-18-0098

67. Ding J, Li J, Wang H, Tian Y, Xie M, He X, et al. Long noncoding RNA CRNDE promotes colorectal cancer cell proliferation via epigenetically silencing DUSP5/CDKN1A expression. *Cell Death Dis* (2017) 8(8):e2997. doi: 10.1038/cddis.2017.328

68. Lai IL, Yang CA, Lin PC, Chan WL, Lee YT, Yen JC, et al. Long noncoding RNA MIAT promotes non-small cell lung cancer proliferation and metastasis through MMP9 activation. *Oncotarget.* (2017) 8(58):98148–62. doi: 10.18632/oncotarget.21465

69. Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, et al. Long noncoding RNA as modular scaffold of histone modification complexes. *Science.* (2010) 329(5992):689–93. doi: 10.1126/science.1192002

70. Long Y, Wang X, Youmans DT, Cech TR. How do lncRNAs regulate transcription? *Sci Adv* (2017) 3(9):eaao2110. doi: 10.1126/sciadv.aao2110

71. Shen W, Zhu M, Wang Q, Zhou X, Wang J, Wang T, et al. DARS-AS1 recruits METTL3/METTL14 to bind and enhance DARS mRNA m(6)A modification and translation for cytoprotective autophagy in cervical cancer. *RNA Biol* (2022) 19 (1):751–63. doi: 10.1080/15476286.2022.2079889

72. Kino T, Hurt DE, Ichijo T, Nader N, Chrousos GP. Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. *Sci Signal* (2010) 3(107):ra8. doi: 10.1126/scisignal.2000568

73. Huo Y, Li Q, Wang X, Jiao X, Zheng J, Li Z, et al. MALAT1 predicts poor survival in osteosarcoma patients and promotes cell metastasis through associating with EZH2. *Oncotarget.* (2017) 8(29):46993–7006. doi: 10.18632/oncotarget.16551

74. Jing Z, Liu Q, Xie W, Wei Y, Liu J, Zhang Y, et al. NCAPD3 promotes prostate cancer progression by up-regulating EZH2 and MALAT1 through STAT3 and E2F1. *Cell Signal* (2022) 92:110265. doi: 10.1016/j.cellsig.2022.110265

75. Wang D, Ding L, Wang L, Zhao Y, Sun Z, Karnes RJ, et al. LncRNA MALAT1 enhances oncogenic activities of EZH2 in castration-resistant prostate cancer. *Oncotarget.* (2015) 6(38):41045–55. doi: 10.18632/oncotarget.5728

76. Zhu Y, Hu H, Yuan Z, Zhang Q, Xiong H, Hu Z, et al. LncRNA NEAT1 remodels chromatin to promote the 5-Fu resistance by maintaining colorectal cancer stemness. *Cell Death Dis* (2020) 11(11):962. doi: 10.1038/s41419-020-03164-8

77. Ma F, Lei YY, Ding MG, Luo LH, Xie YC, Liu XL. LncRNA NEAT1 Interacted With DNMT1 to Regulate Malignant Phenotype of Cancer Cell and Cytotoxic T Cell Infiltration via Epigenetic Inhibition of p53, cGAS, and STING in Lung Cancer. *Front Genet* (2020) 11:250. doi: 10.3389/fgene.2020.00250

78. Dinescu S, Ignat S, Lazar AD, Constantin C, Neagu M, Costache M. Epitranscriptomic signatures in lncRNAs and their possible roles in cancer. *Genes (Basel)* (2019) 10(1):52. doi: 10.3390/genes10010052

79. Cusenza VY, Tameni A, Neri A, Frazzi R. The lncRNA epigenetics: The significance of m6A and m5C lncRNA modifications in cancer. *Front Oncol* (2023) 13:1063636. doi: 10.3389/fonc.2023.1063636

80. Barbieri I, Kouzarides T. Role of RNA modifications in cancer. *Nat Rev Cancer* (2020) 20(6):303–22. doi: 10.1038/s41568-020-0253-2

81. Patil DP, Chen CK, Pickering BF, Chow A, Jackson C, Guttman M, et al. m(6)A RNA methylation promotes XIST-mediated transcriptional repression. *Nature.* (2016) 537(7620):369–73. doi: 10.1038/nature19342

82. Wu Y, Yang X, Chen Z, Tian L, Jiang G, Chen F, et al. m(6)A-induced lncRNA RP11 triggers the dissemination of colorectal cancer cells via upregulation of Zeb1. *Mol Cancer* (2019) 18(1):87. doi: 10.1186/s12943-019-1014-2

83. Ni W, Yao S, Zhou Y, Liu Y, Huang P, Zhou A, et al. Long noncoding RNA GAS5 inhibits progression of colorectal cancer by interacting with and triggering YAP phosphorylation and degradation and is negatively regulated by the m(6)A reader YTHDF3. *Mol Cancer* (2019) 18(1):143. doi: 10.1186/s12943-019-1079-y

84. He RZ, Jiang J, Luo DX. The functions of N6-methyladenosine modification in lncRNAs. *Genes Dis* (2020) 7(4):598–605. doi: 10.1016/j.gendis.2020.03.005

85. Wei W, Huo B, Shi X. miR-600 inhibits lung cancer via downregulating the expression of METTL3. *Cancer Manag Res* (2019) 11:1177–87. doi: 10.2147/CMAR.S181058

86. Du M, Zhang Y, Mao Y, Mou J, Zhao J, Xue Q, et al. MiR-33a suppresses proliferation of NSCLC cells via targeting METTL3 mRNA. *Biochem Biophys Res Commun* (2017) 482(4):582–9. doi: 10.1016/j.bbrc.2016.11.077

87. Yang Z, Li J, Feng G, Gao S, Wang Y, Zhang S, et al. MicroRNA-145 modulates N(6)-methyladenosine levels by targeting the 3'-untranslated mRNA region of the N (6)-methyladenosine binding YTH domain family 2 protein. *J Biol Chem* (2017) 292 (9):3614–23. doi: 10.1074/jbc.M116.749689

88. Long X, Wen F, Li J, Huang X. LncRNA FEZF1-AS1 accelerates multiple myeloma progression by regulating IGF2BP1/BZW2 signaling. *Hematol Oncol* (2023). doi: 10.1002/hon.3157

89. Che F, Ye X, Wang Y, Wang X, Ma S, Tan Y, et al. METTL3 facilitates multiple myeloma tumorigenesis by enhancing YY1 stability and pri-microRNA-27 maturation in m(6)A-dependent manner. *Cell Biol Toxicol* (2022) 39:2033–50. doi: 10.1007/s10565-021-09690-1

90. Huang X, Yang Z, Li Y, Long X. m6A methyltransferase METTL3 facilitates multiple myeloma cell growth through the m6A modification of BZW2. *Ann Hematol* (2023) 102(7):1801–10. doi: 10.1007/s00277-023-05283-6

91. Xu A, Zhang J, Zuo L, Yan H, Chen L, Zhao F, et al. FTO promotes multiple myeloma progression by posttranscriptional activation of HSF1 in an m(6)A-YTHDF2-dependent manner. *Mol Ther* (2022) 30(3):1104–18. doi: 10.1016/j.ymthe.2021.12.012

92. Wang C, Li L, Li M, Wang W, Jiang Z. FTO promotes Bortezomib resistance via m6A-dependent destabilization of SOD2 expression in multiple myeloma. *Cancer Gene Ther* (2023) 30(4):622–8. doi: 10.1038/s41417-022-00429-6

93. Qu J, Hou Y, Chen Q, Chen J, Li Y, Zhang E, et al. RNA demethylase ALKBH5 promotes tumorigenesis in multiple myeloma via TRAF1-mediated activation of NF-kappaB and MAPK signaling pathways. *Oncogene.* (2022) 41(3):400–13. doi: 10.1038/s41388-021-02095-8

94. Yu T, Yao L, Yin H, Teng Y, Hong M, Wu Q. ALKBH5 Promotes Multiple Myeloma Tumorigenicity through inducing m(6)A-demethylation of SAV1 mRNA and Myeloma Stem Cell Phenotype. *Int J Biol Sci* (2022) 18(6):2235–48. doi: 10.7150/ijbs.64943

95. Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, et al. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. *Mol Cell* (2013) 49(1):18–29. doi: 10.1016/j.molcel.2012.10.015

96. Wang Z, He J, Bach DH, Huang YH, Li Z, Liu H, et al. Induction of m(6)A methylation in adipocyte exosomal LncRNAs mediates myeloma drug resistance. *J Exp Clin Cancer Res* (2022) 41(1):4. doi: 10.1186/s13046-021-02209-w

97. Wen S, Wei Y, Zen C, Xiong W, Niu Y, Zhao Y. Long non-coding RNA NEAT1 promotes bone metastasis of prostate cancer through N6-methyladenosine. *Mol Cancer* (2020) 19(1):171. doi: 10.1186/s12943-020-01293-4

98. Qin J, Ke B, Liu T, Kong C, Li A, Fu H, et al. Aberrantly expressed long noncoding RNAs as potential prognostic biomarkers in newly diagnosed multiple myeloma: A systemic review and meta-analysis. *Cancer Med* (2023) 12(3):2199–218. doi: 10.1002/cam4.5135

99. Taiana E, Bandini C, Favasuli VK, Ronchetti D, Silvestris I, Puccio N, et al. Activation of long non-coding RNA NEAT1 leads to survival advantage of multiple myeloma cells by supporting a positive regulatory loop with DNA repair proteins. *Haematologica.* (2023) 108(1):219–33. doi: 10.3324/haematol.2022.281167

100. Wu Y, Wang H. LncRNA NEAT1 promotes dexamethasone resistance in multiple myeloma by targeting miR-193a/MCL1 pathway. *J Biochem Mol Toxicol* (2018) 32(1). doi: 10.1002/jbt.22008

101. Chen YS, Yang WL, Zhao YL, Yang YG. Dynamic transcriptomic m(5) C and its regulatory role in RNA processing. *Wiley Interdiscip Rev RNA* (2021) 12(4):e1639. doi: 10.1002/wrna.1639

102. Ren H, Liu C, Wu H, Wang Z, Chen S, Zhang X, et al. m(5)C Regulator-mediated methylation modification clusters contribute to the immune microenvironment regulation of multiple myeloma. *Front Genet* (2022) 13:920164. doi: 10.3389/fgene.2022.920164

103. Liu X, Wang D, Han S, Wang F, Zang J, Xu C, et al. Signature of m5C-Related lncRNA for Prognostic Prediction and Immune Responses in Pancreatic Cancer. *J Oncol* (2022) 2022:7467797. doi: 10.1155/2022/7467797

104. Pan J, Huang Z, Xu Y. m5C-related lncRNAs predict overall survival of patients and regulate the tumor immune microenvironment in lung adenocarcinoma. *Front Cell Dev Biol* (2021) 9:671821. doi: 10.3389/fcell.2021.671821

105. Bai M, Sun C. M5C-related lncRNA predicts lung adenocarcinoma and tumor microenvironment remodeling: computational biology and basic science. *Front Cell Dev Biol* (2022) 10:885568. doi: 10.3389/fcell.2022.885568

106. Safra M, Sas-Chen A, Nir R, Winkler R, Nachshon A, Bar-Yaacov D, et al. The m1A landscape on cytosolic and mitochondrial mRNA at single-base resolution. *Nature.* (2017) 551(7679):251–5. doi: 10.1038/nature24456

107. Goyal B, Yadav SRM, Awasthee N, Gupta S, Kunnumakkara AB, Gupta SC. Diagnostic, prognostic, and therapeutic significance of long non-coding RNA MALAT1 in cancer. *Biochim Biophys Acta Rev Cancer* (2021) 1875(2):188502. doi: 10.1016/j.bbcan.2021.188502

108. Liu N, Feng S, Li H, Chen X, Bai S, Liu Y. Long non-coding RNA MALAT1 facilitates the tumorigenesis, invasion and glycolysis of multiple myeloma via miR-1271-5p/SOX13 axis. *J Cancer Res Clin Oncol* (2020) 146(2):367–79. doi: 10.1007/s00432-020-03127-8

109. Gu Y, Xiao X, Yang S. LncRNA MALAT1 acts as an oncogene in multiple myeloma through sponging miR-509-5p to modulate FOXP1 expression. *Oncotarget.* (2017) 8(60):101984–93. doi: 10.18632/oncotarget.21957

110. Chen Y, Lin H, Miao L, He J. Role of N7-methylguanosine (m(7)G) in cancer. *Trends Cell Biol* (2022) 32(10):819–24. doi: 10.1016/j.tcb.2022.07.001

111. Han J, Liu Q, Zhou Y, Li D, Wang R. Landscape of internal N7-methylguanosine of long non-coding RNA modifications in resistant acute myeloid leukemia. *BMC Genomics* (2023) 24(1):425. doi: 10.1186/s12864-023-09526-8

112. Yang S, Zhou J, Chen Z, Sun Q, Zhang D, Feng Y, et al. A novel m7G-related lncRNA risk model for predicting prognosis and evaluating the tumor immune microenvironment in colon carcinoma. *Front Oncol* (2022) 12:934928. doi: 10.3389/fonc.2022.934928

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 08 January 2024
DOI 10.3389/fonc.2023.1239042

OPEN ACCESS

EDITED BY
Mario Tiribelli,
University of Udine, Italy

REVIEWED BY
Cosimo Cumbo,
University of Bari Aldo Moro, Italy
Jafar Rezaie,
Urmia University of Medical Sciences, Iran

*CORRESPONDENCE
Olga Mulas
mulasolga@unica.it

†These authors have contributed equally to this work

RECEIVED 12 June 2023
ACCEPTED 27 November 2023
PUBLISHED 08 January 2024

CITATION
Bernardi S, Mulas O, Mutti S, Costa A, Russo D and La Nasa G (2024) Extracellular vesicles in the Chronic Myeloid Leukemia scenario: an update about the shuttling of disease markers and therapeutic molecules. *Front. Oncol.* 13:1239042. doi: 10.3389/fonc.2023.1239042

Extracellular vesicles in the Chronic Myeloid Leukemia scenario: an update about the shuttling of disease markers and therapeutic molecules

Simona Bernardi[1,2†], Olga Mulas[3*†], Silvia Mutti[1,2], Alessandro Costa[3], Domenico Russo[1] and Giorgio La Nasa[3]

[1]Department of Clinical and Experimental Sciences, University of Brescia, Unit of Bone Marrow Transplantation, Azienda Socio Sanitaria Territoriale (ASST) Spedali Civili of Brescia, Brescia, Italy, [2]Lab CREA (Centro di Ricerca Emato-oncologica Associazione italiana contro le leucemie, linfomi e mieloma-AIL), ASST Spedali Civili of Brescia, Brescia, Italy, [3]Department of Medical Sciences and Public Health, University of Cagliari, Hematology Unit, Businco Hospital, Cagliari, Italy

Extracellular vesicles (EVs) are various sets of cell-derived membranous structures containing lipids, nucleic acids, and proteins secreted by both eukaryotic and prokaryotic cells. It is now well recognized that EVs are key intercellular communication mediators, allowing the functional transfer of bioactive chemicals from one cell to another in both healthy and pathological pathways. It is evident that the condition of the producer cells heavily influences the composition of EVs. Hence, phenotypic changes in the parent cells are mirrored in the design of the secreted EVs. As a result, EVs have been investigated for a wide range of medicinal and diagnostic uses in different hematological diseases. EVs have only recently been studied in the context of Chronic Myeloid Leukemia (CML), a blood malignancy defined by the chromosomal rearrangement t(9;22) and the fusion gene BCR-ABL1. The findings range from the impact on pathogenesis to the possible use of EVs as medicinal chemical carriers. This review aims to provide for the first time an update on our understanding of EVs as carriers of CML biomarkers for minimal residual disease monitoring, therapy response, and its management, as well as the limited reports on the use of EVs as therapeutic shuttles for innovative treatment approaches.

KEYWORDS

Chronic Myeloid Leukemia, extracellular vesicles, exosomes, vesicular markers, therapeutic shuttle, TKIs

1 Pathogenesis of Chronic Myeloid Leukemia

Chronic Myeloid Leukemia (CML) is a blood cancer characterized by the uncontrolled growth of myeloid cells at different stages of maturation that may be detected both in the bone marrow (BM) and in the peripheral blood (PB) (1). Classically subdivided into three clinical forms, the chronic (CP), accelerated (AP), and blastic phases (BP), the CML represented the pathfinder for many discoveries in the medical domain (2). The translocation t (9;22), also known as Philadelphia (Ph)-chromosome, was identified as the hallmark of CML (3), and the subsequent identified BCR-ABL1 fusion gene is the central player in the pathogenesis of CML. BCR-ABL1 encodes a 210 KD chimeric protein with constitutively active tyrosine kinase activity that promotes several downstream signaling pathways in neoplastic cells (4). In particular, the expression of this oncoprotein leads to altered adhesion to stromal cells and the extracellular matrix, promoting survival and inhibiting apoptosis (3). In addition, cellular transformation and the acquisition of self-renewal capacity are facilitated. Tyrosine kinase inhibitors (TKIs) are the mainstay of current CML treatment. Thanks to their administration, high remission rates have been recorded, and improvements in patient survival rates have been observed. Current guidelines endorse using imatinib, dasatinib, nilotinib, and bosutinib as frontline treatment options in CML patients (5). On the other hand, third-generation TKI ponatinib and newer asciminib are intended for patients previously treated with two or more TKIs or those harboring the T315I mutation (6). Nevertheless, CML continues to be a significant challenge in clinical practice due to the difficulty in predicting its progression and prognosis and the inter-patients variability in CP's duration and treatment response. Indeed, the presence of the BCR-ABL1 oncoprotein is known to provide for the acquisition of additional genetic abnormalities, likely by increased genomic instability (7). The consequence of this clonal evolution is associated with an increased incidence of relapse, poor prognosis, resistance to TKIs treatment, and, unfortunately, the advancement into blastic crisis (8). The more frequent additional genetic abnormalities detected are duplication of the Ph chromosome, trisomy 8, isochromosome 17, loss of chromosome Y or monosomy 7. In addition, like other hematological malignancies, loss of Tp53 is associated with increased resistance to apoptosis (9, 10). Other important elements in the CML scenario are the leukemic stem cells (LSC) resident in the bone marrow niche. LSC are characterized by the presence of BCR-ABL1 rearrangement and a quiescence that leads to the absence of BCR::ABL1 transcript. The non-transcription of the fusion gene makes LSC undetectable by conventional approaches, such as the quantification of BCR::ABL1 transcript by real-time PCR that is the basis of the minimal residual disease (MRD) monitoring (11, 12). Recently, extracellular vesicles (EVs) and exosomes have generated considerable interest in cancer research (13–15). Increasing evidence suggests that these vesicles are important in regulating immune stimulation or suppression that can drive inflammatory, autoimmune, and infectious disease pathology (16). Given their involvement in disease progression and treatment resistance, EVs have been proven to play an active role in the tumor microenvironment (TME) in the past few years (17, 18). Several findings indicate that they also play a key role in the hematological field and appear to be actively released by LSC. Less is known about their function in CML, especially regarding their potential clinical significance. The purpose of this review is to provide for the first time an overview of the data presented about EVs in CML with a special focus on their role as a shuttle of disease markers and, wherever possible, as new therapeutic approaches.

2 The extracellular vesicles

Extracellular vesicles (EVs) are a heterogeneous group of cell-derived membranous structures secreted by both eukaryotic and prokaryotic cells containing lipids, nucleic acids, and proteins. Chargaff and West firstly observed EVs as procoagulant platelet-derived particles in normal plasma in the mid-40s (19). Their presence in various body fluids was then discovered through several studies during the 1970s-80s (20–22). Concomitantly, other researchers observed their origin from tumor masses. The term exosome was born in the same period, referring to vesicles released by multi-vesicular bodies that fuse with the cell membrane. Finally, in the early 2000s, thanks to the evidence that EVs contain nucleic acids, including RNAs such as microRNA (miRNA), EVs acquired substantially renewed interest as players in the cell-to-cell communication (23, 24). Advancing on these pioneering studies, EVs have been resulted as released by most cell types and isolated from different biological fluids (25, 26).

In 2012 The International Society for Extracellular Vesicles (ISEV) was founded. ISEV, including scientists involved in the study of extracellularly secreted vesicles, is considered the reference for the EVs classification and EVs analysis promotion. Based on the current knowledge, EVs can be roughly classified into two main subtypes regarding their physical characteristics and biogenesis pathway: small-EVs (sEVs) and large-EV. The formers have a diameter that ranges between 30–150 nm and include the so-called exosomes. These vesicles derive from intraluminal vesicles formed by the inward budding of the endosomal membrane during the maturation of multivesicular endosomes (MVEs). These are intermediates within the endosomal system and released through the fusion of MVEs with the cell surface. On the other hand, large-EVs represent a heterogeneous population of microvesicles with a diameter that can reach up to 1000 nm. Large vesicles are generated by the outward budding and fission of the cellular lipid membrane and the subsequent secretion of vesicles into the extracellular space (27). As previously stated, EVs are currently established as pivotal mediators of intercellular communication, capable of functionally transferring bioactive molecules from the cell of origin to another in both physiological and pathological pathways. Indeed, released EVs may interact with the releasing cells, therefore acting as autocrine mediators, and with other cell types located both close and far from the cell of origin. Indeed, they act as paracrine or endocrine mediators. EVs exchange information between cells by shuttling

several types of molecules, such as proteins, lipids, and the above reported nucleic acids, many of which are selectively sorted inside vesicles (28). Among these regulatory molecules, the miRNAs are expressed by all cell types. Identifying miRNA related to hematological diseases opened up new avenues in biomarkers research. MiRNAs are a family of short, noncoding RNAs that participate in the post-transcriptional regulation of gene expression. They modulate the translation of messenger RNAs (mRNAs) through mechanisms based on the binding of complementary sequences to 3'UTR of mRNA. Their expression is a dynamic process that reflects the evolution of the physiological and pathological condition at the cellular level, which could be an innovative tool in the hematological fields. Indeed, it is well known that they play key roles in almost every cellular process (29).

It is clear that EVs composition largely depends on the status of the producer cells and therefore, one can say that phenotypic alterations in the cell of origin are mirrored by the composition of the secreted EVs, both in terms of EVs type and in terms of cargoes. As a consequence, a multitude of therapeutic and diagnostic applications have been explored for EVs (30, 31). Diagnostic applications take advantage of the different information shuttled by the specific EVs, like the presence of a genetic mutation associated with a disease. On the other hand, therapeutic approaches exploit the EVs capacity to carry and release potentially bioactive molecules (32). In the CML context, the main cells with which the leukemic cell communicates via sEVs are hematopoietic and mesenchymal stem cells, myeloid-derived suppressor cells and endothelial cells (ECs) (33). It is well known that tumor-derived EVs have a remarkable impact on the different recipient cells. Indeed, their effect on cellular proliferation and resistance to apoptosis, induction of angiogenesis, evasion from immune response, transfer of mutations, and modulation of the TME sustains their role as central mediators in key cancer processes. Recent studies confirmed the above-cited pro-tumorigenic activity also in the case of CML-derived EVs. In fact, recent data suggest that they could establish an autocrine loop with their producing cells, through a ligand-receptor interaction mediated by the exosome-associate TGF-β1 (34). Taverna et al. underlined that EVs released from CML cells could affect ECs directly by inducing their release of proangiogenic cytokines, such as IL8, thus modulating neovascularization, which plays an important role in the development and progression of CML (35). The same group has also highlighted that exosomal transfer of miR-126 to ECs directly modulates the adhesive and migratory abilities of CML cells themselves. Other groups reported that the communication between CML cells and surrounding BM stromal cells by CML-derived EVs leads to the inhibition of osteogenesis and thus promotes CML progression. Together, they showed that CML-derived EVs reduce the tumor-suppressive miR-320 in donor cells, resulting in enhanced cell growth *in vivo* models (36). Additionally, CML-derived EVs released by *in vitro* models may transfer the BCR::ABL1 mRNA to normal BM cells, inducing BCR–ABL1 ectopic expression. This intercellular transfer of active biomolecules changes cells' behavior and promotes disease progression. The disease progression is partially favored by changes in the TME (37) and the immune system's tolerance. It could be driven by leukemia-derived EVs, as supposed by an Iranian Group who reported that EVs derived by an *in vitro* model of CML drive a tumor-favorable functional performance in T cells (38). This latter evidence has been confirmed by Swatler and colleagues, who demonstrated that leukemic sEVs derived from CML cells promoted leukemia engraftment, associated with an abundance of immunosuppressive T regulatory lymphocytes (Tregs). In the used animal model, the recipient cells changed their transcriptional profile and activated a suppressive activity and effector phenotype by regulating specific receptors' expression (39, 40). For these reasons, the analysis of sEVs' features, cargoes, and their potential roles in pathogenesis investigations, patients' management, and therapy delivery increased the interest of scientists in these small "bullets".

3 sEVs as shuttle of CML markers

As reported above, circulating sEVs cargo has been deeply analyzed to detect potential shuttled leukemia markers (41). Despite the recurring availability of leukemic cells in myeloid leukemias in either BM and/or PB, many groups have conducted studies aiming to improve the sensitivity of the analysis and to reduce the number of invasive and painful BM biopsy (42). Considering the role played by the sEVs and the successful results obtained in the solid tumors by oncologists, these sEVs have also been investigated for their ability to shuttle leukemic biomarkers. DNA, miRNA, mRNA, protein, or lipid profiles associated with different hematologic malignancies are expected to be identified in patients' sEVs (43). In the CML scenario, the recent insight of circulating sEVs as leukemic biomarkers has highlighted their potential for more sensitive liquid biopsy approaches for an accurate MRD monitoring, a TFR optimization, and an optimal evaluation of drug efficacy. In the following sections, we will critically present and discuss the main results of these pivotal aspects of adult CML patient management.

3.1 sEVs for CML MRD monitoring

Thanks to the high efficacy of TKIs targeting BCR-ABL1, the efforts of physicians involved in CML moved from "save the patient" to "monitor the patient" as best as possible. The present strategy for MRD monitoring is based on the quantification of BCR::ABL1 transcript on PB cells, normalized for a reference gene (ABL1 is mainly used). The MRD quantification is internationally standardized and routinely performed by quantitative real-time PCR (RT-qPCR). Two main molecular classes are identifiable. Major Molecular Response (MMR) and Deep Molecular Response (DMR). MMR (also defined as MR3.0) consists of the reduction of the BCR::ABL1 transcript level by at least 3 logs and results in BCR::ABL1/ABL1 ratio < 0,1%. DMR, defined as a BCR::ABL1/ABL1 ratio ≤ 0.01%, can be further subdivided into MR4.0, MR4.5, or MR5.0 when the logarithmic reduction is 4, 4.5, or 5 logs. These reductions are identifiable by BCR::ABL1/ABL1 ratio ≤ 0.01%, ≤ 0.0032%, and ≤ 0.001%, respectively. The sample is considered good

quality when ABL1 transcript copies are more than 10.000 at MR4.0, 32.000 at MR4.5, and 100.000 at MR5 (44). These minimums are essential for the definition of the DMR classes in case of undetectable BCR::ABL1 transcript.

Due to the pivotal importance of MRD monitoring in CML patients, sEVs seemed to be very interesting biological tools to support a sensitive, reliable, and relevant detection of resident active leukemic cells. Some years ago, CML researchers questioned the potential role of CML-derived vesicles as disease biomarkers and new sources for the detection of the BCR::ABL1 transcript. The first method relied on the isolation of EVs from the plasma of CML patients via ExoQuickTM Exosome Precipitation Solution and the identification of BCR::ABL1 transcripts based on nested PCR. Even though only patients in the blast and accelerated phases pre-sent BCR::ABL1 transcript within vesicular cargo, vesicular RNA sequence analysis indicated 99% similarity with human cellular BCR::ABL1 (45). Further studies have been carried out as a result of the development of more sophisticated and potent technologies for sEVs isolation and transcript detection, such as dPCR (46, 47). Specifically, it was reported the feasibility of detecting BCR::ABL1 vesicular transcripts in CP-treated CML patients with undetectable MRD levels by standard monitoring (48). The hypothesis of using sEVs content analysis to enhance the detection of active leukemia cells still present in patients' bodies has been highlighted by this crucial result. Bernardi et al. used a commercial kit immuno-capturing sEVs expressing a pan-cancer antigen to examine the viability of a leukemia-derived sEVs enrichment. Leukemia sEVs enrichment and a BCR::ABL1 transcript detection technique based on dPCR gave the proposed approach a head start (49). The researchers showed for the first time that BCR::ABL1 transcripts could be detected in exosomes circulating in CML patients' PB, even in cases of patients under TKIs treatment and presenting undetectable MRD levels (50). Moreover, these BCR::ABL1-positive exosomes have been reported as useful in determining the molecular remission grade.

Deviating from the classical MRD monitoring strategy based on the cellular BCR::ABL1 transcript quantification, in the last decade the role of miRNAs in various biological developmental processes and the alteration of their expression was found to broadly influence the phenotype of many cancer subtypes. Many studies have identified hundreds of differentially expressed genes at each stage of the disease using the microarray approach on CML cell lines (51, 52). Flamant and colleagues showed an increased expression of miR-150 and miR-146a, and reduced expression of miR-142-3p and miR-199b-5p in CML cells after 2 weeks of TKI treatment, identifying miRNA as easily measurable biomarkers to monitor the response to TKI (53). Indirectly, it may be considered a measure of viable leukemic cells. Similar recently published results demonstrated that a higher miR-150 and miR-146a expression level predict early response rate in imatinib-treated CML patients (54, 55). Other groups described vesicular miR-29b, miR-320a, miR-30a, and miR-30e as overexpressed in a CML cell line. These miRNA showed to play a role of tumor suppressor, reducing cell proliferation and inducing apoptosis by interfering with BCR-ABL1 activated pathways (56, 57). Moreover, the comparison of vesicular miRNAs between CML patients and healthy subjects highlighted a set of these non-coding RNA differentially expressed in CML patients. This evidence suggests they could play a role in the clinical diagnosis, prognostication, and evaluation of treatment response. For example, the expression of miR-506 and mir451a was shown to be noticeably lower in CML patients than in healthy controls. Moreover, the expression is significantly reduced by the leukemic progression in AP and BP (58, 59). On the other hand, miR-21 increased in CML patients with higher expression in the advanced stages of the disease (60). Some of the main relevant differences in vesicular miRNA expression in CML are recapitulated in Table 1.

A very interesting breakthrough in the field of sEVs was directed by Valadi and colleagues in 2007. They first reported that exosomes, along with their lipid and protein cargo, contain a significant amount of nucleic acids, particularly mRNAs (24), which lately resulted in translatable into proteins by recipient cells (64). This means that EVs shuttle genetic information. Of note, the term "exosomes" used in the presented studies refers to the EVs classification valid at the time of publication. These new findings, along with the multiple studies regarding the role of miRNAs as disease markers, open the way for exploring vesicular ribonucleic acids as novel reliable disease biomarkers in MRD monitoring.

3.2 sEVs in TFR optimization

Among CML patients, a number of them may sustain a TKIs therapy discontinuation, after which they may achieve "treatment-

TABLE 1 Differences in vesicular miRNA expression in CML and their potential implication in clinical practice.

miRNA	EVs Source	Expression	Biomarker	Ref
miR-506	Serum	Down-regulation	Diagnostic, Prognostic	(58)
miR-451a	Plasma	Down-regulation	Prognostic	(59)
miR-21	Blood	Up-regulation	Prognostic,treatment response	(60)
miR-146a	Plasma	Up-regulation	Treatment response	(54)
miR-148b	Blood	Down-regulation	Treatment response	(61)
miR-215	Plasma	Down-regulation	Treatment response	(62)
miR-199b	Plasma	Down-regulation	Treatment response	(63)

EVs, Extracellular Vesicles.

free remission" (TFR) (65). Generally, TKIs discontinuation strategy is adopted in patients that present deep and durable (2 or 3 years) molecular response (DMR), as routinely assessed through RT-qPCR. Nevertheless, many clinical trials demonstrated that no more than 50% can maintain TRF (66). In the last years, it has become clear that the intrinsic limitations of RT-qPCR, among which is the reduced precision in the quantification of the low levels of the target (BCR::ABL1 transcript), are to be considered the main culprit in the erroneous selection of patients eligible for a TKIs discontinuation program. As anticipated, the advent of dPCR opened the way for novel MRD monitoring strategies. dPCR was developed to overcome some of the major limitations of conventional amplification technologies, increasing precision, accuracy, and sensitivity. Bernardi and colleagues underlined that dPCR offers an accurate quantification of BCR::ABL1 transcripts in circulating sEVs, even in patients presenting undetectable MRD levels by conventional monitor. Indeed, it has been proven the capacity of this strategy to improve the detectability of cells releasing BCR::ABL1-positive sEVs. Would this approach support the selection of patients eligible for TKIs therapy discontinuation aiming at TFR? (50). Further studies are needed to answer this question, even if the preliminary results reported in other disease settings are very encouraging (67–69). In order to find potential alternative markers for stopping TKIs use, the role of vesicular miRNA has also been studied. In particular, miR-215 expression was downregulated in the research by Kazuma Ohyashiki et al., both at the cellular and sEVs levels, in CML cases with successful imatinib discontinuation (62). The same authors found that the downregulation of miR-148b had similar effects in TFR patients (61). This data indicates that these miRNA may help with immune surveillance in CML patients with safe TKI discontinuation (61, 62).

Regardless, achieving TFR in a CML setting involves the management of several side effects. Musculoskeletal pain is a common symptom following TKIs discontinuation. However, further insight is still needed to determine the potential contributing variables to this clinical condition. The discovery of a potential main character was made possible by analyzing the exosomal miRNA that circulates in CML patients who have stopped using TKIs. TaqMan low-density array was used to profile exosomal miRNAs, and the results showed that exosomal miR140-3p was substantially elevated in CML patients who reported musculoskeletal pain compared to patients who did not ($p = 0.0336$) and healthy controls ($p = 0.0022$). MiR140-3p is thought to have a biological role in inflammation, and CML patients who have experienced symptom relief have substantially lower exosomal levels of the protein. These findings suggest that exosomal miRNA analysis could be used to identify treatment side effects or effectiveness when TKIs are being used (70).

3.3 sEVs for therapy efficacy

Despite the TKIs' above-mentioned remarkable effectiveness in treating CML, a tiny percentage of patients develop drug resistance while receiving TKIs therapy. Physicians are still triggered by it. The BCR-ABL1 protein's acquired point mutations are primarily linked to TKIs resistance, but little is known about how resistance traits can develop in cells lacking these variants. One of the most recent processes, similar to what has been seen in acute myeloid leukemia cells, is the vesicular-mediated transfer of molecules from resistant to sensitive CML cells (71). In particular, high levels of exosomal miR365 have been reported in cases of lower drug sensitivity and lower apoptosis rate. The exposure of sensitive CML cells to exosomes released by resistant and miR365-rich cells induced drug resistance. This process is due to the inhibition of pro-apoptotic proteins in sensitive CML cells (72). In support of these results, the influence of circulating miRNA in CML-derived cells has been demonstrated even when they are not carried by vesicles. Hershkovitz-Rokah et al. demonstrated the capacity of miR-30e to sensitize K562 cells and patient primary cells to imatinib treatment through regulation of cell cycle progression between G1 and S phases (73). MiR-199b targets HES1, a transcription factor involved in the Notch pathway and highly conserved among multicellular organisms. It regulates cell-fate determination during development and maintains adult tissue homeostasis. Expression studies have revealed downregulation of miR-199b in CML patients presenting 9q deletion. A lower level of miR-199b was found in imatinib-resistant patients, suggesting that it could be considered one of the factors for drug resistance (63).

Moreover, an additional study confirmed that exosomes released by imatinib–resistant K562 (K562IR) cells and internalized by imatinib–sensitive cells of the same line (K562IS) could increase the survival of the latter. This phenomenon was observed even in the presence of toxic doses of imatinib (2 μM). K562IR-exosomes characterization led to three specific cell-surface markers, namely, IFITM3, CD146, and CD36, that resulted in upregulation when compared to K562IS-exosomes. The upregulation of these proteins was later verified in the K562IR cells confirming that sEVs mirror the parental cell's features. Flow cytometric analysis further demonstrated the potential of CD146 as a cell surface marker expressed by K562 cells presenting imatinib resistance. These results suggest that exosomes and the related membrane proteins could be potential diagnostic markers of drug resistance in CML patients treated with TKIs (74). Conversely, miR328 has been reported as significantly associated with sensitivity to first-generation TKI in another *in vitro* CML model. For instance, *in vitro* delivery of alkalized exosomes, containing or not miR328 as cargo, elevated endogenous miR328 levels, inducing sensitivity to imatinib. Moreover, endogenous miR328 suppression produced imatinib resistance in the K562 CML cell line (75). Similarly, miR-185 expression sensitizes Ph+ cells to TKIs-induced apoptosis and affects their proliferation rate, partly through a BCR-ABL1-kinase-dependent mechanism. Overall, restoration of miR-185 expression had an evident effect on the survival of patient-derived TKI-insensitive stem/progenitor cells isolated in patients and cultured *in vitro* in the presence of TKIs (76). In addition, the imatinib sensitivity of K562 cell line was tested in another trial administering exosomes released by human umbilical cord MSCs (hUC-MSCs) during the cell culture. Exosomes released by hUC-MSCs alone seem to unaffected cell viability but promote imatinib-induced cell death. Moreover, they activate caspase-9 and caspase-3 more than imatinib alone (77). Lately, Chen X. et al., elucidated the

role of miR-146a-5p/USP6/GLS1 in leukemia and chemoresistance of leukemia cells and confirmed hUC-MSC exosomes capacity to promote imatinib-induced cell apoptosis through miR-145a-5p/USP6. USP6 levels were elevated and related to a poor prognosis in BM aspiration samples from CML patients. Compared to clinical samples that were imatinib-sensitive, USP6 was markedly increased in imatinib-resistant samples. Leukemia cells' apoptosis was dramatically reduced by USP6 overexpression in response to imatinib. Increased GLS1 ubiquitination caused by overexpression of USP6 reduced GLS protein. A mechanistic investigation revealed that miR-146a-5p and GLS1 were both required for USP6 control of the imatinib resistance of CML cells. Through miR-145a-5p/USP6, the administration of hUC-MSCs exosomes increased imatinib-induced cell death. Therefore, through miR-146a-5p and its target GLS1, hUC-MSC exosomes increased imatinib-induced death of K562-R cells by decreasing GLS1 ubiquitination and increasing GLS protein. The research sheds fresh information on the role of miR-146a-5p/USP6/GLS1 signaling in leukemia chemoresistance (78).

Little is known about the role of second-generation TKIs. Although no data are reported on the role of vesicles in this context, direct expression of miRNA still appears to have an important place in the interaction of other TKIs besides imatinib. This also supports what above reported and commented. Indeed, the combined expression of different miRNAs was recently investigated in CML cells exposed to nilotinib. Particularly, miR-145 and miR-708 expressions were associated as a predictive indicator of nilotinib response at the treatment-naïve state. In addition, higher expressions of miR-150 and decreased levels of miR-185 were found in nilotinib non-responders, compared with nilotinib responders (79). Liu et al. described one of the possible mechanisms through which dasatinib could be able to overcome imatinib resistance. Their reports highlighted that dasatinib promotes cellular apoptosis by downregulation of Akt/mTOR pathway activities. Moreover, dasatinib prevents exosomal release through the downregulation of beclin-1 and Vps34-dependent autophagic activity. These results suggest distinct dasatinib-induced activation of apoptotic response and exosome generation in CML cells resistant to first-generation TKI (80). Hence, the synergy of exosomes and TKIs may be considered an effective approach to improve the response rate during CML treatment and provide an interesting basis for new therapeutic strategies designed for chemoresistant/target therapy-resistance leukemia.

All of the cited mechanisms are summarized in Figure 1.

4 EVs as shuttle of therapeutic molecules

Despite the success of TKIs-based therapy, the exploration of exosome-based therapy, which combines the vesicles with both TKIs and unusual molecules, was surprisingly more prevalent in CML. The target of CML blasts was reported in an outstanding work using modified exosomes loaded with imatinib. HEK293T cell line transfected with plasmids encoding the exosomal protein Lamp2b fused to a portion of interleukin 3 (IL3) was used. The researchers selected this protein because it is known that the IL3 receptor is overexpressed on the surface of CML blasts. It was found that imatinib and BCR-ABL1-silencing RNA could be delivered to CML cells by exosomes produced by the transfected cells. In sensitive and resistant models, as well as *in vitro* and *in vivo* models, this ability resulted in reduced leukemia cell proliferation. For example, in a mouse model, imatinib-laden IL3-exosomes significantly reduced the tumor burden when compared to

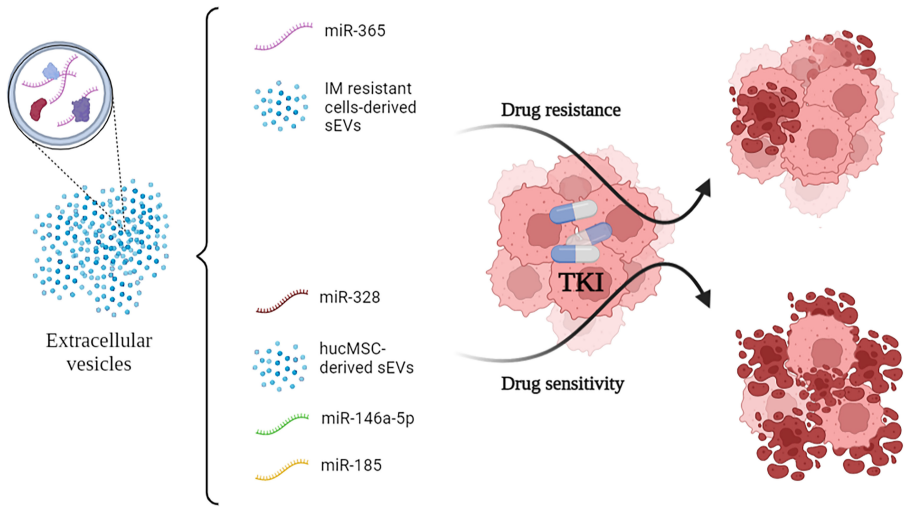

FIGURE 1
Small-EVs and some of their encapsulated miRNA show opposite effects on the efficacy of TKI administration to CML cells. In particular, vesicular miR-365 drives resistance to TKI in the recipient cells. Conversely, miR-328, miR-146a-5p and miR-185 drive sensitivity to TKI. TKI, Tyrosine Kinase Inhibitors.

imatinib-free IL3-exosomes, regular exosomes loaded with imatinib, and imatinib alone ($p < 0.0005$) (81). Similarly, CML exosomes exposed to a TGF-β1 receptor inhibitor or a specific neutralizing TGF-β1 antibody significantly reverse the proliferation of CML cells compared to those exposed to TGF-β1 enriched LAMA84 exosomes (34). These pivotal data strongly support the application of exosomes as specific drug delivery tools even in CML, as observed in TKIs-resistant cells. The authors achieved these impressive results after many evidences obtained by using exosomes for delivery of unconventional molecules *in vitro* models of CML. In particular, the authors reported the impact of curcumin on CML exosome composition (82) and the use of common lemon-juice-derived small vesicles. The latter were able to suppress leukemia proliferation in xenograft models using NOD/SCID mice subcutaneously inoculated with CML cells. On the other hand, alkalized exosomes have been shown to block miR328 lysosomal degradation and thus sensitize CML cells to imatinib (75). In addition, the exosomes specifically reached the leukemic site within the mice model and activated apoptotic cell processes (83). Recently, Cochran et al. showed that Natural Killer-derived exosomes (NKexo) were able to maintain the anti-leukemia capacity of their donor NK-cells, NKexo resulted in cytotoxic against malignant hematopoietic cell lines (K562 and Jurkat), thus acting as a potential acellular therapeutic modality (84). Results showed that low doses of NK3.3 EVs inhibited the growth of K562 cells over 72 h, while high doses of NK3.3 EVs were cytotoxic. These findings were verified by Samara's group's latest study (85), which additionally provided a more comprehensive analysis of the time- and dose-dependent antileukemic activity of NKexo, on a wider variety of leukemia cell lines and ex-vivo models derived from patients' samples. Firstly, they showed that NKexo (20 μg) have the ability to increase apoptosis rates by up to 64.37 (± 11.7%) across all biomodels, including K562. In contrast, healthy-donor PB Mononuclear Cells presented no alteration, suggesting a selective cytotoxic effect targeting leukemia cells. Moreover, NKexo cytolytic activity via the release of cytotoxic effectors was confirmed, and a reduction in cell count, ranging between 65% and 84%, was seen in all leukemia cell lines tested, including K652. Finally, the clonogenic potential of treatment-naïve CML-derived cells was significantly reduced by 20 μg of NKexo during a 14 days long cell culture. The relative colony-formation efficiency of CML cells was reduced by an average of 28 ± 14% ($p \leq 0.005$). Similarly, umbilical cord mesenchymal cell-derived exosomes were proven able to promote Imatinib-induced apoptosis in K562-R cells via miR-146a-5p and its target USP6, which suppress GLS1 ubiquitination, causing an increase in GLS protein (78). Zang et al. recently demonstrated that another source of mesenchymal cell-derived exosomes, such as human BM, could inhibit the proliferation of CML cells *in vitro* via miR-15a and arrest the cell cycle in the G0/G1 phase. On the other hand, the same authors found that these mesenchymal exosomes promoted the proliferation and decreased the sensitivity of CML cells to TKIs, resulting in drug resistance in the xenograft tumor model (86). Finally, to assess innovative therapeutic approaches, another very interesting strategy was evaluated to increase treatment outcomes in CML patients. Indeed the authors developed a sophisticated liposome conjugated with Begelomab (anti-CD26) loaded with venetoclax to target CD26+ CML LSCs/progenitor cells selectively. They proved that the CD26+ LSCs/progenitor cells could be eliminated after antigen binding and drug release without any side effects on CD26− cells (87).

5 Conclusions

In this review, we have brought together the main knowledge about extracellular vesicles, comprehensively addressing various crucial CML aspects. CML has been the first disease for which a targeted therapy was identified, paving the way for novel treatments in other hematological fields. Over the course of its history, there has been a gradual improvement in monitoring and new goals of treatment, such as TFR, have been attained. Despite few data available, if compared to other cancers, EVs could have multiple applications in CML. Undoubtedly, many efforts have been made to evaluate the role of EVs in treatment response monitoring and encouraging results have been observed in MRD monitoring. It is fascinating to see the possible application of these extracellular bodies into TKIs-resistant disease and their role, such as shuttle for other specific drugs. On the other hand, the lack of standardization, and the large variability in EVs, imply that their use is still limited to often speculative valuations. Further research is needed to understand their role. In summary, this review could be an essential source of knowledge for future studies about EVs as a crucial mediator for new therapeutic strategies in CML.

Author contributions

Conceptualization, SB and OM; data curation, SB, OM, SM, AC, DR and GL; writing—original draft preparation, SB, OM, SM, and AC; writing—review & editing, SB, OM, SM, AC, DR and GL, supervision, SB, OM, DR and GL. All authors have read and agreed to the published version of the manuscript.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The APC was funded by Department of Medical Sciences and Public Health, University of Cagliari and Dipartimento di Scienze Cliniche e Sperimentali, Università degli studi di Brescia, viale Europa 11, 25123, Brescia.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Goldman JM, Melo JV. Targeting the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* (2001) 344:1084–6. doi: 10.1056/NEJM200104053441409

2. Hochhaus A, Dreyling M. ESMO guidelines working group chronic myelogenous leukemia: ESMO clinical recommendations for the diagnosis, treatment and follow-up. *Ann Oncol Off J Eur Soc Med Oncol* (2008) 19 Suppl 2:ii63–64. doi: 10.1093/annonc/mdn091

3. Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. *Nat Rev Cancer* (2005) 5:172–83. doi: 10.1038/nrc1567

4. Perrotti D, Jamieson C, Goldman J, Skorski T. Chronic myeloid leukemia: mechanisms of blastic transformation. *J Clin Invest.* (2010) 120:2254–64. doi: 10.1172/JCI41246

5. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, et al. European leukemiaNet 2020 recommendations for treating chronic myeloid leukemia. *Leukemia* (2020) 34:966–84. doi: 10.1038/s41375-020-0776-2

6. Cortes JE, Kim D-W, Pinilla-Ibarz J, le Coutre P, Paquette R, Chuah C, et al. A phase 2 trial of ponatinib in philadelphia chromosome-positive leukemias. *N Engl J Med* (2013) 369:1783–96. doi: 10.1056/NEJMoa1306494

7. Muller AJ, Young JC, Pendergast AM, Pondel M, Landau NR, Littman DR, et al. BCR first exon sequences specifically activate the BCR/ABL tyrosine kinase oncogene of philadelphia chromosome-positive human leukemias. *Mol Cell Biol* (1991) 11:1785–92. doi: 10.1128/mcb.11.4.1785-1792.1991

8. Skorski T. Genomic instability: the cause and effect of BCR/ABL tyrosine kinase. *Curr Hematol Malig. Rep* (2007) 2:69–74. doi: 10.1007/s11899-007-0010-6

9. Cortes JE, Talpaz M, Giles F, O'Brien S, Rios MB, Shan J, et al. Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imatinib mesylate therapy. *Blood* (2003) 101:3794–800. doi: 10.1182/blood-2002-09-2790

10. O'Dwyer ME, Mauro MJ, Blasdel C, Farnsworth M, Kurilik G, Hsieh Y-C, et al. Clonal evolution and lack of cytogenetic response are adverse prognostic factors for hematologic relapse of chronic phase CML patients treated with imatinib mesylate. *Blood* (2004) 103:451–5. doi: 10.1182/blood-2003-02-0371

11. Vetrie D, Helgason GV, Copland M. The leukaemia stem cell: similarities, differences and clinical prospects in. *Nat Rev Cancer* (2020) 20:158–73. doi: 10.1038/s41568-019-0230-9

12. Arrigoni E, Del Re M, Galimberti S, Restante G, Rofi E, Crucitta S, et al. Concise review: chronic myeloid leukemia: stem cell niche and response to pharmacologic treatment. *Stem Cells Transl Med* (2018) 7:305–14. doi: 10.1002/sctm.17-0175

13. Ahmadi M, Mahmoodi M, Shoaran M, Nazari-Khanamiri F, Rezaie J. Harnessing normal and engineered mesenchymal stem cells derived exosomes for cancer therapy: opportunity and challenges. *Int J Mol Sci* (2022) 23:13974. doi: 10.3390/ijms232213974

14. Rezaie J, Etemadi T, Feghhi M. The distinct roles of exosomes in innate immune responses and therapeutic applications in cancer. *Eur J Pharmacol* (2022) 933:175292. doi: 10.1016/j.ejphar.2022.175292

15. Ahmadi M, Jafari R, Mahmoodi M, Rezaie J. The tumorigenic and therapeutic functions of exosomes in colorectal cancer: opportunity and challenges. *Cell Biochem Funct* (2021) 39:468–77. doi: 10.1002/cbf.3622

16. Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. *Nat Rev Immunol* (2014) 14:195–208. doi: 10.1038/nri3622

17. Szczepanski MJ, Szajnik M, Welsh A, Whiteside TL, Boyiadzis M. Blast-derived microvesicles in sera from patients with acute myeloid leukemia suppress natural killer cell function via membrane-associated transforming growth factor-beta1. *Haematologica* (2011) 96:1302–9. doi: 10.3324/haematol.2010.039743

18. Caivano A, Laurenzana I, De Luca L, La Rocca F, Simeon V, Trino S, et al. High serum levels of extracellular vesicles expressing Malignancy-related markers are released in patients with various types of hematological neoplastic disorders. *Tumour Biol J Int Soc Oncodevelopmental Biol Med* (2015) 36:9739–52. doi: 10.1007/s13277-015-3741-3

19. Chargaff E, West R. The biological significance of the thromboplastic protein of blood. *J Biol Chem* (1946) 166:189–97.

20. Stegmayr B, Ronquist G. Promotive effect on human sperm progressive motility by prostasomes. *Urol. Res* (1982) 10:253–7. doi: 10.1007/BF00255932

21. Dalton AJ. Microvesicles and vesicles of multivesicular bodies versus "Virus-like" Particles. *J Natl Cancer Inst* (1975) 54:1137–48. doi: 10.1093/jnci/54.5.1137

22. Ronquist G, Brody I, Gottfries A, Stegmayr B. An mg2+ and ca2+-stimulated adenosine triphosphatase in human prostatic fluid. *Andrologia* (1978) 10:261–72. doi: 10.1111/j.1439-0272.1978.tb03030.x

23. Ratajczak J, Miekus K, Kucia M, Zhang J, Reca R, Dvorak P, et al. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia* (2006) 20:847–56. doi: 10.1038/sj.leu.2404132

24. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-Mediated Transfer of mRNAs and microRNAs Is a Novel Mechanism of Genetic Exchange between Cells. *Nat Cell Biol* (2007) 9:654–9. doi: 10.1038/ncb1596

25. Mahbubfam S, Rezaie J, Nejati V. Crosstalk between exosomes signaling pathway and autophagy flux in senescent human endothelial cells. *Tissue Cell* (2022) 76:101803. doi: 10.1016/j.tice.2022.101803

26. Shaban SA, Rezaie J, Nejati V. Exosomes derived from senescent endothelial cells contain distinct pro-angiogenic miRNAs and proteins. *Cardiovasc Toxicol* (2022) 22:592–601. doi: 10.1007/s12012-022-09740-y

27. Yáñez-Mó M, Siljander PR-M, Andreu Z, Zavec AB, Borràs FE, Buzas EI, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell. Vesicles* (2015) 4:27066. doi: 10.3402/jev.v4.27066

28. van der Pol E, Böing AN, Harrison P, Sturk A, Nieuwland R. Classification, functions, and clinical relevance of extracellular vesicles. *Pharmacol Rev* (2012) 64:676–705. doi: 10.1124/pr.112.005983

29. Ciafrè SA, Galardi S. microRNAs and RNA-binding proteins. *RNA Biol* (2013) 10:934–42. doi: 10.4161/rna.24641

30. Rezaie J, Nejati V, Mahmoodi M, Ahmadi M. Mesenchymal stem cells derived extracellular vesicles: A promising nanomedicine for drug delivery system. *Biochem Pharmacol* (2022) 203:115167. doi: 10.1016/j.bcp.2022.115167

31. Almohammai A, Rahbarghazi R, Keyhanmanesh R, Rezaie J, Ahmadi M. Asthmatic condition induced the activity of exosome secretory pathway in rat pulmonary tissues. *J Inflamm Lond Engl* (2021) 18:14. doi: 10.1186/s12950-021-00275-7

32. Bernardi S, Farina M. Exosomes and extracellular vesicles in myeloid neoplasia: the multiple and complex roles played by these Magic bullets. *Biology* (2021) 10:105. doi: 10.3390/biology10020105

33. Jurj A, Pasca S, Teodorescu P, Tomuleasa C, Berindan-Neagoe I. Basic knowledge on BCR-ABL1-positive extracellular vesicles. *biomark Med* (2020) 14:451–8. doi: 10.2217/bmm-2019-0510

34. Raimondo S, Saieva L, Corrado C, Fontana S, Flugy A, Rizzo A, et al. Chronic myeloid leukemia-derived exosomes promote tumor growth through an autocrine mechanism. *Cell Commun Signal CCS* (2015) 13:8. doi: 10.1186/s12964-015-0086-x

35. Taverna S, Flugy A, Saieva L, Kohn EC, Santoro A, Meraviglia S, et al. Role of exosomes released by chronic myelogenous leukemia cells in angiogenesis. *Int J Cancer* (2012) 130:2033–43. doi: 10.1002/ijc.26217

36. Gao X, Wan Z, Wei M, Dong Y, Zhao Y, Chen X, et al. Chronic Myelogenous Leukemia Cells Remodel the Bone Marrow Niche via Exosome-Mediated Transfer of miR-320. *Theranostics* (2019) 9:5642–56. doi: 10.7150/thno.34813

37. Jiang Y-H, Liu J, Lin J, Li S-Q, Xu Y-M, Min Q-H, et al. K562 Cell-Derived Exosomes Suppress the Adhesive Function of Bone Marrow Mesenchymal Stem Cells via Delivery of miR-711. *Biochem Biophys Res Commun* (2020) 521:584–9. doi: 10.1016/j.bbrc.2019.10.096

38. Jafarzadeh N, Gholampour MA, Alivand M-R, Kavousi S, Arzi L, Rad F, et al. CML derived exosomes promote tumor favorable functional performance in T cells. *BMC Cancer* (2021) 21:1002. doi: 10.1186/s12885-021-08734-3

39. Swatler J, Dudka W, Bugajski L, Brewinska-Olchowik M, Kozlowska E, Piwocka K. Chronic myeloid leukemia-derived extracellular vesicles increase foxp3 level and suppressive activity of thymic regulatory T cells. *Eur J Immunol* (2020) 50:606–9. doi: 10.1002/eji.201848051

40. Swatler J, Turos-Korgul L, Brewinska-Olchowik M, De Biasi S, Dudka W, Le BV, et al. 4-1BBL-containing leukemic extracellular vesicles promote immunosuppressive effector regulatory T cells. *Blood Adv* (2022) 6:1879–94. doi: 10.1182/bloodadvances.2021006195

41. Gholipour E, Sarvarian P, Samadi P, Talebi M, Movassaghpour A, Motavalli R, et al. Exosome: from leukemia progression to a novel therapeutic approach in leukemia treatment. *BioFactors Oxf. Engl* (2020) 46:698–715. doi: 10.1002/biof.1669

42. Boyiadzis M, Whiteside TL. Plasma-derived exosomes in acute myeloid leukemia for detection of minimal residual disease: are we ready? *Expert Rev Mol Diagn.* (2016) 16:623–9. doi: 10.1080/14737159.2016.1174578

43. Boyiadzis M, Whiteside TL. The emerging roles of tumor-derived exosomes in hematological Malignancies. *Leukemia* (2017) 31:1259–68. doi: 10.1038/leu.2017.91

44. Cumbo C, Anelli L, Specchia G, Albano F. Monitoring of minimal residual disease (MRD) in chronic myeloid leukemia: recent advances. *Cancer Manage Res* (2020) 12:3175–89. doi: 10.2147/CMAR.S232752

45. Kang K-W, Jung J-H, Hur W, Park J, Shin H, Choi B, et al. The potential of exosomes derived from chronic myelogenous leukaemia cells as a biomarker. *Anticancer Res* (2018) 38:3935–42. doi: 10.21873/anticanres.12679

46. Soverini S, Bernardi S, Galimberti S. Molecular testing in CML between old and new methods: are we at a turning point? *J Clin Med* (2020) 9:3865. doi: 10.3390/jcm9123865

47. Zanaglio C, Bernardi S, Gandolfi L, Farina M, Re F, Polverelli N, et al. RT-qPCR versus digital PCR: how do they impact differently on clinical management of chronic myeloid leukemia patients? *Case Rep Oncol* (2020) 13:1263–9. doi: 10.1159/000510440

48. Bernardi S, Malagola M, Polverelli N, Russo D. Exosomes in chronic myeloid leukemia: are we reading a new reliable message? *Acta Haematol* (2020) 143:509–10. doi: 10.1159/000505088

49. Bernardi S, Malagola M, Zanaglio C, Polverelli N, Dereli Eke E, D'Adda M, et al. Digital PCR improves the quantitation of DMR and the selection of CML candidates to TKIs discontinuation. *Cancer Med* (2019) 8:2041–55. doi: 10.1002/cam4.2087

50. Bernardi S, Foroni C, Zanaglio C, Re F, Polverelli N, Turra A, et al. Feasibility of tumor–derived exosome enrichment in the onco–hematology leukemic model of chronic myeloid leukemia. *Int J Mol Med* (2019) 44:2133–44. doi: 10.3892/ijmm.2019.4372

51. Ohmine K, Ota J, Ueda M, Ueno S, Yoshida K, Yamashita Y, et al. Characterization of stage progression in chronic myeloid leukemia by DNA microarray with purified hematopoietic stem cells. *Oncogene* (2001) 20:8249–57. doi: 10.1038/sj.onc.1205029

52. Oehler VG, Yeung KY, Choi YE, Bumgarner RE, Raftery AE, Radich JP. The derivation of diagnostic markers of chronic myeloid leukemia progression from microarray data. *Blood* (2009) 114:3292–8. doi: 10.1182/blood-2009-03-212969

53. Flamant S, Ritchie W, Guilhot J, Holst J, Bonnet M-L, Chomel J-C, et al. Micro-RNA response to imatinib mesylate in patients with chronic myeloid leukemia. *Haematologica* (2010) 95:1325–33. doi: 10.3324/haematol.2009.020636

54. Habib EM, Nosiar NA, Eid MA, Taha AM, Sherief DE, Hassan AE, et al. Circulating miR-146a expression predicts early treatment response to imatinib in adult chronic myeloid leukemia. *J Investig Med Off Publ. Am Fed. Clin Res* (2021) 69:333–7. doi: 10.1136/jim-2020-001563

55. Habib EM, Nosiar NA, Eid MA, Taha AM, Sherief DE, Hassan AE, et al. MiR-150 expression in chronic myeloid leukemia: relation to imatinib response. *Lab Med* (2022) 53:58–64. doi: 10.1093/labmed/lmab040

56. Li Y, Wang H, Tao K, Xiao Q, Huang Z, Zhong L, et al. miR-29b suppresses CML cell proliferation and induces apoptosis via regulation of BCR/ABL1 protein. *Exp Cell Res* (2013) 319:1094–101. doi: 10.1016/j.yexcr.2013.02.002

57. Liu Y, Song Y, Ma W, Zheng W, Yin H. Decreased microRNA-30a levels are associated with enhanced ABL1 and BCR-ABL1 expression in chronic myeloid leukemia. *Leuk. Res* (2013) 37:349–56. doi: 10.1016/j.leukres.2012.12.003

58. Chen N, Meng Z, Song J, Kong L, Zhang Y, Guo S, et al. miR-506 in patients with chronic myeloid leukemia and its effect on apoptosis of K562 cells. *Am J Transl Res* (2021) 13:9413–20. doi: 10.2217/fon-2018-0741

59. Zhang J, Jiang Y, Han X, Roy M, Liu W, Zhao X, et al. Differential expression profiles and functional analysis of plasma miRNAs associated with chronic myeloid leukemia phases. *Future Oncol* (2019) 15:763–76. doi: 10.2217/fon-2018-0741

60. Mirza MAB, Guru SA, Abdullah SM, Rizvi A, Saxena A. microRNA-21 expression as prognostic and therapeutic response marker in chronic myeloid leukaemia patients. *Asian Pac. J Cancer Prev APJCP* (2019) 20:2379–83. doi: 10.31557/APJCP.2019.20.8.2379

61. Ohyashiki JH, Ohtsuki K, Mizoguchi I, Yoshimoto T, Katagiri S, Umezu T, et al. Downregulated microRNA-148b in circulating PBMCs in chronic myeloid leukemia patients with undetectable minimal residual disease: A possible biomarker to discontinue imatinib safely. *Drug Des Devel. Ther* (2014) 8:1151–9. doi: 10.2147/DDDT.S66812

62. Ohyashiki K, Umezu T, Katagiri S, Kobayashi C, Azuma K, Tauchi T, et al. Downregulation of plasma miR-215 in chronic myeloid leukemia patients with successful discontinuation of imatinib. *Int J Mol Sci* (2016) 17:570. doi: 10.3390/ijms17040570

63. Joshi D, Chandrakala S, Korgaonkar S, Ghosh K, Vundinti BR. Down-regulation of miR-199b associated with imatinib drug resistance in 9q34.1 deleted BCR/ABL positive CML patients. *Gene* (2014) 542:109–12. doi: 10.1016/j.gene.2014.03.049

64. Zhang J, Li S, Li L, Li M, Guo C, Yao J, et al. Exosome and exosomal microRNA: trafficking, sorting, and function. *Genomics Proteomics Bioinf* (2015) 13:17–24. doi: 10.1016/j.gpb.2015.02.001

65. Russo D, Garcia-Gutierrez JV, Soverini S, Baccarani M. Chronic myeloid leukemia prognosis and therapy: criticisms and perspectives. *J Clin Med* (2020) 9:1709. doi: 10.3390/jcm9061709

66. Mahon F-X, Réa D, Guilhot J, Guilhot F, Huguet F, Nicolini F, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre stop imatinib (STIM) trial. *Lancet Oncol* (2010) 11:1029–35. doi: 10.1016/S1470-2045(10)70233-3

67. Raza A, Khan AQ, Inchakalody VP, Mestiri S, Yoosuf ZSKM, Bedhiafi T, et al. Dynamic liquid biopsy components as predictive and prognostic biomarkers in colorectal cancer. *J Exp Clin Cancer Res CR* (2022) 41:99. doi: 10.1186/s13046-022-02318-0

68. Yu W, Hurley J, Roberts D, Chakrabortty SK, Enderle D, Noerholm M, et al. Exosome-based liquid biopsies in cancer: opportunities and challenges. *Ann Oncol Off J Eur Soc Med Oncol* (2021) 32:466–77. doi: 10.1016/j.annonc.2021.01.074

69. Bergantim R, Peixoto da Silva S, Polónia B, Barbosa MAG, Albergaria A, Lima J, et al. Detection of measurable residual disease biomarkers in extracellular vesicles from liquid biopsies of multiple myeloma patients-A proof of concept. *Int J Mol Sci* (2022) 23:13686. doi: 10.3390/ijms232213686

70. Asano M, Umezu T, Katagiri S, Kobayashi C, Tauchi T, Gotoh M, et al. Up-regulated exosomal miRNA-140-3p in CML patients with musculoskeletal pain associated with discontinuation of tyrosine kinase inhibitors. *Int J Hematol* (2017) 105:419–22. doi: 10.1007/s12185-017-2199-z

71. Bouvy C, Wannez A, Laloy J, Chatelain C, Dogné J-M. Transfer of Multidrug Resistance among Acute Myeloid Leukemia Cells via Extracellular Vesicles and Their microRNA Cargo. *Leuk. Res* (2017) 62:70–6. doi: 10.1016/j.leukres.2017.09.014

72. Min Q-H, Wang X-Z, Zhang J, Chen Q-G, Li S-Q, Liu X-Q, et al. Exosomes derived from imatinib-resistant chronic myeloid leukemia cells mediate a horizontal transfer of drug-resistant trait by delivering miR-365. *Exp Cell Res* (2018) 362:386–93. doi: 10.1016/j.yexcr.2017.12.001

73. Hershkovitz-Rokah O, Modai S, Pasmanik-Chor M, Toren A, Shomron N, Raanani P, et al. MiR-30e induces apoptosis and sensitizes K562 cells to imatinib treatment via regulation of the BCR-ABL protein. *Cancer Lett* (2015) 356:597–605. doi: 10.1016/j.canlet.2014.10.006

74. Hrdinova T, Toman O, Dresler J, Klimentova J, Salovska B, Pajer P, et al. Exosomes released by imatinib–resistant K562 cells contain specific membrane markers, IFITM3, CD146 and CD36 and increase the survival of imatinib–sensitive cells in the presence of imatinib. *Int J Oncol* (2021) 58:238–50. doi: 10.3892/ijo.2020.5163

75. Dong Y, Lin Y, Gao X, Zhao Y, Wan Z, Wang H, et al. Targeted blocking of miR328 lysosomal degradation with alkalized exosomes sensitizes the chronic leukemia cells to imatinib. *Appl Microbiol Biotechnol* (2019) 103:9569–82. doi: 10.1007/s00253-019-10127-3

76. Lin H, Rothe K, Chen M, Wu A, Babaian A, Yen R, et al. The miR-185/PAK6 axis predicts therapy response and regulates survival of drug-resistant leukemic stem cells in CML. *Blood* (2020) 136:596–609. doi: 10.1182/blood.2019003636

77. Liu Y, Song B, Wei Y, Chen F, Chi Y, Fan H, et al. Exosomes from mesenchymal stromal cells enhance imatinib-induced apoptosis in human leukemia cells via activation of caspase signaling pathway. *Cytotherapy* (2018) 20:181–8. doi: 10.1016/j.jcyt.2017.11.006

78. Chen X, Chen Y, Zhang M, Cheng H, Mai H, Yi M, et al. HucMSC Exosomes Promoted Imatinib-Induced Apoptosis in K562-R Cells via a miR-145a-5p/USP6/GLS1 Axis. *Cell Death Dis* (2022) 13:92. doi: 10.1038/s41419-022-04531-3

79. Yen R, Grasedieck S, Wu A, Lin H, Su J, Rothe K, et al. Identification of key microRNAs as predictive biomarkers of nilotinib response in chronic myeloid leukemia: A sub-analysis of the ENESTxtnd clinical trial. *Leukemia* (2022) 36:2443–52. doi: 10.1038/s41375-022-01680-4

80. Liu J, Zhang Y, Liu A, Wang J, Li L, Chen X, et al. Distinct dasatinib-induced mechanisms of apoptotic response and exosome release in imatinib-resistant human chronic myeloid leukemia cells. *Int J Mol Sci* (2016) 17:531. doi: 10.3390/ijms17040531

81. Bellavia D, Raimondo S, Calabrese G, Forte S, Cristaldi M, Patinella A, et al. Interleukin 3- receptor targeted exosomes inhibit *in vitro* and *in vivo* chronic myelogenous leukemia cell growth. *Theranostics* (2017) 7:1333–45. doi: 10.7150/thno.17092

82. Taverna S, Fontana S, Monteleone F, Pucci M, Saieva L, De Caro V, et al. Curcumin Modulates Chronic Myelogenous Leukemia Exosomes Composition and Affects Angiogenic Phenotype via Exosomal miR-21. *Oncotarget* (2016) 7:30420–39. doi: 10.18632/oncotarget.8483

83. Raimondo S, Naselli F, Fontana S, Monteleone F, Lo Dico A, Saieva L, et al. Citrus limon-derived nanovesicles inhibit cancer cell proliferation and suppress CML xenograft growth by inducing TRAIL-mediated cell death. *Oncotarget* (2015) 6:19514–27. doi: 10.18632/oncotarget.4004

84. Cochran AM, Kornbluth J. Extracellular vesicles from the human natural killer cell line NK3.3 have broad and potent anti-tumor activity. *Front Cell Dev Biol* (2021) 9:698639. doi: 10.3389/fcell.2021.698639

85. Samara A, Anbar M, Shapira S, Zemlyansky A, Zozovsky A, Raanani P, et al. Using natural killer cell-derived exosomes as a cell-free therapy for leukemia. *Hematol Oncol* (2022) 41(3):487–498. doi: 10.1002/hon.3111

86. Zhang X, Yang Y, Yang Y, Chen H, Tu H, Li J. Exosomes from bone marrow microenvironment-derived mesenchymal stem cells affect CML cells growth and promote drug resistance to tyrosine kinase inhibitors. *Stem Cells Int* (2020) 2020:8890201. doi: 10.1155/2020/8890201

87. Houshmand M, Garello F, Stefania R, Gaidano V, Cignetti A, Spinelli M, et al. Targeting chronic myeloid leukemia stem/progenitor cells using venetoclax-loaded immunoliposome. *Cancers* (2021) 13:1311. doi: 10.3390/cancers13061311

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 26 February 2024
DOI 10.3389/fonc.2024.1339620

OPEN ACCESS

EDITED BY
Michael Liew,
ARUP Laboratories, United States

REVIEWED BY
Prajish Iyer,
City of Hope National Medical Center, United States
Antonio Giovanni Solimando,
University of Bari Aldo Moro, Italy

*CORRESPONDENCE
Elias Hobeika
elias.hobeika@uni-ulm.de

RECEIVED 16 November 2023
ACCEPTED 06 February 2024
PUBLISHED 26 February 2024

CITATION
Schmid VK and Hobeika E (2024) B cell receptor signaling and associated pathways in the pathogenesis of chronic lymphocytic leukemia.
Front. Oncol. 14:1339620.
doi: 10.3389/fonc.2024.1339620

B cell receptor signaling and associated pathways in the pathogenesis of chronic lymphocytic leukemia

Vera K. Schmid and Elias Hobeika*

Institute of Immunology, Ulm University, Ulm, Germany

B cell antigen receptor (BCR) signaling is a key driver of growth and survival in both normal and malignant B cells. Several lines of evidence support an important pathogenic role of the BCR in chronic lymphocytic leukemia (CLL). The significant improvement of CLL patients' survival with the use of various BCR pathway targeting inhibitors, supports a crucial involvement of BCR signaling in the pathogenesis of CLL. Although the treatment landscape of CLL has significantly evolved in recent years, no agent has clearly demonstrated efficacy in patients with treatment-refractory CLL in the long run. To identify new drug targets and mechanisms of drug action in neoplastic B cells, a detailed understanding of the molecular mechanisms of leukemic transformation as well as CLL cell survival is required. In the last decades, studies of genetically modified CLL mouse models in line with CLL patient studies provided a variety of exciting data about BCR and BCR-associated kinases in their role in CLL pathogenesis as well as disease progression. BCR surface expression was identified as a particularly important factor regulating CLL cell survival. Also, BCR-associated kinases were shown to provide a crosstalk of the CLL cells with their tumor microenvironment, which highlights the significance of the cells' milieu in the assessment of disease progression and treatment. In this review, we summarize the major findings of recent CLL mouse as well as patient studies in regard to the BCR signalosome and discuss its relevance in the clinics.

KEYWORDS

chronic lymphocytic leukemia (CLL), B cell receptor (BCR) signaling, IGHV, CD79a/b (Igα/Igβ), PI3K/AKT, SYK, BTK

1 The complex role of the BCR signaling in CLL

Among all types of adult leukemia, chronic lymphocytic leukemia (CLL) is the most prevalent lymphoproliferative disease. It is distinguished by the culmination of characteristically 19- and CD5-positive malignant B cells in the bone marrow, blood, spleen and other lymphoid tissues (1–3). CLL displays a highly heterogeneous clinical

course, ranging from fast progression with poor outcomes to an indolent course of disease with a good prognosis and regular life expectancy (4). Consistent with this, the selection of a suitable therapy relies on common parameters such as lymphocyte doubling time and the clinical stage of the disease. To date, no single agent to treat CLL was detected (3, 4).

The B cell antigen receptor (BCR) complex consists of an immunoglobulin (Ig) transmembrane protein that is associated with two signal-transmitting subunits, named CD79a (Igα) and CD79b (Igβ) (5). It is critical for normal B cell maturation and survival that the BCR has the capacity to create signals through the Igα/Igβ signaling heterodimer at vital stages during B cell development (6). Moreover, BCR-mediated signaling plays an essential role in the pathogenesis of CLL, as evidenced by multiple sources. First of all, CLL cells retain the surface expression of the BCR, which is a common characteristic among neoplastic B cell malignancies (7). This is based on the fact that malignant B cells derive advantages from the pro-survival signals that are initiated as a result of BCR activation. Second, there is genetic proof across various groups indicating that BCR expression is required for both CLL development (8, 9) and persistence (10, 11). Third, B cell malignancies often exhibit BCR signaling dysregulation and pathways triggered downstream of the BCR were shown to be highly activated in CLL cases (12). BCR-mediated signaling is the primary operating pathway in CLL cells which was identified through gene expression profiling data, particularly in the lymph node (LN) microenvironment, which is thought to support growth and survival of CLL cells (13). Last and most importantly, targeted therapy using BCR pathway inhibitors is a promising approach to treat CLL (14). This is evidenced by the exceptional clinical efficacy of inhibitors targeting BCR signaling, such as ibrutinib (Bruton's tyrosine kinase (BTK) inhibitor) or idelalisib (Phosphatidylinositol 3-kinase (PI3K) inhibitor), in reversing the CLL disease phenotype suggests an enormous importance of BCR-derived survival signals for CLL cell persistence (15–18).

In more than 30% of cases, patient-derived CLL cells express similar, or even identical, BCRs with corporate stereotypic features and sequence similarities (19, 20). According to the expressed stereotypic BCR heavy chain, CLL cells are classified into distinct CLL subsets. Each subset displays common genomic abnormalities (21) as well as highly homogeneous clinical and biological properties (22). Altogether, the IGHV-D-J gene recombination pattern and the amino acid constitution of the heavy chain variable complementarity determining region 3 (HCDR3) categorize these stereotyped CLL cases into 19 major subgroups (1, 22, 23). Very recently, a detailed study on BCR stereotypy reported that not only 30% but even 41% of all CLL cases can be categorized into stereotypic subgroups with overall 29 major subsets (20). Most interestingly, the clinical outcome of CLL is determined by the molecular specifics of the stereotyped interactions between BCRs (24). For example, tightly bound and long-lasting BCR-BCR crosslinking interactions in the CLL subset #4 lead to B cell anergy. This is a clinically inactive state which is specifically observed in CLL clones of the subgroup #4 (24, 25).. In contrast, subgroup #2 CLL cases have a more aggressive course of disease due to a low-affinity and rapidly dissolving BCR-BCR interaction, leading to an enhanced signaling activity by the BCR (24).

This significant limitation in the CLL Ig gene repertoire proposes that BCR recognition of a restricted set of antigen epitopes results in the selection and expansion of B cell clones that ultimately enter the pathogenesis of CLL. Epidemiological studies did show that several infectious diseases can be linked to the development of CLL, and CLL-associated Igs react with a variety of viruses or pathogens, suggesting a pathogen-induced CLL development (26–28). In an Eμ-TCL1 mouse study, however, an accelerated development of leukemia could not be observed due to an acute or chronic infection. Preferably, a BCR-mediated autoantigen recognition resulted in the pathogenesis of CLL (8). Interestingly, the murine CLL cells preferred the selection of specific light chains that allowed BCR cross-reaction with a large number of autoantigens (8). This is consistent with the hypothesis, based on evidence obtained in CLL patients, that light chains in combination with defined heavy chains are important for the formation of the leukemic BCR specificity (29, 30). Iacovelli et al. observed that autonomous BCR signaling as well as low-affinity BCR interactions with self-antigens were actively selected during leukemia development in Eμ-TCL1 mice, implying a crucial involvement of these two factors in the pathogenesis of CLL (9). Furthermore, in this model, autoantigen-induced BCR signaling resulted in a more aggressive course of CLL (9). Similarly, a correlation between the response to BCR binding and shorter survival was reported in CLL patient analyses (31). In PtC-reactive Eμ-TCL1 leukemic cells, the response to autoantigen stimulation also resulted in a more aggressive disease (32). Therefore, aberrant autoantigen-induced responses induce an accelerated CLL progression, underlining the great variance in the clinical course of CLL. However, not only low-affinity but also high-affinity BCR-antigen cross-linking cooperates with autonomic BCR-BCR interactions in triggering CLL. For instance, high-affinity binding between three stereotypically mutated CLL subset BCRs and the Fc portion of human IgG was reported, as well as high-affinity binding to the fungal antigen β- (1, 6)-glucan (33, 34).

1.1 CLL-associated mutations in the IGHV and IGLV genes

On the basis of the somatic hypermutation (SHM) status in the variable region of the Ig heavy-chain (IGHV) gene, CLL can be categorized into two main types of disease: the unmutated CLL (U-CLL) and the mutated CLL (M-CLL) (35). While in U-CLL cases, the IGHVs show > 98% identity to the germline Ig sequence, the IGHVs of M-CLL show less than 98% homology to the germline sequence. In particular, this IGHV-mutation-based classification represents a strong prognostic marker for CLL. In general, U-CLL cases are associated with a more aggressive form of CLL relative to the mainly indolent course of disease in M-CLL patients, which also experience a longer progression-free survival (PFS) (35, 36). The CLL-Ig repertoire is characterized by the high representation of particularly selected IGHV-coding genes termed IGHV1-69, IGHV3-21, IGHV3-23, IGHV3-7 and IGHV4-34 (37, 38). The

IGHV1-69 gene is most frequently selected in the U-CLL group, whereas the IGHV3-21, IGHV3-23, IGHV3-7, and IGHV4-34 genes are typically associated with a high mutational burden (37, 38). Among the CLL subsets expressing stereotyped HCDR3 sequences, Murray et al. observed recurrent amino acid modifications in the IGHV domain, especially in those expressing IGHV4-34 and IGHV3-21 genes, which show unique structures of SHM (39). Since the described mutations are represented in low frequency among non-CLL IGHV domains, they can be considered as CLL-specific (39). CLL research mainly focuses on studying IGHV sequence structures. However, increasing evidence suggests that the Ig light chain (IGLV) sequence has effects on the clinical course and the outcome of CLL as well [reviewed in (40)]. Lately, the IGLV3-21 gene was identified as a prognostic marker for CLL with a poor prognosis, independent of the corresponding heavy-chain (41). Compared to the overall incidence of CLL (7%), increased recurrence of the IGLV3-21 gene (28%) was observed in high-risk CLL cohorts (41). Furthermore, a specific mutant form of the IGLV3-21, the IGLV3-21^{G110R}, was highlighted to play an important role in the pathogenesis and prognosis of CLL. This mutation increases the probability of homotypic BCR interactions, resulting in autonomous BCR signaling (42). Thus, IGLV3-21^{R110}–expressing CLL cells represent a definite subset of CLL with poor prognosis, irrespective of the IGHV mutational status (42).

The self-activation of CLL-derived BCRs is a significant factor in the development of CLL. This is accomplished through the interaction of CLL-derived BCRs with specific BCR epitopes that are unique to certain subsets. This interaction results in the activation of BCR-mediated pro-survival signaling within CLL cells.

2 BCR-mediated autonomous signaling in CLL

Autonomous, antigen-independent BCR signaling was identified as the mechanistic basis of malignant BCR signaling in most of the CLL cases (9, 43). Self-activation of the CLL-derived BCRs is a significant factor in the progression of CLL pathogenesis. This autonomous signaling is induced by the BCRs' ability to interact with their own defined BCR epitopes that are unique to certain CLL subsets. This interaction results in the activation of BCR-mediated pro-survival signaling within CLL cells (9, 43). This antigen-independent signaling is enabled by an intermolecular cross-link of an oncogenic HCDR3 domain with unique motifs located between the FR2 and FR3 domains within the Ig molecule (43). Each CLL case may acquire specific autoreactive BCRs through certain affinity maturation processes, including the incorporation of distinct SHMs and class-switch recombination (24, 39). Structural analysis of the CLL subset #2 and #4 BCRs revealed the origin of the G110R mutation, which is crucial for homotypic BCR interaction, by a nonsynonymous SHM of the G110 residue in the IGLJ germline segment of the BCR (24). CLL patients carrying the IGLV3-21*01 light chain allele exhibit a higher risk of generating CLL. This specific allele facilitates the acquisition of the malignant G110R mutation, which promotes strong BCR-BCR interaction initiating self-directed BCR signaling (42). Autonomous signaling causes higher basal Calcium (Ca^{2+}) signaling and increased activity of signaling factors downstream of the BCR such as BTK, the spleen tyrosine kinase (SYK), and the phosphatidylinositol 3-kinase (PI3K) (44). Most importantly, reversion of the R110 residue into G110 abolishes BCR autonomous signaling (24).

3 BCR-mediated downstream signaling in CLL

3.1 The BCR signaling subunits Igα and Igβ

The BCR is associated with a signaling heterodimer that consists of two subunits, Igα and Igβ. The Igα/Igβ subunit is required for a proper membrane transport of the Igs for BCR surface expression. Moreover, the mediation of BCR signaling by the Igα/Igβ heterodimer is essential for the maturation, differentiation and survival of B cells (6, 45). The Igα/Igβ subunits are implicated in the BCR complex formation and stabilization. Furthermore, Igα and Igβ facilitate assembly and steadiness of BCR, promote IgM transport to cell surface and increase BCR surface expression levels by regulating its glycosylation (45, 46). In CLL samples, defective glycosylation and subsequent impaired folding of the IgM and CD79a chains leads to impaired BCR assembly as well as reduced surface membrane (sm)IgM expression (47). It was revealed that CLL cells expressing low CD79b protein levels also exhibit reduced expression levels of IgM-BCR complexes. The cytokine IL-4, however, is able to restore CD79b and smIgM expression and is thereby enhancing the activation of BCR-mediated survival signaling in CLL cells (48).

Recently, our group demonstrated that an induced loss of the Igα subunit in CLL cells of a Eμ-TCL1 mouse model, results in an almost complete loss of the diseased cells, indicating a crucial involvement of the BCR in the persistence of CLL cells (10).

Similarly, we could show that Tam-induced deletion of the intracellular Igβ signaling domain in isolated CLL B cells of mb1-CreER^{T2};Igβ$^{\Delta c/fl}$;Eμ-TCL1 mice leads to a significant CLL cell regression within 8 weeks (Figures 1A, B). In these mice, efficient deletion of the Igβ-encoding gene could be monitored by an induced GFP expression (49) (Figure 1D). GFP^{+} cells with Igβ-tail-deficiency maintained IgM BCR surface expression (Figure 1C) whereas their viability *in vivo* and *in vitro* was reduced (Figures 1A, E). This indicates that the survival, as well as the progression of CLL, depends on the functionality of BCR to generate signals via the Igα/Igβ heterodimer, making it an essential factor.

Furthermore, we were interested if Igβ signaling tail is essential for CLL progression. Thus, our group tested whether B cells with a constitutive loss of the Igβ signaling tail in the early pro B cell stage are able to develop CLL in an Eμ-TCL1 mouse model. Indeed, constitutive deletion of the Igβ signaling tail in B cells resulted in CLL outbreak of the Igβ$^{\Delta c/\Delta c}$;Eμ-TCL1 mice at an age of 12-14 months. The development of the disease was indicated by the

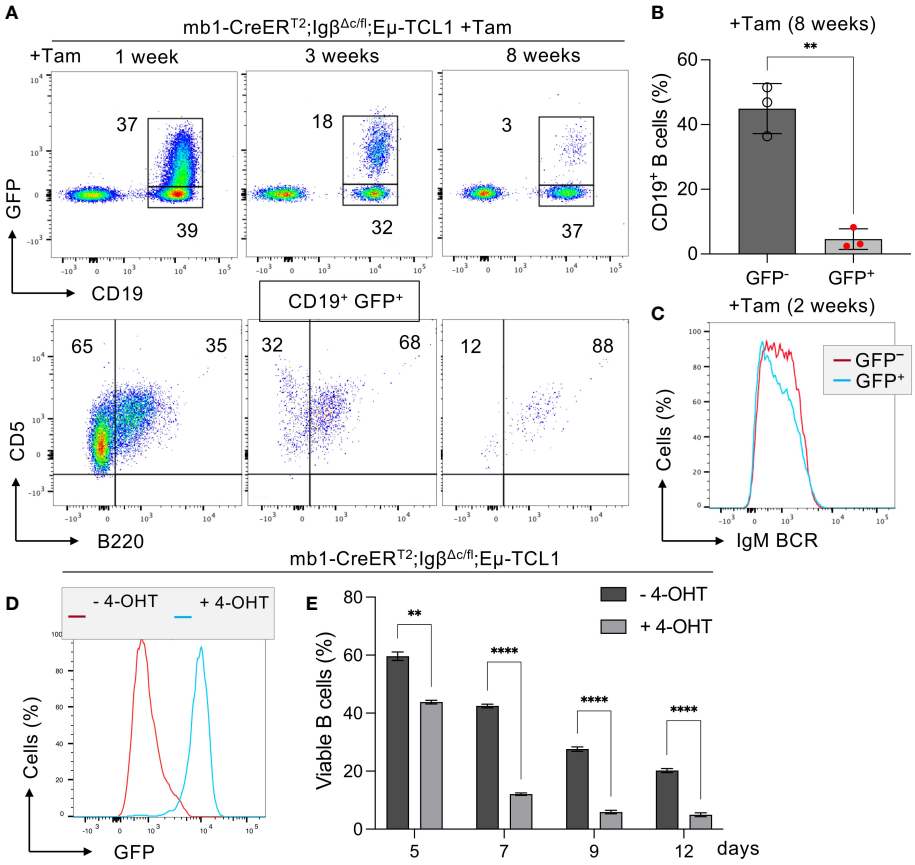

FIGURE 1
Igβ signaling tail deficiency in Eμ-TCL1 mice leads to CLL cell reduction. **(A)** Flow cytometry assessment was performed on B cells from the peripheral blood (PBL) of diseased mb1-CreER^{T2};Igβ$^{Δc/fl}$;Eμ-TCL1 mice, 1 week (left), 3 weeks (middle), plus 8 weeks (right) following the initiation of Tamoxifen (Tam) treatment. The dot plots of the anti-CD19 versus GFP staining are shown. The CD19$^+$GFP$^+$ gated region indicates Igβ tail deficient B cells, while the CD19$^+$GFP$^-$ gated region marks the Igβ tail sufficient B cell population. The B220 vs CD5 staining of the CD19$^+$GFP$^+$ gated cells is depicted below. The B220$^-$CD5$^+$ population represents diseased CLL cells, while the B220$^+$CD5$^+$ gated region exhibits healthy cells. The average relative frequency of the cells within the gate is indicated by the numbers in the dot plots. The data is presentable for three independent mouse analyses. **(B)** Eight weeks after administering Tam treatment to mb1CreER^{T2};Igβ$^{Δc/fl}$;Eμ-TCL1 mice, the percentage of B cells in the CD19$^+$GFP$^+$ and CD19$^+$GFP$^-$ B cell populations was quantified. The graphs display the respective average percentage of B cells ± SEM, while p-values were determined by a Student's t-test (two-tailed; ** $p < 0.01$). The cell count for each group consists of data from three mice. **(C)** After two weeks of Tam treatment, the fluorescence intensity of IgM BCR expression in CD19$^+$GFP$^-$ (red) or CD19$^+$GFP$^+$ (blue) B cells of mb1-CreER^{T2};Igβ$^{Δc/fl}$;Eμ-TCL1 CLL mice was determined. The results presented in the histogram are representative of three self-contained experiments. **(D)** Flow cytometry was used to analyze the expression of GFP in mb1-CreER^{T2};Igβ$^{Δc/fl}$;Eμ-TCL1 CLL cells that were either treated with 4-OHT *in vitro* (+4-OHT blue) or kept untreated (-4-OHT red) for 5 days (5d). The fluorescence intensity of CD19$^+$ B cells was determined and indicated in histograms. The data is presentable of three self-sufficient experiments. **(E)** The survival of B cells in mb1-CreER^{T2};Igβ$^{Δc/fl}$;Eμ-TCL1 CLL cells was statistically analyzed on day 5, 7, 9, and 12 following *in vitro* 4-OHT treatment (+4-OHT; light grey). The control remained without treatment (-4-OHT; dark grey). The graphs display the mean ± SEM and p-values were determined by the Student's t-test (two-tailed; **** $p < 0.0001$; ** $p < 0.01$). The results of three independent analyses are presented.

accumulation of an increased number of CD5$^+$B220^{low} CLL B cells in the spleen of the mouse in combination with splenomegaly (Figures 2A, C, D). Efficient deletion of the Igβ tail was validated by flow cytometry (Figure 2B). The malignant transformation of B cells in Igβ$^{Δc/Δc}$;Eμ-TCL1 mice despite their Igβ-tail deficiency indicates that expression of the Igβ-tail is not essential for the pathogenesis and persistence of CLL. In addition, CLL cells of Igβ$^{Δc/Δc}$;Eμ-TCL1 mice were not susceptible to anti-Igβ antibody treatment compared to CLL cells that originated from conventional Eμ-TCL1 mice (Figure 3) indicating that the CLL cells survive independently of Igβ-tail signaling. So, it is possible that the CLL cells found a way to circumvent Igβ-tail deficiency via deregulation of specific BCR-regulated pathways. However, the susceptibility of Igβ-tail sufficient CLL cells to anti-Igβ antibody treatment suggests a potential clinical efficacy of anti-Igβ antibodies in CLL treatment. However, a clinical phase I trial of polatuzumab vedotin, an anti-Igβ antibody fused to a microtubule-disrupting drug named monomethyl auristatin E, did not show any clinical responses in CLL (50). The missing effect is probably caused by the low or absent Igβ surface expression levels observed in CLL patients. This also explains the lack of Igβ-targeting chimeric antigen receptor T cell studies in CLL therapy, although they show high efficacy in other B cell lymphomas, such as the diffuse large B cell lymphoma (DLBCL) (51). Interestingly, mutations in the extracellular and transmembrane regions of the

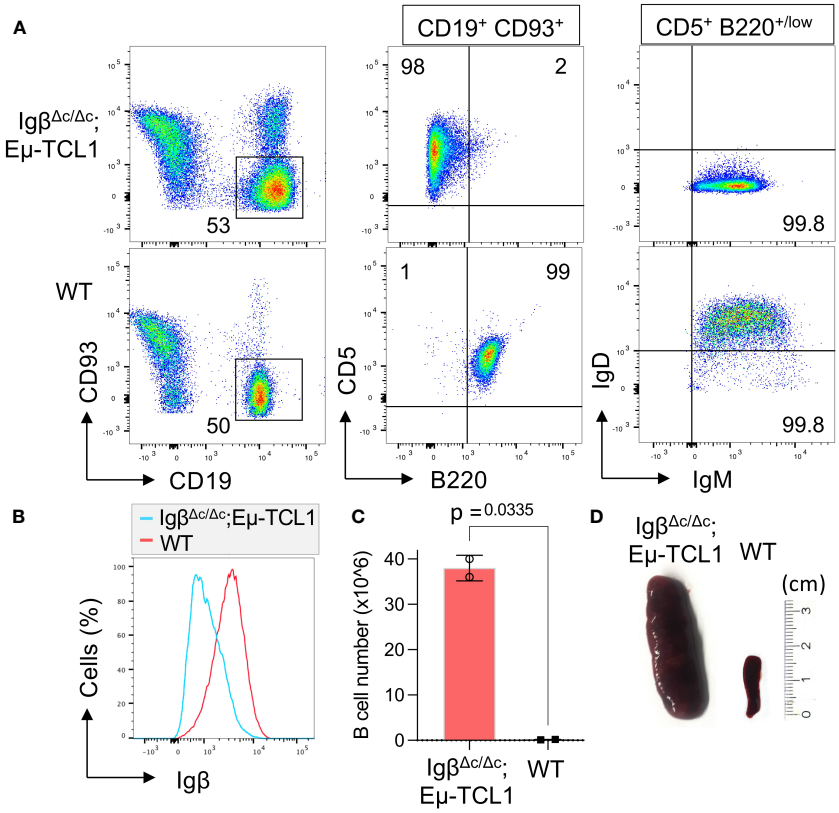

FIGURE 2
Igβ-tail deficiency in a Eμ-TCL1 mouse model results in CLL development. **(A)** Flow cytometry analysis was conducted on B cells isolated from the spleen of 14-month-old $Igβ^{Δc/Δc}$;Eμ-TCL1 mice and WT control mice. The dot plot depicts the B220 vs CD5 staining of $CD19^{+}CD93^{-}$ gated mature B cells, and the IgM vs IgD staining of $CD5^{+}B220^{low}$ CLL cells of $Igβ^{Δc/Δc}$;Eμ-TCL1 mice or on normal $CD5^{-}$ $B220^{+}$ B cells of WT mice. The data presented is representative of three self-contained mouse analyses. **(B)** Flow cytometry was used to analyze isolated splenic B cells from $Igβ^{Δc/Δc}$;Eμ-TCL1 mice and WT mice. The fluorescent intensity of Igβ expression is represented in a histogram. **(C)** The absolute number of B cells in the peritoneal cavity of 14-month-old $Igβ^{Δc/Δc}$;Eμ-TCL1 mice and the control mice of the same age were quantified. The graphs represent the average count ± SEM. A two-tailed Student's t-test was conducted to obtain the p-values. The cell count for each group comprises two mice. **(D)** The images show the spleen of a mb1- $Igβ^{Δc/Δc}$;Eμ-TCL1 mouse and a WT mouse.

Igβ-encoding gene B29 were detected in CLL patients, which show aberrant BCR signaling (52). However, no mutations of the Igα-encoding gene were observed (45). It might be possible that the mutations in the B29 gene play a role in CLL oncogenesis. So far, only the Igβ subunit of the BCR complex was targeted for therapy of B cell diseases. However, recently a synergistic potential of combined Igα-targeted and Igβ-targeted therapy of B cell leukemia was observed. Showing high antitumor activity in DLBCL, this method may also be an option in the future treatment of CLL (53).

The Igα/Igβ heterodimer forms the center of the intricate BCR signaling network with essential functional implications for both normal and leukemic CLL cells. Overall, we could show that the expression of a functional BCR complex is essential for the survival of CLL cells (Figure 1), CLL development, however, could take place despite restricted BCR signaling in Igβ signaling tail deficient B cells (Figure 2). It might be interesting to identify to what extent the detected mutations of Igβ modulate BCR signaling or play a role in CLL leukemogenesis. Studies focusing on the mechanism of Igα/Igβ ubiquitination and glycosylation in CLL may also uncover another layer of BCR signal regulation.

3.2 The Src family kinase LYN

BCR signaling is initiated by the enzymatic activation of the receptor-associated Src family kinases (SFKs), like LYN, FYN, LCK and BLK. Activated SFKs stimulate the phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) located in the cytoplasmic Igα/Igβ signaling subunit of the BCR. This results in the recruitment and activation of tandem Src homology 2 (SH2) domain-containing effectors, like SYK, which cause the initiation of several BCR downstream signaling pathways (Figure 4) (54, 55). The LCK/YES novel kinase (LYN) is distinct from other SFKs in its additional capability to induce phosphorylation of the immunoreceptor tyrosine-based inhibitory motif (ITIM) of inhibitory surface receptors, which recruit tyrosine phosphatases like SHP-1/2 and PP2A. These phosphatases attenuate the B cell activation response triggered by the BCR (56). SHP-1, for instance, counteracts the phosphorylation of the Igα ITAMs and the BCR signaling effector SYK (57). LYN, as a key regulator of the BCR signaling pathway, is overexpressed in CLL patients, and elevated LYN protein levels correlate with a shorter treatment-free survival (58). The increased activity of the LYN

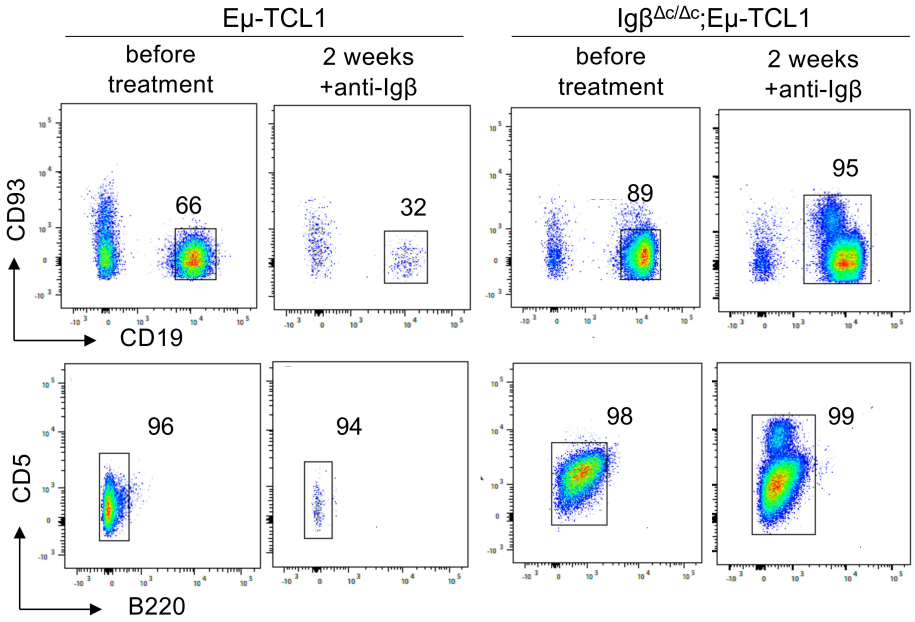

FIGURE 3
Anti-Igβ treatment does not affect the progression of CLL cells lacking Igβ-tail. CLL cell survival in Eμ-TCL1 and $Igβ^{Δc/Δc}$;Eμ-TCL1 mice was analyzed using flow cytometry one day before and two weeks after administering an anti-Igβ antibody. The dot plot illustrates the staining of anti-B220 vs anti-CD5 mature B cells after gating on the $CD19^{+}CD93^{-}$ cell population. CLL cells can be identified by expression of the characteristic markers $CD19^{+}CD93^{-}CD5^{+}B220^{low}$.

kinase observed in CLL cells is also associated with defects in apoptosis mediated by interactions of LYN with the procaspase-8 (59) or SHP-1 (60). Thus, SHP-1 is also found to be expressed at low levels in CLL cells compared to the expression in normal B cells (60).

Although LYN is well-known to balance BCR signaling, LYN activation seems to be dispensable in the development of CLL, since B cell specific gain- and loss-of-function mutations of LYN showed no significant changes in CLL progression in Eμ-TCL1 mice (61, 62). However, several studies suggest an emerging new role of LYN as a crucial regulator within the CLL tumor microenvironment supporting leukemic cell growth and CLL progression (61, 63, 64). For example, LYN-deficiency in macrophages reduces their capability to support CLL cell survival (61). Furthermore, LYN controls the stromal fibroblast polarization, which was shown to support CLL cell survival and leukemic progression. Genetic *Lyn* deletion in stromal cells, for instance, results in reduced expression of c-JUN. This transcription factor is required to induce Thrombospondin-1 expression, which impairs CLL viability by binding to CD47 (64). Thus, the efficacy of LYN inhibition in CLL is to some extent based on an emerging new function of LYN in regulating the tumor microenvironment and the dialog between leukemic cells and bystander cells.

Targeting LYN *in vitro* using the Src/c-Abl tyrosine kinase inhibitor dasatinib blocks CLL cell proliferation and triggers apoptosis in isolated CLL cells (65). Moreover, as a result of dasatinib treatment, reduced BCR-downstream signaling activation and a block in the anti-apoptotic MCL-1-dependent increase in CLL cell survival was observed (66). However, the clinical LYN-targeting drug dasatinib shows by far less effectiveness in the treatment of CLL patients compared to other BCR pathway inhibitors, that will be described later. In a phase II clinical trial, dasatinib treatment achieved partial responses in 3 out of 15 patients (20%; 90% CI 6-44%), and nodal response in 9 patients (60%), indicating only partial success in a small population of patients with relapsed and refractory CLL (67). These findings question the importance of LYN in CLL development and progression. However, since dasatinib has a wide target spectrum it is not a precise tool for evaluating functional relevance of LYN in CLL. CLL studies using different LYN-targeting inhibitors would offer additional insights into the role of LYN in the treatment of CLL.

3.3 The spleen tyrosine kinase family

3.3.1 SYK in 'tonic' BCR signaling

The spleen tyrosine kinase is part of the SYK family of cytoplasmic non-receptor tyrosine kinases. It is a key component in the BCR-mediated signal transmission and regulates numerous physiological functions in B cells. Recruitment of SYK to phosphorylated ITAM sequences leads to the phosphorylation of more ITAM tyrosines of adjacent BCRs. This generates a positive feedback loop amplifying BCR signal transduction (57). Moreover, SYK directly phosphorylates and activates BTK and mediates PI3K activation by the phosphorylation of its adaptor CD19 (55). The activation of BCR signaling by SYK is counteracted by the tyrosine phosphatase SHP-1 (Figure 4) (57).

Gene expression of SYK along with its downstream signaling pathways is significantly enhanced in CLL cells (68, 69).

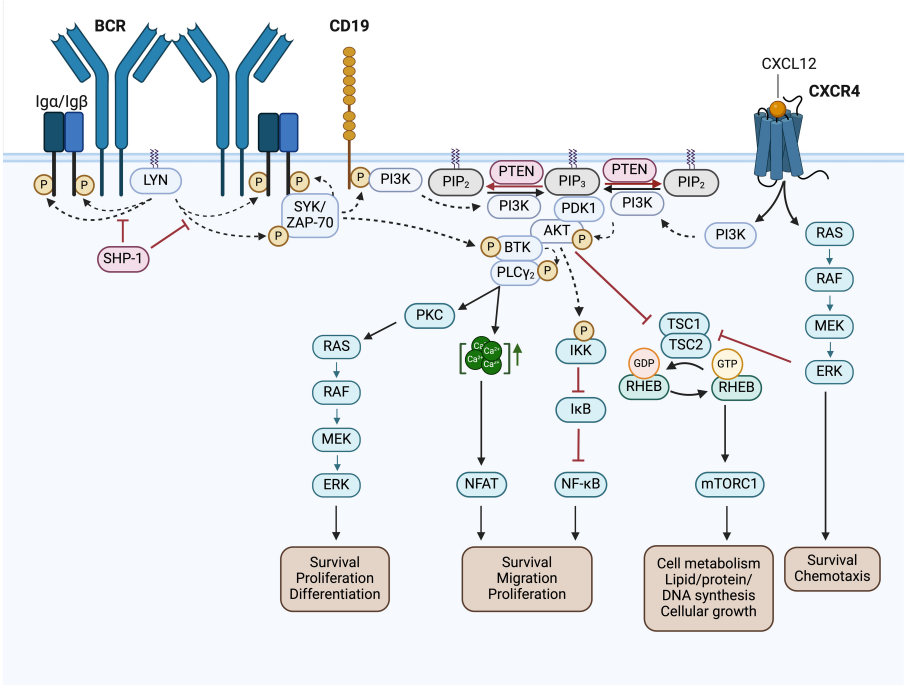

FIGURE 4
BCR signaling pathway and BCR-associated CXCR4 signaling in CLL. Activated Src family kinases (SFKs), such as LYN, stimulate the phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) located in the cytoplasmic Igα/Igβ signaling subunit of the BCR. This results in the recruitment and activation of SH2 domain-containing effectors, like SYK and ZAP-70, which phosphorylate BTK and CD19. SHP-1 inhibits the phosphorylation of the Igα ITAMs and SYK. Phosphorylated CD19 recruits the PI3K to the cell membrane, where it phosphorylates PIP_2 to generate PIP_3. Thereby, PI3K creates an essential docking platform for PH domain-containing signaling factors, such as PDK1, BTK and AKT. Binding to PIP_3 results in membrane recruitment and activation of PDK1, BTK and AKT, which mediate the initiation of several BCR downstream signaling cascades, such as RAS/RAF/MEK/ERK signaling, NFAT, NF-κB and mTORC1 signaling. The phosphatase PTEN represses PI3K signaling by PIP_3 dephosphorylation, generating PIP_2. NFAT is activated by increased cytoplasmic Ca^{2+} concentrations, which are induced by PLCγ. NF-κB is retained in an inactive state by the inhibitor IκB. Phosphorylation of IKK leads to IκBs phosphorylation and degradation, finally resulting in NF-κB activation. TSC1/2 inhibits RHEB GTPase activity, which is required to induce mTORC1 activation. AKT- or ERK-mediated inhibition of TSC2, results in mTORC1 activation. In addition, binding of CXCL12 to CXCR4 induces CLL cell migration, survival and chemotaxis via the activation of the downstream signaling pathways MAPK/ERK, PI3K/AKT, PLCγ/Ca^{2+} and NF-κB. This figure was created with BioRender.com. BCR, B cell receptor; LYN, LCK/YES novel kinase; SYK, spleen tyrosine kinase; ZAP-70, CD3ζ-chain-associated protein of 70 kDa; SHP-1, Src homology region 2 domain-containing phosphatase-1; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; PIP_2, phosphatidylinositol-4,5-bisphosphate; PIP_3, phosphatidylinositol-3,4,5-triphosphate; PDK1, 3-phosphoinositide-dependent protein kinase 1; PKB/AKT, protein kinase B; BTK, Bruton's tyrosine kinase; PLCγ, phospholipase Cγ; PKC, protein kinase C; CXCL12, chemokine C-X-C motif ligand 12; CXCR4, C-X-C motif chemokine receptor; RAS, RAF, Rat sarcoma protein family; MEK; ERK, extracellular-signal-regulated kinase; IKK, IκB kinase complex; IκB, inhibitor of nuclear factor κB; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NFAT, nuclear factor of activated T-cells; TSC1/2, tuberous sclerosis complex 1/2; RHEB, Ras homolog enriched in brain; mTOR complex mTORC1, mechanistic target of rapamycin; Ca^{2+}, Calcium-Ion; GTP, Guanosine-5′-triphosphate; GDP, Guanosindiphosphat; P, phosphorylation.

Interestingly, SYK expression in U-CLL cells is increased compared to leukemic cells harboring mutated IGHV domain genes (69). Additionally, SYK is constitutively phosphorylated on activating tyrosines (68). However, to date, no mutations of SYK were detected in patients with CLL (70). Impaired BCR signaling was associated with CLL progression, making SYK a prospective therapeutic target in treating the disease. Preclinical studies in CLL cell lines displayed an effective block in the BCR signaling mediated basal activity of several pro-survival factors after SYK inhibition. These factors include AKT, the extracellular signal-regulated kinases (ERK), plus the anti-apoptotic factor MCL-1, which causes apoptosis of malignant CLL cells (68). Moreover, SYK was observed to be essential in integrin signaling, α-tubulin phosphorylation and CXCL12-mediated polarization of B lymphocytes (71). Thus, inhibition of SYK activity results in markedly reduced migration of the CLL cells toward CXCL12, a key homing attractor. Furthermore, SYK inhibition reduces the adhesion to VCAM-1, an important stromal integrin ligand, and decreases the secretion of CCL4 and CCL3 in CLL cells (72). This disruption of the interaction between the CLL microenvironment and the surrounding stroma through SYK inhibitors attenuates the integrin-/chemokine-mediated protective stromal survival effects in CLL (72). In addition, SYK inhibitors were shown to abrogate CD40 ligand-induced blastogenesis and CLL cell proliferation but not the proliferation of normal B lymphocytes (73).

3.3.1.1 SYK-targeting inhibitors

In clinical trials, the SYK inhibitors fostamatinib disodium (74), entospletinib (75) and cerdulatinib (76), for example, did show selective CLL growth-inhibitory effects. Fostamatinib is the first reported SYK inhibitor (also known as R788), that is metabolized to R406 *in vivo*. In a Eμ-TCL1 murine CLL model, fostamatinib was

found to selectively inhibit the growth of the leukemic B cell population, which resulted in significantly prolonged the animal survival (77). Fostamatinib showed an overall response rate (ORR) of 54.5% [only partial response (PR)] and a median progression-free survival (PFS) of 6.4 months (95% CI, 2.2-7.1) in CLL patients participating in a clinical phase I/II study (74). Entospletinib (GS-9973), like other classes of drugs inhibiting BCR signals, disrupts the cellular interactions with the tumor microenvironment and causes a redistribution of CLL cells, which clinically manifests by LN depletion and transient lymphocytosis (75). In an entospletinib phase II trial, patients with relapsed/refractory (R/R) CLL showed an ORR of 61% (95% CI, 44.5%-75.8%; all partial responses) and a median PFS of 13.8 months (95% CI, 7.7 months to not reached) (Table 1) (75). However, in another phase II study later on with R/R CLL patients that received prior treatment with a BCR inhibitor, the ORR of entospletinib was only 32.7% (95% CI, 21.7-45.3%) with a PFS of 5.6 months (95% CI, 3.7-8.3) (Table 1) (78). Recently, a phase I/II clinical trial of the CD20-targeting drug obinutuzumab in combination with entospletinib in patients with R/R CLL was completed with a promising outcome. Among the 21 R/R-CLL participants that received ≥1 prior therapy, the ORR was 67% (95% CI, 43-85%) with 14% (95% CI, 3-36%) achieving a complete response (CR), and 53% a partial response (PR). Median PFS was 27.5 months (95% CI, 16 months – not reached) (Table 1) (79). Cerdulatinib (PRT062070), an inhibitor of SYK and the Janus kinases JAK1/3, inhibits BCR- along with IL4-mediated signaling in CLL cells and reduces CCL3/CCL4 production to overcome stromal support (96). Moreover, cerdulatinib effectively induces apoptosis and inhibits the proliferation of ibrutinib-resistant CLL cells protected by the tumor microenvironment (97). Cerdulatinib treatment in a phase I study resulted in a restricted response in 3 out of 8 R/R CLL patients, demonstrating promising antitumor activity (76). A phase IIa study (NCT01994382) continued to assess cerdulatinib's tolerability and efficacy in patients affected by R/R B cell lymphomas, including CLL, and displayed an ORR of 61% (80).

Altogether, by effectively blocking BCR downstream signaling activity and by disrupting the protective interactions with the CLL microenvironment, SYK inhibition represents a promising strategy for treating R/R-CLL. The best SYK-targeting efficacy in CLL treatment was reached by the SYK inhibitor entospletinib in combination with the CD20-targeting drug obinutuzumab.

3.3.2 ZAP-70 - a prognostic marker in CLL

The two SYK family members SYK and the CD3ζ-chain-associated protein of 70 kDa (ZAP-70) are structurally homologous and also have a similar functional role in initiating proximal receptor signaling with slight differences, for example in their dependency on Src-family kinases for their catalytic activation (98). Although ZAP-70 was first solely described in T cells, it is also expressed partially in B-CLL cases and was found in other B cell malignancies (99). Although the activation of ZAP-70 was observed to be not quite efficient in CLL cells, the kinase is able to enhance BCR signaling independent of the phosphorylation status of its activating tyrosines (100). It was shown that ZAP-70 constitutively promotes gene expression, protein synthesis as well as microenvironment interactions in CLL cells. ZAP-70 mediated tonic BCR signaling induces an enhanced transcription of the genes coding for the proto-oncogene MYC and the T cell chemokines CCL3/CCL4. These chemokines stimulate the recruitment of T cells into proliferation centers, where they provide a supportive microenvironment. Thereby, ZAP-70 improves CLL cell fitness to survive and proliferate and further drives disease progression (101). This tonic BCR signaling is solely present in U-CLL patients and relies on the ability of ZAP-70 to stimulate the activation of AKT (102). Aberrant ZAP-70 expression in CLL correlates with an unmutated IGHV gene status the selection of unmutated IGHV region genes (103), the expression of a typically self-reactive BCR (43) and a poor clinical outcome (102). In contrast, B CLL cells lacking ZAP-70 expression are mainly anergic, lose BCR responsiveness, and generally result in a more indolent course of disease (104). Hence, ZAP-70 is used as a reliable prognostic marker for CLL (103). Recent findings suggest that ZAP-70 largely suppresses SYK-mediated BCR-signaling and rather redirects BCR-SYK-mediated signaling from Ca^{2+}-NFAT signaling toward the activation of the PI3K signaling pathway (105). This helps B cell clones to escape the NFAT-induced anergic state followed by negative selection that would typically cause elimination of autoreactive or pre-oncogenic B cells. Thus, expression of ZAP-70 in B cells allows sustained signaling induced by autoreactive BCRs, thereby facilitating malignant BCR-mediated B cell transformation (105).

Most interestingly, ZAP-70 not only mediates constitutive BCR signaling, but recently was also found to regulate chemokine-mediated signaling. For example, the CCL19- and CCL21-induced cell migration of U-CLL cells is regulated by the function of ZAP-70 to enhance CCR7 signaling (102). This new data was also presented in the ASH meeting 2023 (102). It provides important new explanations for the enhanced CLL cell fitness in ZAP-70 positive CLL and the more aggressive clinical course of the disease. To what extent this activity of ZAP70 is linked to the expression of unmutated IGHV region genes in CLL still requires clarification.

3.4 PI3K/AKT signaling in CLL

The phosphatidylinositol 3-kinase (PI3K) transduces signals from the BCR, chemokine receptors, plus adhesion receptors, thereby promoting the development, survival, chemotaxis as well as the cytoskeletal rearrangement of B cells (54, 106). By generating the lipid phosphatidylinositol-3,4,5-triphosphate (PIP_3), PI3K creates an essential docking platform for PH domain-containing signaling factors, such as BTK, the 3-phosphoinositide-dependent protein kinase 1 (PDK1), or the protein kinase B (PKB, also known as AKT). Binding to PIP_3 results in membrane recruitment and activation of the named signaling factors, which mediate the initiation of several BCR downstream signaling cascades (Figure 4) (12, 55). BCR-dependent signaling via the PI3K-AKT-axis is believed to provide the essential "tonic signal", that is required for malignant transformation and progression of CLL cells (107). Recently, AKT was found to be overactivated in high-risk CLL and in more than 50% of CLL patients having Richter's

TABLE 1 Outcome of selected clinical trials using BCR signaling targeting inhibitors.

Target	Agent	Phase	Patient [*n*]	Response	Trial information
LYN	Dasatinib	II	15	PR 20% (90% CI, 6-44%), nodal response 60% (13% CR; 47% PR)	Relapsed fludarabine-treated CLL patients, 73% with high-risk del(11q) or del(17p) (67)
SYK	Fostamatinib	I/II	11	mPFS 6.4 months (95% CI, 2.2-7.1), ORR 54.5% (PR)	R/R CLL patients (74)
	Entospletinib	II	49	ORR 32.7% (95% CI, 21.7-45.3%), NR 48.8%, mPFS 5.6 months (95% CI, 3.7-8.3)	R/R CLL patients, which received prior therapy with a BCR signaling inhibitor (78)
	Entospletinib	II	41	ORR 61% (95% CI, 44.5%-75.8%), only PRs, mPFS 13.8 months (95% CI, 7.7 months - not reached)	R/R CLL patients (75)
	Entospletinib + Obinutuzumab	I/II	21	ORR 67% (95% CI, 43-85%), CR 14% (95% CI, 3-36%), PR 53%, mPFS 27.5 months (95% CI, 16 months - not reached)	R/R CLL patients that received ≥1 prior therapy (79)
	Cerdulatinib	IIa	28	ORR 61%	R/R CLL patients, median No. of 3 prior therapies (80)
PI3K	Idelalisib	I	54	ORR 72% (95% CI, 58.4%-83.5%), PR 39%, PRwL 33%, mPFS 15.8 months	R/R CLL patients, median No. of 5 prior therapies, unmutated IGHV 91%, del (17p)/ mutTP53 24% (81).
	Idelalisib + Rituximab	III	110	ORR 85.5% (95% CI, 77.5-91.5%), CR 0.9%, PR 84.5%, mPFS 20.3 months (95% CI, 17.3 to 26.3 months), OS 40.6 months (95% CI, 28.5-57.3 months)	R/R CLL patients, median No. of 3 prior therapies, unmutated IGHV 83.6%, del(17p)/mutTP53 43.2% (17)
	Idelalisib + Rituximab + Bendamustine	III	207	ORR 70% (95% CI, 63-76%), CR 1.4%, PR 68.6%, mPFS 20.8 months (95% CI, 16.6-26.4 months; HR = 0.33)	R/R CLL patients, unmutated IGHV 84%, del(17p)/mutTP53 33% (82).
	Duvelisib	III	160	ORR 74%, CR 0.6%, PR 72.5%, PRwL 0.6%, mPFS 13.3 months (HR = 0.52)	R/R CLL patients, median No. of 2 prior therapies, del(17p)/mutTP53 19% (18).
BTK	Ibrutinib	Ib/II	31	ORR 87%, CR 35%, PR 45%, PRwL 6%, mPFS NR	First-line treated patients, unmutated IGHV 48%, del(17p) 6% (16).
	Ibrutinib	Ib/II	101	ORR 89%, CR 10% PR 76%, PRwL 3%, mPFS 52 months (95% CI, 38–70)	R/R CLL patients, median No. of 4 prior therapies, unmutated IGHV 78%, del(17p) 34% (16)
	Ibrutinib + Venetoclax	III	260	ORR 95.4% (95% CI, 92.1-97.6%), CR 71.5% (95% CI, 65.6%, 76.9%), mPFS NR, 3-year PFS rate 97.2% (HR = 0.13; 95% CI, 0.07-0.24)	First-line treatment, treatment-naive CLL, unmutated IGHV 56.9%, del(11q) 20.6%, del (13q) 31.4% (83).
	Acalabrutinib	I/II	99	ORR 97%, CR 7%, PR 90%, mPFS NR, estimated 48-month PFS rate 96% (95% CI, 89-98%)	TN CLL, unmutated IGHV 62%, mutTP53 18%, del(17p) 10% (84).
	Acalabrutinib	Ib/II	134	ORR 94% (95% CI, 89-97%), CR 4%, PR 84%, PRwL 6%, mPFS NR, estimated 45-month PFS 62% (95% CI, 51-71%)	R/R CLL patients, median No. of 2 prior therapies, unmutated IGHV 73%, del(17p) 23%, del(11q) 18% (85).
	Acalabrutinib	II	60	ORR 78% (95% CI, 66-88%), CR 8%, PR 65%, PRwL 5%, mPFS NR, 36-month PFS 58% (95% CI, 42-71%) and OS rate 78% (95% CI, 65-87%)	ibrutinib-intolerant R/R CLL patients, median No. of 2 prior therapies, del(17p) 28% (86)
	Zanubrutinib	III	109	ORR 94.5%, CR 3.7%, PR 87.2%, PRwL 3.7%, mPFS NR, 18-month PFS 88.6% (95% CI: 79.0–94.0) and OS rate 95.1% (95% CI: 88.4–98.0)	treatment-naïve CLL with del(17p) 100% (87).
	Zanubrutinib	I/II	192	ORR 91%, CR 8%, PR 83%, mPFS 61.4 (95% CI, 40.5 – NR) months, 36-month PFS 72.9% and OS 80%	R/R CLL patients, unmutated IGHV 40.6%, del(17p) 14.6%, mutTP53 50%, del(11q) 22% (88).
	Zanubrutinib + Obinutuzumab	Ib	45	ORR (TN) 100%, 30% CR, 70% PR; ORR (R/R) 92%, 28% CR, 64% PR; mPFS NR	R/R CLL patients (56%), TN CLL (44%) (89)

(Continued)

TABLE 1 Continued

Target	Agent	Phase	Patient [*n*]	Response	Trial information
	Tirabrutinib	I	28	ORR 96%, estimated mPFS 38.5 months, median OS 44.9 months	R/R CLL patients, median No. of 4 prior therapies (not BTKi), unmutated IGHV 84%, mutTP53 52%, del(17p) 36% (90, 91).
	Tirabrutinib + Idelalisib + Obinutuzumab	II	30	ORR 93.3% (95% CI, 80.5-98.8%), CR 6.7%, PR 86.7, mPFS NR (90% CI, 22.3-NR), 24-month PFS 80.6% (90% CI, 41.1%-94.9%), and OS 96.7% (90% CI, 83.9%-99.3%)	R/R CLL, unmutated IGHV 63%, del(17p)/ mutTP53 33% (92).
	Pirtobrutinib	I/II	247	ORR 82.2% (95% CI, 76.8-86.7), CR 1,6%, PR 71,7%, PRwL 8.9%, mPFS 22.1 months (95% CI, 19.6-27.4)	R/R CLL patients, median No. of 3 prior therapies (all BTKi), mutBTK^{C481} 37.8%, mutPLCγ_2 8%, del(17p)/mutTP53 46.6% (93)
	Pirtobrutinib	I/II	100	ORR 79.0% (95% CI, 69.7-86.5%), PR 70%, PRwL 9%, mPFS 16.8 months (95% CI, 13.2-18.7)	R/R CLL patients, median No. of 3 prior therapies, 100% prior BTKi and BCL2i treatment (93)
	Nembtabrutinib	I	29	ORR 75% (PR), mPFS NR (95% CI, 16.7 months – NR) for patients treated at 65mg once daily (n = 8)	R/R CLL, median No. of 5 prior therapies, mutBTK^{C481} 82.8%, mutTP53 38%, del(17p) 24% (94).
	Nembtabrutinib	I/II	57	ORR 56% (95% CI, 42 – 69%), CR 3.5%, PR 26.3% PRwL 26.3%, mPFS 26.3 months (95% CI, 10.1 - NR)	R/R CLL, median No. of 4 prior therapies (cBTKi 95%, cBTKi + BCL2i 42%, mutBTK^{C481} 65%, del(17p) 33%, mutTP53 32% (95).
	Vecabrutinib	Ib	30	(1/30) PR 0.4%, (11/30) stable disease 37%.	R/R CLL, median No. of 4 prior therapies, mutBTK^{C481} 55.2%, del(17p)/ mutTP53 73.3%

ORR, overall response rate; CR, complete remission; PR, partial remission/response; PRwL, partial response with lymphocytosis; mPFS, median progression-free survival; OS, median overall survival; NR, not reached; CI, confidence interval; HR, hazard ratio; R/R, relapsed refractory; TN, treatment-naïve.

transformation (RT), a highly aggressive form of lymphoma that is developed by 2 – 10% of patients during the clinical progression of CLL (108). Moreover, Kohlhaas et al. identified constitutive AKT activation as a driver of CLL to initiate RT through enhanced Notch signaling of the RT CLL cells with the T cells of their tumor microenvironment (108). The phosphatase and tensin homolog (PTEN) is a tumor suppressor that antagonizes PI3K/AKT signaling. PTEN represses PI3K signaling by PIP_3 dephosphorylation, which leads to cell cycle arrest as well as apoptosis (12, 54). In CLL patients, PTEN expression was shown to be downmodulated. Furthermore, genetic deletion of *Pten* results in significantly accelerated CLL development in Eμ-TCL1 mice, which underlines its crucial involvement in malignant transformation (10). In line with this, allelic variances in the *Pten* gene-containing locus 10q23.3 could be identified in CLL patients as well as a total loss of heterozygosity. However, no direct genetic *Pten* mutations were found (109). Furthermore, a decreased PTEN expression is associated with a poor CLL prognosis (110), indicating an essential role of PTEN downregulation in the leukemogenesis and progression of CLL.

3.4.1 PI3K-targeting inhibitors

The PI3K is categorized into three different classes (I, II, III). The PI3Ks of class I can be subdivided into four isoforms: PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ. The isoforms PI3Kγ plus PI3Kδ are expressed in CLL cells and have distinct important functions in regulating BCR signaling, cell migration as well as CLL cell adhesion to stromal cells (111). Idelalisib, a selective PI3Kδ inhibitor, suppresses BCR-mediated signaling as well as CLL cell interactions with the protective tumor microenvironment (112). This causes CLL cell mobilization, resulting in transient lymphocytosis and size reduction of the LNs (113). In a phase I clinical study with idelalisib, a consistent decrease of AKT phosphorylation, reduced secretion of stroma-derived factors (CD40L, CCL2, CXCL13, tumor necrosis factor (TNF)-α) as well as CLL-derived chemokines such as CCL3, CCL4, CCL17 and CCL22 could be observed. Moreover, Idelalisib treatment exhibits a beneficial safety profile and induces a fast and stable disease reduction in R/R CLL patients with poor prognosis, that received a median of 5 prior therapies (81). The ORR of this study reached 72% (95% CI, 58.4%-83.5%), while 39% of the patients had a PR, and 33% showed treatment-induced lymphocytosis. The overall median PFS was 15.8 months, but 32 months with the higher (now recommended) dose of ≥150 mg (81). In patients with R/R CLL, combined therapy of idelalisib and rituximab (a CD20-tageting antibody, frequently used in CLL therapy) results in a higher median overall survival (OS) compared to rituximab therapy alone (17, 114). The OS was 40.6 months (95% CI, 28.5 - 57.3 months) and 34.6 months (95% CI, 16.0 months – not reached (NR)) for idelalisib-rituximab-treated and placebo-rituximab-treated patients, respectively (more data in Table 1) (17). However, relative to the placebo group, idelalisib increased the

incidence of grade ≥ 3 diarrhea, grade ≥ 3 colitis and grade ≥ 3 pneumonitis to 16.4%, 8.2% and 6.4%, respectively (17). A different combined therapy of the chemotherapy drug bendamustine, rituximab, and idelalisib indicates improved the median PFS relative to bendamustine-rituximab combined treatment in R/R CLL patients (PFS 20.8 (95% CI, 16.6 – 26.4%) vs 11.1 (8.9 – 11.1%) months; hazard ratio (HR) = 0.33 (95% CI, 0.25 – 0.44%). For additional results see Table 1. However, a higher risk of infection and generally higher incidence of serious adverse events were observed in the idelalisib-treated group (82). By now, some novel PI3K inhibitors have been developed, including copanlisib (115), duvelisib (116), and umbralisib (117). Based on promising results in a phase III trial (ORR 74%, mPFS 13.3 months (HR = 0.52)), the PI3Kδ and PI3Kγ dual inhibitor duvelisib was approved by the FDA for the treatment of R/R CLL in the year 2018 (18).

Despite the striking success of PI3Kδ inhibitors in CLL therapy, development of resistance upon idelalisib treatment was observed in several patients (81, 114). Recently, hyperactivated insulin-like growth factor1 receptor (IGF1R) signaling was described as a possible mechanism of PI3Kδ inhibitor resistant CLL cells, suggesting IGF1R-targeted treatment as an effective strategy to overcome PI3Kδ inhibitor resistance (118, 119).

In general, the PI3K-AKT signaling axis represents a promising therapeutic target providing an alternative strategy in the treatment of high-risk R/R-CLL. According to recently published data, especially the patients refractory to prior ibrutinib treatment tend to show a more favorable response to idelalisib therapy (120). However, the increased risk of infection and the higher incidence of serious adverse events observed in combination clinical trials comprising ibrutinib treatment (17, 82) questions the tolerability of this PI3K-targeting drug.

3.4.2 AKT-targeting inhibitors

Another promising drug, named OSU-T315, targets the PI3K-AKT signaling axis in a different way: it specifically prevents AKT activation by blocking AKT membrane recruitment without modifying the activation status of receptor-associated kinases. With the disruption of AKT recruitment to lipid rafts, OSU-T315 targets CLL cell survival and triggers caspase-dependent CLL cell apoptosis. *In vitro*, OSU-T315 evidences potential therapeutic effectiveness in high-risk CLL patients with unmutated IGVH, del (17p13.1) or resistance to ibrutinib. Moreover, treatment with OSU-T315 significantly prolonged the survival of TCL1 mice (121). This AKT-targeting inhibitor presents an outstanding novel mechanism in the therapy of CLL and possibly also other B-cell malignancies. Further investigations in a phase I/II clinical trial would provide interesting insights in the tolerability and efficacy of this agent in the treatment of R/R CLL.

3.5 The Bruton's tyrosine kinase

The Bruton's tyrosine kinase (BTK), a Tec family kinase, is considered a key regulator of (oncogenic) BCR signaling, critical for the pathogenesis and progression of CLL cells (122). BTK is activated downstream of the BCR via PH domain-mediated membrane recruitment to PIP_3, followed by phosphorylation, either by SYK or an SFK (123). This results in phospholipase C γ2 ($PLC\gamma_2$) activation, which in turn induces the activation of downstream MAPK signaling pathway and the transcription factor nuclear factor of activated T-cells (NFAT). Thereby, BTK links the BCR to its downstream signaling effectors (Figure 4) (55). Due to chronic BCR signaling, most CLL cell clones show increased BTK expression as well as constitutive phosphorylation compared to non-malignant B cells (124–126). Beyond its classical role in mediating BCR signaling, BTK also has some other molecular effects. As a key regulator of CXC-chemokine receptor 4 and 5 signaling, BTK controls B cell migration in response to the so-called homeostatic chemokines CXCL12 and 13, as well as tissue homing, integrin-mediated adhesion, homeostasis or cellular retention in supportive lymphoid niches (127). The survival and relapse of CLL cells are thought to partially depend on the interaction of leukemic cells with their tumor microenvironment along with the LN-resident CLL cells (128). Thus, functional inhibition of BTK in primary CLL cells strongly reduces BCR- plus chemokine-controlled retention of leukemic B cells in their protective tumor microenvironment (129). In addition, BTK was shown to function in monocyte/macrophage cell populations, which represent a relevant component of the CLL tumor microenvironment (130). Altogether, BTK provides a promising therapeutic target.

3.5.1 BTK inhibitor ibrutinib

The BTK inhibitor ibrutinib has revolutionized the treatment of CLL patients. In February 2014, ibrutinib was approved by the FDA and nowadays is preferred as first-line therapy for the majority of CLL patients. In a clinical phase Ib/II study, ibrutinib showed high efficacy both, in first-line treatment settings with an ORR of 87% (CR 35%, PR 45%) and in the treatment of R/R CLL (ORR 89%; CR 10% PR 76%) (16). The median PFS was not reached (95% CI, not estimable (NE)-NE) in CLL patients with first-line treatment and 52 months (95% CI, 38–70) in R/R CLL patients. The estimated PFS rate of 7 years was 83% with first-line treatment and 34% with treatment for R/R CLL (16). Ibrutinib covalently interacts with the active site of BTK at cysteine 481 and thereby prevents the signal transmission to BTK-downstream survival pathways such as mitogen-activated protein kinase (MAPKs), PI3K or nuclear factor-κB signaling (126, 131). This results in reduced CLL cell proliferation and apoptosis (126, 131). Increasing evidence indicates a crucial inhibitory role of ibrutinib on constituents of the CLL microenvironment (132). For example, ibrutinib effectively blocks the secretion of survival factors (such as BAFF, CD40L, IL-4, IL-6, TNF-α) and inhibits fibronectin binding, as well as the cellular interaction with the stroma. Thereby, the dialog of the tumor cells with the microenvironment is interrupted (126). In addition, ibrutinib treatment causes a decrease in $CD4^+$ and Th17 T cells together with a diminished expression of activation markers on T cells (132). This may be a side-effect of ibrutinib's ability to also inhibit other kinases such as the interleukin-2-inducible kinase (ITK). ITK is a BTK homolog that plays a role in the activation of T cells as well as natural killer cells (133). Ibrutinib also inhibits

chemokine-mediated cellular migration and reduces the production of BCR-induced chemokines such as CCL3 and CCL4 in CLL cells. This results in early transient lymphocytosis together with a reduction in disease progression (134). In ibrutinib-treated CLL patients, the transient lymphocytosis correlates with a size reduction of the spleen and LNs, followed by a rise of leukemic cells in the blood (135). Ibrutinib is supposed to prevent the interaction between CLL cells and microenvironmental stroma cells that support the propagation, maintenance, as well as the resistance of malignant CLL cells (136, 137). By doing so, ibrutinib initiates CLL cell evasion from protective niches, leading to the apoptosis of CLL cells due to a lack of stromal support (132, 138). The most common primary reasons for CLL patients to discontinue ibrutinib treatment were disease progression (first-line, 6%; R/R, 38%) and adverse events (first-line, 26%; R/R, 23%) (16). Although ibrutinib shows already great efficacy with a high ORR in monotherapy, several ongoing clinical studies are currently aiming to discover a combination therapy that increases the efficacy and tolerability of an ibrutinib-monotherapy in the treatment of CLL. In a recent phase III study, presented at the ASH meeting 2023, a significantly improved response in CLL patients treated with a combination of ibrutinib and the BCL2 inhibitor venetoclax was observed with a 3-year PFS rate of 97.2% and an ORR of 95.4% (95% CI, 92.1-97.6%) (Table 1). Severe adverse effects were reported in 51% (83). With these results, the combination of ibrutinib and venetoclax indicates superior clinical efficacy and suggests a strong synergy of BCL2 and BCR-dependent pathways. Consequently, ibrutinib-venetoclax seem to be a promising combination for a successful first-line treatment in combatting CLL.

3.5.2 CLL patient's resistance to ibrutinib

Despite its huge clinical effectiveness, resistance and/or relapse of CLL in patients receiving ibrutinib therapy was frequently observed. The majority of ibrutinib-resistant CLL patients (~ 85%) acquired mutations in the BTK or PLCγ_2 expressing genes. Especially, the BTK^{C481S} mutation is frequently described. It disables ibrutinib's capacity to irreversibly bind BTK, culminating in poor clinical outcomes (139). The R665W and L845F mutations of PLCγ_2, which were identified in ibrutinib-resistant patients, are hypermorphic and induce BCR signaling independent of the BTK activity (140). Both PLCγ_2 mutants are highly sensitive to activation via the Rho GTPase RAC2, suggesting an important role of RAC2 in activating PLCγ_2 in a BTK-independent manner (141). Furthermore, SYK and LYN, respectively, were shown to play a role in inducing mutant PLCγ_2 activity, since inhibition of either SYK or LYN impairs mutant PLCγ_2-mediated signaling (142). Another study showed that CLL-specific PLCγ_2 mutants such as PLCγ_2^{S707Y} are still responsive to a catalytical inactive BTK variant with reduced sensitivity to covalent BTK inhibitors. This activity of noncatalytic BTK may constitute a primary CLL resistance to active-site BTK inhibitors (143). To overcome ibrutinib resistance in CLL treatment, several second-generation BTK inhibitors were generated and extensively studied to evaluate their tolerability plus efficacy in patients with R/R CLL (reviewed in (144)).

3.5.3 Novel covalent BTK inhibitors

3.5.3.1 Acalabrutinib

Acalabrutinib (ACP-196) also binds irreversibly to the BTK C481 active site, however, with a higher selectivity compared to ibrutinib since it is just weakly interacting with the TEC kinase and shows no inhibition of ITK or EGFR, resulting in less adverse effects (145, 146). Among all new-generation BTK inhibitors, acalabrutinib is presently the most advanced drug in clinical development and demonstrates an impressive ORR of 97% (90% PR; 7% CR) in treatment-naïve CLL. In this phase I/II clinical study, the median PFS of acalabrutinib-treated CLL patients was not reached and the 48-month PFS was estimated to 96% (95% CI, 89-98%) (Table 1). Serious adverse events were reported in 38% of the CLL patients (84). In R/R CLL patients, acalabrutinib treatment reached an ORR of 94% (95% CI, 89-97%; 4% CR; 84% PR), and an estimated 45-month PFS of 62% (95% CI, 51-71%) (85). In an ongoing phase II study, acalabrutinib presently showed high efficacy and safety in most R/R CLL patients unable to tolerate ibrutinib with an ORR of 78% (95% CI, 66-88%), and an estimated 36-month PFS rate of 58% (95% CI, 42-71%) (Table 1). Related to aclarubicin treatment, severe adverse events were experienced by 17% of the patients (86). Another phase III clinical study compared the efficacy of acalabrutinib relative to idelalisib-rituximab or bendamustine-rituximab treatment in R/R CLL patients. The patients treated with acalabrutinib reached a significantly increased 12-month PFS of 88% (95% CI, 81-92%) compared to idelalisib-rituximab or bendamustine-rituximab treatment (68%; 95% CI, 59-75%) (147).

3.5.3.2 Zanubrutinib

Zanubrutinib (BGB-3111) is another covalent BTK inhibitor that irreversibly binds C481 in the BTK active site. Compared to ibrutinib, zanubrutinib exhibits a higher selectivity. Initial data of clinical studies indicate a beneficial activity and safety profile of zanubrutinib in CLL patients, in monotherapy or combined with obinutuzumab (87, 89). At an average follow-up of 18.2 months in a clinical phase III trial, zanubrutinib therapy in treatment-naïve CLL patients with del(17p) mutation achieved an ORR of 94.5% (CR 3.7%, PR 87.2%, PRwL 3.7%), and the 18-month PFS rate was estimated to 88.6% (95% CI: 79.0–94.0) (Table 1) (87). In R/R CLL, the ORR of zanubrutinib was 91% (CR8%, PR 83%), with a median PFS of 61.4 months 61.4 (95% CI, 40.5 – NR), and a 36-month PFS and OS of 72.9% and 80%, respectively. Severe adverse effects were reported in 56.9% (Table 1) (88). ORR of the Zanubrutinib plus obinutuzumab combination therapy was 100% (n = 20; 30% CR, 70% PR) in treatment-naïve CLL patients and 92% (n = 23; 28% CR, 64% PR) in patients with R/R CLL. The median follow-up was 29 months, median PFS was not reached and serious adverse events were reported in 49% of the patients (89).

3.5.3.3 Tirabrutinib

Tirabrutinib (ONO/GS-4059) covalently inhibits BTK by preventing Tyr223 auto-phosphorylation. Similar to acalabrutinib and zanubrutinib, tirabrutinib was well tolerated in a first ongoing phase I clinical evaluation in patients with R/R CLL, showing an estimative median PFS of 38.5 and 44.9 months overall survival

(90). Like in the other BTK inhibitors, a large number of patients (82%) exhibit transient CLL cell lymphocytosis (91). Two phase II trials in R/R CLL patients currently assesses the efficacy and safety of tirabrutinib in combination with entospletinib or idelalisib, without or with the addition of obinutuzumab (NCT02983617 and NCT02968563). Initial data shows high efficacy and tolerability in relapsed CLL patients treated with a combination of tirabrutinib, idelalisib and obinutuzumab with an ORR of 93.3% (95% CI, 80.5-98.8%), and a 24-month PFS and OS of 80.6% (90% CI, 41.1%–94.9%) and 96.7% (90% CI, 83.9%–99.3%), respectively. Serious treatment-emerged adverse events were experienced in 36.7% (92).

3.5.4 Novel non-covalent BTK inhibitors

Noncovalent (reversible) BTK inhibitors differ from the previously mentioned compounds in noncovalently binding BTK, resulting in selective inhibitory effects regardless of a C481S BTK mutation (148). Noncovalent BTK-inhibitory drugs were generated in order to successfully improve the treatment in R/R CLL patients with BTK-inhibitor resistance bearing a BTK C481S mutation. In preclinical trials, the non-covalent BTK inhibitors pirtobrutinib (LOXO-305) (149), nemtabrutinib (ARQ-351) (150), vecabrutinib (SNS-062) (151), and fenebrutinib (GDC-0853) (152), inhibited BCR signaling in BTK C481 mutant cells and/or in animal models.

3.5.4.1 Pirtobrutinib

The non-covalent, orally available, BTK inhibitor Pirtobrutinib (LOXO-305) reversibly blocks the ATP binding site on BTK. It is highly selective with a more than 300-fold selectivity for BTK in 98% of tested kinases (153). Recently, a phase I/II clinical study revealed promising effectiveness of pirtobrutinib in the therapy of patients with heavily pretreated CLL. Patients that received prior BTK inhibitor treatment achieved an ORR of 82.2% (95% CI, 76.8-86.7; CR 1,6%, PR 71,7%, PRwL 8.9%), with a median PFS of 22.1 months (95% CI, 19.6-27.4). An ORR of 79.0% (95% CI, 69.7-86.5%; PR 70%, PRwL 9%) and a median PFS of 16.8 months (95% CI, 13.2-18.7) was observed in the subgroup of patients who had previously received both a BTK inhibitor and a BCL2 inhibitor (Table 1) (93). 37.8% (84/222) of the treated patients exhibit a BTKC481 mutation. Notably, the ORR of patients with a BTKC481 mutation was with 89% (95% CI, 90-95%) significantly higher compared to the ORR of CLL patients without BTKC481 mutation (74%; 95% CI, 64-82%). Moreover, pirtobrutinib is well tolerated in most CLL patients. Only 2.6% discontinued pirtobrutinib therapy due to treatment-related adverse events (93). An ongoing phase III clinical trial is currently comparing the efficacy of pirtobrutinib in combination with venetoclax and rituximab to the standard therapy venetoclax-rituximab in previously treated R/R CLL patients (NCT04965493).

3.5.4.2 Nembtabrutinib

Nemtabrutinib (ARQ-351) is a reversible, non-covalent BTK inhibitor that binds and inhibits the kinase activity of BTK independent of the C481 residue. As a result, nemtabrutinib targets both, normal BTK and the C481-mutated forms of BTK. In preclinical studies, nembtabrutinib increased survival over ibrutinib in Eμ-TCL1 mouse models and was able to suppresses BCR-induced activation of PLCγ_2 and BTK-C481S mutants, occurring in patients with clinical resistance to ibrutinib (150). In a first-in-human phase I clinical study, nembtabrutinib showed preliminary efficacy in a patient population with advanced R/R CLL. An ORR of 75% (PR) was reached by 8 CLL patients treated at 65mg once daily (n = 8), including 6 patients with a BTK mutation. Median PFS was not reached (95% CI, 16.7 months – NR) (Table 1) (94). Another phase I/II study of nemtabrutinib in R/R B-cell malignancies reported an ORR of 56% (95% CI, 42 – 69%; CR 3.5%, PR 26.3% PRwL 26.3%) with a median PFS of 26.3 months (95% CI, 10.1 - NR) (Table 1) (95). Notably, 95% of CLL patients received prior treatment with a covalent BTK inhibitor (cBTKi) and 42% had prior cBTKi and BCL2 inhibitor therapy. Moreover, 63% of the CLL study population exhibited a BTK-C481S mutation (95). In general, nembtabrutinib shows promising efficacy in the treatment of high-risk CLL patients with clinical resistance/relapse to previous therapy and successfully targets BTK-C481 mutations facilitating CLL cell resistance to several covalent BTK inhibitors. Moreover, the clinical safety was manageable with 11.4% (94) and 13% (95) of patients, respectively, that discontinued treatment due to treatment-related adverse events. An ongoing, open-label, phase III trial is presently investigating the safety and efficacy of nemtabrutinib in combination with venetoclax as second-line or later therapy for R/R CLL patients (NCT05947851).

3.5.4.3 Vecabrutinib

In a preclinical characterization using an Eμ-TCL1 mouse model, vecabrutinib (SNS-062) significantly reduced tumor burden and improved animal survival (151). However, first results of a phase Ib clinical study (NCT03037645) in CLL patients with or without BTK mutation reveal that vecabrutinib did not translate to such a strong response as expected. In general, vecabrutinib was well-tolerated, but merely resulted in modest clinical benefit, with 0.4% PR (1/30) and 37% (11/30) of the CLL patients having a stable disease (Table 1) (154). Despite strong preclinical evidence, the efficacy of vecabrutinib was not sufficient to combat CLL in refractory patients. Consequently, the clinical development and evaluation of vecabrutinib in the treatment of B-cell malignancies was terminated (154).

3.5.5 Emerging resistance to novel BTK inhibitors

Despite the promising results of the novel noncovalent BTK inhibitors, mechanisms of resistance to these drugs could already be observed. Similar to ibrutinib-resistance, mutations in PLCγ_2 and BTK are the predominant resistance mechanisms to acalabrutinib treatment. BTK C481 mutations occurred in 43% of acalabrutinib-treated CLL patients at the time of disease progression, T474I mutation in 21% and PLCγ_2 mutations were found 29% (155). On-target BTK mutations (e.g. A428D, V416L, T474I, M437R, L528W) and PLCγ_2 mutations allow CLL cells to escape the BTK inhibitory effects in CLL patients treated with the noncovalent inhibitor pirtobrutinib (156). Recent data, presented at the ASH meeting 2023, show that in all CLL patients that had a BTK C481S

mutation prior to pirtobrutinib therapy, the C481S mutation declines on pirtobrutinib treatment. However, at disease progression this mutation is replaced by a T474I BTK mutation in 3 of 5 patients, while the BTK L528W mutation, that inactivates kinase function is observed in one of 5 patients (157). The kinase-inactive mutation BTK L528W was also enriched in CLL patients, which acquired resistance to the second-generation BTK inhibitor zanubrutinib (158). Similarly, BTK plus PLCγ_2 mutations, including mutations in the BTK amino acids L528, A428, and V416, were discovered in REC-1 mantle cell lymphoma (MCL) cell lines, which are resistant to vecabrutinib, pirtobrutinib, and fenebrutinib. Interestingly, only REC-1 cells resistant to nemtabrutinib acquired no BTK or PLCγ_2 mutation, suggesting a different mechanism in the development of resistance (159).

3.6 MAPK signaling

The mitogen-activated protein kinase (MAPK) signaling pathway is activated downstream of the BCR and plays a pivotal role in the regulation of cell differentiation, proliferation, survival, plus cell migration (160). Nearly half of CLL patients show activated MAPK signaling, suggesting a pathogenic role of this pathway in CLL (161). In addition, several studies identified activated MAPK signaling as a key oncogenic driver of CLL development and progression, with approximately 5 – 8% of CLL patients harboring at least a single genetic mutation in this pathway. Mutations of the MAPK signaling pathway include the RAS genes (NRAS, KRAS), BRAF, and the novel putative driver MAP2K1 (162, 163). Furthermore, CLL patients carrying such mutations frequently correlate with an aggressive course of disease, exhibiting adverse biological characteristics like an increased CD49d, ZAP-70 expression, trisomy 12 or unmutated IGHV regions and also show a significantly shorter treatment-free survival (163, 164).

Despite its activation in CLL, targeting MAPK signaling does not show significant effects on CLL viability. Paradoxically, MAPK signaling inhibitors promote MAPK signaling activity, reduce the expression of negative modulators of their pathway, and augment AKT-mediated signaling (165). In line with this, genetic deletion or inhibition of the inhibitory phosphatases DUSP1 or DUSP6, which are negatively regulating MAPK signaling, results in reduced CLL cell survival. Apoptosis following DUSP1/6 inhibition was also evident in drug-resistant CLL (166). This appears surprising since MAPK activity is actually well-known for its effects in promoting cell survival. But the B cell-specific physiological effects of the MAPK pathway vary to a great extent. For example, according to the cell type and stimuli, activation of a specific MAPK, named extracellular signal-regulated kinase (ERK), can also trigger apoptotic processes causing cell death (167). Similarly, active ERK1/2 is also associated with cell death during the B cell negative selection, which serves to avert autoimmunity (168). In these cases, acute activation of MAPK signaling promotes the aggregation of mitochondrial reactive oxygen species, thereby inducing cell death mediated by the DNA damage response (167, 168). This phenomenon was also observed after DUSP1/6 inhibition in CLL (166), which supports an important role of DUSP1/6-mediated negative regulation of MAPK signaling in CLL cells survival and proposes DUSP1/6 inhibition along with subsequent over-active MAPK signaling as a potential new CLL therapy approach. In contrast, the MEK1/2 inhibitor binimetinib in monotherapy or combined with the BCL-2 inhibitor venetoclax shows great effectiveness in causing CLL cell death (169). Besides, CLL cells with trisomy 12 are susceptible to ERK and MEK inhibition (170). Furthermore, the ERK inhibitor rulixertinib reduces ERK phosphorylation in MAPK-mutant CLL clones (163). This suggests a dual role of MAPK activity in CLL, which may depend on the CLL subset/cell type.

3.7 NF-κB signaling

The nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) is retained in an inactive state by inhibitor proteins (IκB). Activation of the IκB kinase complex (IKK) leads to IκBs phosphorylation and subsequent degradation, which finally results in NF-κB translocation into the nucleus to induce target gene transcription (Figure 4) (171, 172). In B cells, NF-κB mediated transcription can be activated by several upstream signaling pathways including inflammatory cytokines, BCRs, toll-like receptors (TLR) or TNF receptors, like the B cell-activating factor receptor (BAFFR) or CD40 ligand. Activated NF-κB controls multiple processes, such as differentiation, cell cycle progression, and survival (171). Moreover, NF-κB signaling is found to be constitutively activated in CLL patients, indicating that aberrant NF-κB activation plays a crucial role in CLL pathogenesis and progression (13). In CLL cells, activation of the BCR successively increased the NF-κB mediated transcription, indicated by a 23% higher detectable target gene expression (173). Moreover, a few recurrently mutated genes involved in the activation of NF-κB were observed in CLL at a low frequency (> 5%) (i.e. mutations in MYD88, BIRC3, NFKBIE) (174–177). The mutated myeloid differentiation primary response 88 (MYD88) protein imitates constitutively active TLR signaling and thus intensifies BCR–mediated NF-κB signaling (177). The loss-of-function mutation in the gene coding for the baculoviral IAP repeat containing 3 (BIRC3) prevents negative regulation of the MAP3K14/NF-κB inducing kinase (NIK), a key activator of NF-κB signaling (174). The mutation in the inhibitory IκBε molecule encoding gene NFKBIE is associated with significantly enhanced NF-κB activation and is most frequently found in poor-prognostic subgroups of CLL (176). It was shown that cross-talk of CLL cells with the tumor microenvironment results in NF-κB activation, which provides pro-survival signals to the malignant CLL clones by increasing the expression of various anti-apoptotic genes (13, 178). Thus, NF-κB activity correlates with a dismal CLL outcome and represents an essential mechanism of CLL resistance (13, 178). Most interestingly, resistance mutations to BTK inhibitors were revealed to arise only through NF-κB and not via PI3K-RAS-MAPK-mediated signaling of the BCR pathway. Following BTK inhibition, CLL cells only select gain-of-function alterations that are mediated by NF-κB signaling, highlighting BTK's significant

role in BCR-induced activation of NF-κB (179). Hence, targeting NF-κB signaling represents a potential future therapeutic approach to overcome CLL resistance which is supported by NF-κB survival signaling originating from the protective tumor microenvironment. A first *in vitro* study shows promising effects in targeting the NF-κB inducing kinase (NIK) by the inhibitor CW15337 in CLL cell clones (180).

4 Tumor microenvironment and BCR-associated pathways in CLL

In addition to the signals originating from the BCR, CLL cell survival depends on various co-stimulatory signals, which can occur through direct cellular interactions or via soluble factors. These signals are essential for CLL cell survival by facilitating proliferation as well as migration and homing of the malignant cells to protective niches where they are able to undergo cell division. One BCR-associated pathway with continuously rising importance in regulating migration and homing of CLL is the CXCR4 signaling pathway.

4.1 CXCR4 signaling

The C-X-C motif chemokine receptor 4 (CXCR4) regulates the movement of B cells toward the chemokine C-X-C motif ligand 12 (CXCL12), its corresponding chemokine ligand. Interaction of CXCL12 with CXCR4 triggers the activation of several downstream pathways such as MAPK/ERK, PI3K/AKT, PLCγ/Ca^{2+} and NF-κB signaling (Figure 4) (181, 182). For CLL cells, CXCR4 expression is critical for the migration toward specific niches, where the leukemic cells are protected by a survival- and growth-promoting microenvironment (183). This is why in CLL patients, CXCR4 is upregulated and associated with adverse prognosis. In line with this, CLL patients exhibiting low CXCR4 levels are associated with good prognosis as well as a significantly decreased risk of disease progression (184). An oncogenic hyperactivated form of CXCR4 in Eμ-TCL1 mice was described to collaborate with TCL1 in accelerating the progression of CLL (182). Upon ibrutinib treatment, a downmodulation of CXCR4 expression levels and CXCR4 signal inhibition in CLL cells could be identified in Eμ-TCL1 mice (185). Lately, it was reported that CRISPR-Cas9 induced disruption of CXCR4 signaling significantly affects not only the migration and homing of CLL cells with RT, but also reduces cell growth in murine and patient-derived xenograft models and impairs BCR-mediated signaling (186). This is a first evidence that targeting the CXCR4 pathway possibly represent a potent new therapeutic target in CLL patients with or without RT. Regarding the emerging importance of CXCR4 in CLL progression and survival, CXCR4 inhibitors are presently investigated in clinical studies (187).

In addition, it was revealed that according to CXCR4 expression in combination with CD5 (a surface molecule characteristically expressed on CLL B cells), CLL clones can be defined into subgroups varying in the time elapsed since the last cell division (also called "age"): the newly originated, proliferative fraction (PF; $CXCR4^{Dim}CD5^{Bright}$), the double dim fraction (DDF; $CXCR4^{Dim}CD5^{Dim}$), the intermediate fraction (IF; $CXCR4^{Int}CD5^{Int}$), the double bright fraction (DBF; $CXCR4^{Bright}CD5^{Bright}$) and the resting fraction (RF; $CXCR4^{Bright}CD5^{Dim}$) (188–190). The last-born cells are thought to enter the circulation as PFs and from there transition to either a low CD5 (DDF) or a high CXCR4 (IF and DBF) phenotype, eventually converging into RFs (190). Besides, the fractions also differ in smIgM and smIgD BCR densities, since young cells of the PF show high IgM/IgD surface expression, whereas cells with low IgM/IgD expression were the oldest (RF) (190). While in CLL patients the peripheral blood mainly consists of quiescent CLL cells of the RF, the CLL cells residing in LNs are actively proliferating cells of the PF ($CXCR4^{Dim}CD5^{Bright}$) (189, 191). In compliance, gene expression analyses revealed that LNs are specific sites, where the upregulation of genes associated with BCR activation as well as CLL cell proliferation takes place (13). Furthermore, the high proliferation rate in LNs correlates with aggressive disease, rapid lymphocyte doubling, and shorter treatment-free survival compared to CLL with low growth rates (13, 191). Most interestingly, the different intraclonal fractions show variable susceptibility to CLL therapy, since older CXCR4-positive CLL cells (RF and DBF) were observed to be less susceptible to *in vivo* inhibition by ibrutinib relative to younger cells (190). This points out the necessity to develop new treatment strategies specifically targeting all points in the life cycle of the various intraclonal fractions of a CLL clone.

5 BCR signaling in the CLL cell metabolism

Deregulated cellular energy metabolism is a well-known hallmark of cancer. Like all malignant cells, CLL cells make adaptations to meet their increased metabolic needs. The mechanistic target of rapamycin (mTOR) complex is crucial for the coordination of energy, oxygen, nutrient and growth factor availability in the cell as well as the regulation of cellular growth and survival (192). mTOR forms two structurally and functionally unique complexes, mTORC1 and mTORC2. Although both complexes are crucial mediators of cellular metabolism, solely mTORC1 is directly activated by nutrient, oxygen, and energy availability, which is ultimately resulting in DNA, protein, and lipid synthesis as well as cellular growth (193). By inducing the activation of mTORC1, BCR signaling is (among others) directly implicated in the regulation of B cell metabolism. The tuberous sclerosis complex 1/2 (TSC1/2) negatively regulates mTORC1 by inhibiting RHEB GTPase activity, which is required to induce mTORC1 activation. BCR-mediated activation of the MAPK and PI3K/AKT signaling cascades leads to the phosphorylation and inhibition of TSC2, which ultimately results in the activation of mTORC1 (194).

In CLL, BCR signaling was found to regulate cellular metabolism via the PI3K/AKT/mTOR signaling axis. Genetic deletion as well as

inhibition of PI3Kδ results in a significant reduction of the metabolic flux in CLL cells (195). Moreover, metabolic flux analysis of 140 CLL patients revealed that patients that are diseased with the more aggressive form of U-CLL exhibit significantly higher glycolytic activity compared to M-CLL patients. These results indicate that the IGHV mutational status of CLL cells is directly linked to their glycolytic activity, most likely by BCR-mediated signaling (196). Similar to the IGHV mutational status, glycolytic capacity was found to be a reliable predictor of overall survival in CLL patients (196). Moreover, the proliferative drive in CLL cells is associated with high MYC and mTOR activity promoting mitochondrial biogenesis and leading to increased oxidative phosphorylation (OXPHOS). Increased MYC-mTOR-OXPHOS activity cooperates to drive cell growth and meet the increased energy needs in CLL (197). Due to the increased PI3K/AKT/mTOR pathway activity in CLL, studies targeting PI3K/AKT/mTOR signaling indicated pro-apoptotic effects in the treatment of CLL and other B cell leukemias (198). Thus, PI3K/AKT/mTOR signaling may present a future therapeutic target in the treatment of CLL, probably also in combination with already existing therapeutics. For example, combined mTOR and electron transport chain inhibition were found to synergistically counteract venetoclax resistance in CLL (199). This synergistic effect may provide an opportunity to enhance the efficacy of the BCL2 inhibitor venetoclax, which is frequently used in the treatment of CLL.

6 Conclusion

Our understanding of the CLL pathogenesis regarding BCR-mediated signaling, tumor microenvironment, and co-stimulatory signals has markedly improved during the last years. Furthermore, the range of therapeutic options to treat CLL has considerably increased. The development of next-generation drugs targeting BCR signaling crucial for CLL cell pathogenesis and survival has significantly ameliorated the clinical course of CLL patients. Several inhibitors targeting BCR downstream signaling pathways are already in clinical use and show high efficacy in CLL therapy (16–18, 74). Among all therapeutics targeting BCR-signaling, BTK-targeting inhibitors show the most beneficial clinical responses in the treatment of CLL. With an ORR of 95.4% (95% CI, 92.1-97.6%), a CR of 71.5% (95% CI, 65.6%, 76.9%) and a median 3-year PFS rate of 97.2% (HR = 0.13; 95% CI, 0.07-0.24), the combination of the BTK inhibitor ibrutinib with the BCL2 inhibitor venetoclax indicates superior clinical efficacy and suggests a strong synergy of BCL2 and BCR-dependent pathways (83). In most cases, disease progression is a result of specifically acquired mutations that allow the CLL cell to escape the inhibitory mechanism of the therapeutic agent. Due to constant research and improvement of the current therapeutics, novel drug combinations as well as next-generation inhibitors are also able to partially overcome therapy resistance in refractory CLL. Reversible, non-covalent BTK-inhibitors, such as pirtobrutinib or nemtabrutinib for instance, successfully target CLL cells exhibiting a BTK-C481 mutation and show promising efficacy in the treatment of CLL patients that have developed clinical resistance to ibrutinib therapy (93, 94). However, despite the immense progress in CLL research, no agent has clearly demonstrated efficacy in R/R CLL patients in the long run. The emerging importance of the tumor-supporting microenvironment in CLL progression and survival represents a novel point of action in the treatment of CLL. CXCR4-targeting inhibitors may present a promising mechanism to enhance the efficacy of existing treatment options by preventing CLL cell migration to these protective niches. Moreover, the variable susceptibility to CLL therapy of the different intraclonal fractions (187) points out the necessity to develop new treatment strategies specifically targeting all points in the life cycle of a CLL clone. Thus, continuous research in the field of CLL to understand the molecular mechanisms of leukemic transformation as well as CLL cell survival is of utmost importance to identify new drug targets or combinations and mechanisms of drug action in the future.

Author contributions

VS: Writing – review & editing. EH: Supervision, Writing – review & editing.

Funding

This work was supported by the German Research Foundation (DFG) through the SFB1074 (Experimental Models and Clinical Translation in Leukemia) projects A9/A10 and the DFG project (HO 5859/2-1).

Acknowledgments

We thank Hassan Jumaa for proofreading this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Fabbri G, Dalla-Favera R. The Molecular Pathogenesis of Chronic Lymphocytic Leukaemia. *Nat Rev Cancer*. (2016) 16:145–62. doi: 10.1038/nrc.2016.8

2. Stevenson FK, Forconi F, Kipps TJ. Exploring the Pathways to Chronic Lymphocytic Leukemia. *Blood*. (2021) 138:827–35. doi: 10.1182/blood.2020010029

3. Bosch F, Dalla-Favera R. Chronic Lymphocytic Leukaemia: From Genetics to Treatment. *Nat Rev Clin Oncol*. (2019) 16:684–701. doi: 10.1038/s41571-019-0239-8

4. Chiorazzi N, Chen SS, Rai KR. Chronic Lymphocytic Leukemia. *Cold Spring Harb Perspect Med*. (2021) 11(2):a035220. doi: 10.1101/cshperspect.a035220

5. Levit-Zerdoun E, Becker M, Pohlmeyer R, Wilhelm I, Maity PC, Rajewsky K, et al. Survival of Igα-Deficient Mature B Cells Requires Baff-R Function. *J Immunol*. (2016) 196:2348–60. doi: 10.4049/jimmunol.1501707

6. Kraus M, Alimzhanov MB, Rajewsky N, Rajewsky K. Survival of Resting Mature B Lymphocytes Depends on Bcr Signaling *Via* the Igalpha/Beta Heterodimer. *Cell*. (2004) 117:787–800. doi: 10.1016/j.cell.2004.05.014

7. Koehrer S, Burger JA. Chronic Lymphocytic Leukemia: Disease Biology. *Acta Haematol*. (2024) 147:8–21. doi: 10.1159/000533610

8. Jiménez de Oya N, De Giovanni M, Fioravanti J, Übelhart R, Di Lucia P, Fiocchi A, et al. Pathogen-Specific B-Cell Receptors Drive Chronic Lymphocytic Leukemia by Light-Chain- Dependent Cross-Reaction with Autoantigens. *EMBO Mol Med*. (2017) 9:1482–90. doi: 10.15252/emmm.201707732

9. Iacovelli S, Hug E, Bennardo S, Duehren-von Minden M, Gobessi S, Rinaldi A, et al. Two Types of Bcr Interactions Are Positively Selected During Leukemia Development in the Eμ-Tcl1 Transgenic Mouse Model of Cll. *Blood*. (2015) 125:1578–88. doi: 10.1182/blood-2014-07-587790

10. Schmid VK, Khadour A, Ahmed N, Brandl C, Nitschke L, Rajewsky K, et al. B Cell Antigen Receptor Expression and Phosphatidylinositol 3-Kinase Signaling Regulate Genesis and Maintenance of Mouse Chronic Lymphocytic Leukemia. *Haematologica*. (2022) 107(8):1796–814. doi: 10.3324/haematol.2021.279924

11. Chakraborty S, Martines C, Porro F, Fortunati I, Bonato A, Dimishkovska M, et al. B- Cell Receptor Signaling and Genetic Lesions in Tp53 and Cdkn2a/Cdkn2b Cooperate in Richter Transformation. *Blood*. (2021) 138:1053–66. doi: 10.1182/blood.2020008276

12. Seda V, Mraz M. B-Cell Receptor Signalling and Its Crosstalk with Other Pathways in Normal and Malignant Cells. *Eur J Haematol*. (2015) 94:193–205. doi: 10.1111/ejh.12427

13. Herishanu Y, Pérez-Galán P, Liu D, Biancotto A, Pittaluga S, Vire B, et al. The Lymph Node Microenvironment Promotes B-Cell Receptor Signaling, Nf-Kappab Activation, and Tumor Proliferation in Chronic Lymphocytic Leukemia. *Blood*. (2011) 117:563–74. doi: 10.1182/blood-2010-05-284984

14. Hallek M, Al-Sawaf O. Chronic Lymphocytic Leukemia: 2022 Update on Diagnostic and Therapeutic Procedures. *Am J Hematol*. (2021) 96:1679–705. doi: 10.1002/ajh.26367

15. Laurenti L, Efremov DG. Therapeutic Targets in Chronic Lymphocytic Leukemia. *Cancers (Basel)*. (2020) 12(11):3259. doi: 10.3390/cancers12113259

16. Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum K, et al. Ibrutinib Treatment for First-Line and Relapsed/Refractory Chronic Lymphocytic Leukemia: Final Analysis of the Pivotal Phase Ib/Ii Pcyc-1102 Study. *Clin Cancer Res*. (2020) 26:3918–27. doi: 10.1158/1078-0432.CCR-19-2856

17. Sharman JP, Coutre SE, Furman RR, Cheson BD, Pagel JM, Hillmen P, et al. Final Results of a Randomized, Phase Iii Study of Rituximab with or without Idelalisib Followed by Open-Label Idelalisib in Patients with Relapsed Chronic Lymphocytic Leukemia. *J Clin Oncol*. (2019) 37:1391–402. doi: 10.1200/JCO.18.01460

18. Flinn IW, Hillmen P, Montillo M, Nagy Z, Illés Á, Etienne G, et al. The Phase 3 Duo Trial: Duvelisib Vs Ofatumumab in Relapsed and Refractory Cll/Sll. *Blood*. (2018) 132:2446–55. doi: 10.1182/blood-2018-05-850461

19. Stevenson FK, Forconi F, Packham G. The Meaning and Relevance of B-Cell Receptor Structure and Function in Chronic Lymphocytic Leukemia. *Semin Hematol*. (2014) 51:158–67. doi: 10.1053/j.seminhematol.2014.05.003

20. Agathangelidis A, Darzentas N, Hadzidimitriou A, Brochet X, Murray F, Yan XJ, et al. Stereotyped B-Cell Receptors in One-Third of Chronic Lymphocytic Leukemia: A Molecular Classification with Implications for Targeted Therapies. *Blood*. (2012) 119:4467–75. doi: 10.1182/blood-2011-11-393694

21. Strefford JC, Sutton LA, Baliakas P, Agathangelidis A, Malčíková J, Plevova K, et al. Distinct Patterns of Novel Gene Mutations in Poor-Prognostic Stereotyped Subsets of Chronic Lymphocytic Leukemia: The Case of Sf3b1 and Subset 2. *Leukemia*. (2013) 27:2196–9. doi: 10.1038/leu.2013.98

22. Stamatopoulos K, Agathangelidis A, Rosenquist R, Ghia P. Antigen Receptor Stereotypy in Chronic Lymphocytic Leukemia. *Leukemia*. (2017) 31:282–91. doi: 10.1038/leu.2016.322

23. Gerousi M, Laidou S, Gemenetzi K, Stamatopoulos K, Chatzidimitriou A. Distinctive Signaling Profiles with Distinct Biological and Clinical Implications in Aggressive Cll Subsets with Stereotyped B-Cell Receptor Immunoglobulin. *Front Oncol*. (2021) 11:771454. doi: 10.3389/fonc.2021.771454

24. Minici C, Gounari M, Ubelhart R, Scarfo L, Duhren-von Minden M, Schneider D, et al. Distinct Homotypic B-Cell Receptor Interactions Shape the Outcome of Chronic Lymphocytic Leukaemia. *Nat Commun*. (2017) 8:15746. doi: 10.1038/ncomms15746

25. Ntoufa S, Papakonstantinou N, Apollonio B, Gounari M, Galigalidou C, Fonte E, et al. B Cell Anergy Modulated by Tlr1/2 and the Mir-17∼92 Cluster Underlies the Indolent Clinical Course of Chronic Lymphocytic Leukemia Stereotyped Subset 4. *J Immunol*. (2016) 196:4410–7. doi: 10.4049/jimmunol.1502297

26. Hwang KK, Trama AM, Kozink DM, Chen X, Wiehe K, Cooper AJ, et al. Ighv1-69 B Cell Chronic Lymphocytic Leukemia Antibodies Cross-React with Hiv-1 and Hepatitis C Virus Antigens as Well as Intestinal Commensal Bacteria. *PloS One*. (2014) 9:e90725. doi: 10.1371/journal.pone.0090725

27. Steininger C, Widhopf GF, Ghia EM, Morello CS, Vanura K, Sanders R, et al. Recombinant Antibodies Encoded by Ighv1-69 React with Pul32, a Phosphoprotein of Cytomegalovirus and B-Cell Superantigen. *Blood*. (2012) 119:2293–301. doi: 10.1182/blood-2011-08-374058

28. Kostareli E, Hadzidimitriou A, Stavroyianni N, Darzentas N, Athanasiadou A, Gounari M, et al. Molecular Evidence for Ebv and Cmv Persistence in a Subset of Patients with Chronic Lymphocytic Leukemia Expressing Stereotyped Ighv4-34 B-Cell Receptors. *Leukemia*. (2009) 23:919–24. doi: 10.1038/leu.2008.379

29. Hadzidimitriou A, Darzentas N, Murray F, Smilevska T, Arvaniti E, Tresoldi C, et al. Evidence for the Significant Role of Immunoglobulin Light Chains in Antigen Recognition and Selection in Chronic Lymphocytic Leukemia. *Blood*. (2009) 113:403–11. doi: 10.1182/blood-2008-07-166868

30. Kostareli E, Sutton LA, Hadzidimitriou A, Darzentas N, Kouvatsi A, Tsaftaris A, et al. Intraclonal Diversification of Immunoglobulin Light Chains in a Subset of Chronic Lymphocytic Leukemia Alludes to Antigen-Driven Clonal Evolution. *Leukemia*. (2010) 24:1317–24. doi: 10.1038/leu.2010.90

31. Cesano A, Perbellini O, Evensen E, Chu CC, Cioffi F, Ptacek J, et al. Association between B-Cell Receptor Responsiveness and Disease Progression in B-Cell Chronic Lymphocytic Leukemia: Results from Single Cell Network Profiling Studies. *Haematologica*. (2013) 98:626–34. doi: 10.3324/haematol.2012.071910

32. Chen SS, Batliwalla F, Holodick NE, Yan XJ, Yancopoulos S, Croce CM, et al. Autoantigen Can Promote Progression to a More Aggressive Tcl1 Leukemia by Selecting Variants with Enhanced B-Cell Receptor Signaling. *Proc Natl Acad Sci U.S.A*. (2013) 110:E1500–7. doi: 10.1073/pnas.1300616110

33. Hoogeboom R, van Kessel KP, Hochstenbach F, Wormhoudt TA, Reinten RJ, Wagner K, et al. A Mutated B Cell Chronic Lymphocytic Leukemia Subset That Recognizes and Responds to Fungi. *J Exp Med*. (2013) 210:59–70. doi: 10.1084/jem.20121801

34. Hoogeboom R, Wormhoudt TA, Schipperus MR, Langerak AW, Dunn-Walters DK, Guikema JE, et al. A Novel Chronic Lymphocytic Leukemia Subset Expressing Mutated Ighv3- 7-Encoded Rheumatoid Factor B-Cell Receptors That Are Functionally Proficient. *Leukemia*. (2013) 27:738–40. doi: 10.1038/leu.2012.238

35. Gupta SK, Viswanatha DS, Patel KP. Evaluation of Somatic Hypermutation Status in Chronic Lymphocytic Leukemia (Cll) in the Era of Next Generation Sequencing. *Front Cell Dev Biol*. (2020) 8:357. doi: 10.3389/fcell.2020.00357

36. Giudice ID, Foà R. Another Step Forward in the 20-Year History Of. *Haematologica*. (2019) 104:219–21. doi: 10.3324/haematol.2018.207399

37. Fais F, Ghiotto F, Hashimoto S, Sellars B, Valetto A, Allen SL, et al. Chronic Lymphocytic Leukemia B Cells Express Restricted Sets of Mutated and Unmutated Antigen Receptors. *J Clin Invest*. (1998) 102:1515–25. doi: 10.1172/JCI3009

38. Baliakas P, Agathangelidis A, Hadzidimitriou A, Sutton LA, Minga E, Tsanousa A, et al. Not All Ighv3-21 Chronic Lymphocytic Leukemias Are Equal: Prognostic Considerations. *Blood*. (2015) 125:856–9. doi: 10.1182/blood-2014-09-600874

39. Murray F, Darzentas N, Hadzidimitriou A, Tobin G, Boudjogra M, Scielzo C, et al. Stereotyped Patterns of Somatic Hypermutation in Subsets of Patients with Chronic Lymphocytic Leukemia: Implications for the Role of Antigen Selection in Leukemogenesis. *Blood*. (2008) 111:1524–33. doi: 10.1182/blood-2007-07-099564

40. Datta M, Jumaa H. Immunoglobulin Gene Sequence as an Inherited and Acquired Risk Factor for Chronic Lymphocytic Leukemia. *Cancers (Basel)*. (2022) 14 (13):3045. doi: 10.3390/cancers14133045

41. Stamatopoulos B, Smith T, Crompot E, Pieters K, Clifford R, Mraz M, et al. The Light Chain Iglv3-21 Defines a New Poor Prognostic Subgroup in Chronic Lymphocytic Leukemia: Results of a Multicenter Study. *Clin Cancer Res*. (2018) 24:5048–57. doi: 10.1158/1078-0432.CCR-18-0133

42. Maity PC, Bilal M, Koning MT, Young M, van Bergen CAM, Renna V, et al. Iglv3-21*01 Is an Inherited Risk Factor for Cll through the Acquisition of a Single-Point Mutation Enabling Autonomous Bcr Signaling. *Proc Natl Acad Sci U.S.A*. (2020) 117:4320–7. doi: 10.1073/pnas.1913810117

43. Dühren-von Minden M, Übelhart R, Schneider D, Wossning T, Bach MP, Buchner M, et al. Chronic Lymphocytic Leukaemia Is Driven by Antigen-Independent Cell-Autonomous Signalling. *Nature*. (2012) 489:309–12. doi: 10.1038/nature11309

44. Muggen AF, Pillai SY, Kil LP, van Zelm MC, van Dongen JJ, Hendriks RW, et al. Basal Ca(2+) Signaling Is Particularly Increased in Mutated Chronic Lymphocytic Leukemia. *Leukemia*. (2015) 29:321–8. doi: 10.1038/leu.2014.188

45. Tkachenko A, Kupcova K, Havranek O. B-Cell Receptor Signaling and Beyond: The Role of Igα (Cd79a)/Igβ (Cd79b) in Normal and Malignant B Cells. *Int J Mol Sci*. (2023) 25(1):10. doi: 10.3390/ijms25010010

46. Huse K, Bai B, Hilden VI, Bollum LK, Våtsveen TK, Munthe LA, et al. Mechanism of Cd79a and Cd79b Support for Igm+ B Cell Fitness through B Cell Receptor Surface Expression. *J Immunol*. (2022) 209:2042–53. doi: 10.4049/jimmunol.2200144

47. Vuillier F, Dumas G, Magnac C, Prevost MC, Lalanne AI, Oppezzo P, et al. Lower Levels of Surface B-Cell-Receptor Expression in Chronic Lymphocytic Leukemia Are Associated with Glycosylation and Folding Defects of the Mu and Cd79a Chains. *Blood*. (2005) 105:2933–40. doi: 10.1182/blood-2004-09-3643

48. Guo B, Zhang L, Chiorazzi N, Rothstein TL. Il-4 Rescues Surface Igm Expression in Chronic Lymphocytic Leukemia. *Blood*. (2016) 128:553–62. doi: 10.1182/blood-2015-11-682997

49. Casola S, Cattoretti G, Uyttersprot N, Koralov SB, Seagal J, Segal J, et al. Tracking Germinal Center B Cells Expressing Germ-Line Immunoglobulin Gamma1 Transcripts by Conditional Gene Targeting. *Proc Natl Acad Sci U.S.A*. (2006) 103:7396–401. doi: 10.1073/pnas.0602353103

50. Palanca-Wessels MC, Czuczman M, Salles G, Assouline S, Sehn LH, Flinn I, et al. Safety and Activity of the Anti-Cd79b Antibody-Drug Conjugate Polatuzumab Vedotin in Relapsed or Refractory B-Cell Non-Hodgkin Lymphoma and Chronic Lymphocytic Leukaemia: A Phase 1 Study. *Lancet Oncol*. (2015) 16:704–15. doi: 10.1016/S1470-2045(15)70128-2

51. Ormhøj M, Scarfò I, Cabral ML, Bailey SR, Lorrey SJ, Bouffard AA, et al. Chimeric Antigen Receptor T Cells Targeting Cd79b Show Efficacy in Lymphoma with or without Cotargeting Cd19. *Clin Cancer Res*. (2019) 25:7046–57. doi: 10.1158/1078-0432.CCR-19-1337

52. Gordon MS, Kato RM, Lansigan F, Thompson AA, Wall R, Rawlings DJ. Aberrant B Cell Receptor Signaling from B29 (Igbeta, Cd79b) Gene Mutations of Chronic Lymphocytic Leukemia B Cells. *Proc Natl Acad Sci U.S.A*. (2000) 97:5504–9. doi: 10.1073/pnas.090087097

53. Seon BK, Okazaki M, Duzen J, Matsuno F, Goey AKL, Maguire O. Identification of Unique Molecular Heterogeneity of Human Cd79, the Signaling Component of the Human B Cell Antigen Receptor (Bcr), and Synergistic Potentiation of the Cd79-Targeted Therapy of B Cell Tumors by Co-Targeting of Cd79a and Cd79b. *Leuk Res*. (2024) 136:107436. doi: 10.1016/j.leukres.2024.107436

54. Wen Y, Jing Y, Yang L, Kang D, Jiang P, Li N, et al. The Regulators of Bcr Signaling During B Cell Activation. *Blood Sci*. (2019) 1:119–29. doi: 10.1097/BS9.0000000000000026

55. Profitós-Pelejà N, Santos JC, Marín-Niebla A, Roué G, Ribeiro ML. Regulation of B-Cell Receptor Signaling and Its Therapeutic Relevance in Aggressive B-Cell Lymphomas. *Cancers (Basel)*. (2022) 14(4):860. doi: 10.3390/cancers14040860

56. Zonta F, Pagano MA, Trentin L, Tibaldi E, Frezzato F, Trimarco V, et al. Lyn Sustains Oncogenic Signaling in Chronic Lymphocytic Leukemia by Strengthening Set-Mediated Inhibition of Pp2a. *Blood*. (2015) 125:3747–55. doi: 10.1182/blood-2014- 12-619155

57. Alsadeq A, Hobeika E, Medgyesi D, Kläsener K, Reth M. The Role of the Syk/Shp-1 Kinase-Phosphatase Equilibrium in B Cell Development and Signaling. *J Immunol*. (2014) 193:268–76. doi: 10.4049/jimmunol.1203040

58. Wang YH, Fan L, Wang L, Zhang R, Zou ZJ, Fang C, et al. Expression Levels of Lyn, Syk, Plcγ2 and Erk in Patients with Chronic Lymphocytic Leukemia, and Higher Levels of Lyn Are Associated with a Shorter Treatment-Free Survival. *Leuk Lymphoma*. (2013) 54:1165–70. doi: 10.3109/10428194.2012.736983

59. Zonta F, Pagano MA, Trentin L, Tibaldi E, Frezzato F, Gattazzo C, et al. Lyn-Mediated Procaspase 8 Dimerization Blocks Apoptotic Signaling in B-Cell Chronic Lymphocytic Leukemia. *Blood*. (2014) 123:875–83. doi: 10.1182/blood-2013-02-485540

60. Tibaldi E, Brunati AM, Zonta F, Frezzato F, Gattazzo C, Zambello R, et al. Lyn-Mediated Shp-1 Recruitment to Cd5 Contributes to Resistance to Apoptosis of B-Cell Chronic Lymphocytic Leukemia Cells. *Leukemia*. (2011) 25:1768–81. doi: 10.1038/leu.2011.152

61. Nguyen PH, Fedorchenko O, Rosen N, Koch M, Barthel R, Winarski T, et al. Lyn Kinase in the Tumor Microenvironment Is Essential for the Progression of Chronic Lymphocytic Leukemia. *Cancer Cell*. (2016) 30:610–22. doi: 10.1016/j.ccell.2016.09.007

62. Kohlhas V, Hallek M, Nguyen PH. Constitutive Activation of Lyn Kinase Enhances Bcr Responsiveness, but Not the Development of Cll in Eμ-Tcl1 Mice. *Blood Adv*. (2020) 4:6106–16. doi: 10.1182/bloodadvances.2020002584

63. de Oliveira TD, Vom Stein A, Rebollido-Rios R, Lobastova L, Lettau M, Janssen O, et al. Stromal Cells Support the Survival of Human Primary Chronic Lymphocytic Leukemia (Cll) Cells through Lyn-Driven Extracellular Vesicles. *Front Med (Lausanne)*. (2022) 9:1059028. doi: 10.3389/fmed.2022.1059028

64. Vom Stein AF, Rebollido-Rios R, Lukas A, Koch M, von Lom A, Reinartz S, et al. Lyn Kinase Programs Stromal Fibroblasts to Facilitate Leukemic Survival *Via* Regulation of C-Jun and Thbs1. *Nat Commun*. (2023) 14:1330. doi: 10.1038/s41467-023- 36824-2

65. Veldurthy A, Patz M, Hagist S, Pallasch CP, Wendtner CM, Hallek M, et al. The Kinase Inhibitor Dasatinib Induces Apoptosis in Chronic Lymphocytic Leukemia Cells in Vitro with Preference for a Subgroup of Patients with Unmutated Igvh Genes. *Blood*. (2008) 112:1443–52. doi: 10.1182/blood-2007-11-123984

66. McCaig AM, Cosimo E, Leach MT, Michie AM. Dasatinib Inhibits B Cell Receptor Signalling in Chronic Lymphocytic Leukaemia but Novel Combination Approaches Are Required to Overcome Additional Pro-Survival Microenvironmental Signals. *Br J Haematol*. (2011) 153:199–211. doi: 10.1111/j.1365-2141.2010.08507.x

67. Amrein PC, Attar EC, Takvorian T, Hochberg EP, Ballen KK, Leahy KM, et al. Phase Ii Study of Dasatinib in Relapsed or Refractory Chronic Lymphocytic Leukemia. *Clin Cancer Res*. (2011) 17:2977–86. doi: 10.1158/1078-0432.CCR-10-2879

68. Gobessi S, Laurenti L, Longo PG, Carsetti L, Berno V, Sica S, et al. Inhibition of Constitutive and Bcr-Induced Syk Activation Downregulates Mcl-1 and Induces Apoptosis in Chronic Lymphocytic Leukemia B Cells. *Leukemia*. (2009) 23:686–97. doi: 10.1038/leu.2008.346

69. Buchner M, Fuchs S, Prinz G, Pfeifer D, Bartholomé K, Burger M, et al. Spleen Tyrosine Kinase Is Overexpressed and Represents a Potential Therapeutic Target in Chronic Lymphocytic Leukemia. *Cancer Res*. (2009) 69:5424–32. doi: 10.1158/0008-5472.CAN-08-4252

70. Philippen A, Diener S, Zenz T, Dohner H, Stilgenbauer S, Mertens D. Syk Carries No Activating Point Mutations in Patients with Chronic Lymphocytic Leukaemia (Cll). *Br J Haematol*. (2010) 150:633–6. doi: 10.1111/j.1365-2141.2010.08244.x

71. Matsusaka S, Tohyama Y, He J, Shi Y, Hazama R, Kadono T, et al. Protein-Tyrosine Kinase, Syk, Is Required for Cxcl12-Induced Polarization of B Cells. *Biochem Biophys Res Commun*. (2005) 328:1163–9. doi: 10.1016/j.bbrc.2005.01.076

72. Buchner M, Baer C, Prinz G, Dierks C, Burger M, Zenz T, et al. Spleen Tyrosine Kinase Inhibition Prevents Chemokine- and Integrin-Mediated Stromal Protective Effects in Chronic Lymphocytic Leukemia. *Blood*. (2010) 115:4497–506. doi: 10.1182/blood-2009-07-233692

73. Parente-Ribes A, Skånland SS, Bürgler S, Os A, Wang D, Bogen B, et al. Spleen Tyrosine Kinase Inhibitors Reduce Cd40l-Induced Proliferation of Chronic Lymphocytic Leukemia Cells but Not Normal B Cells. *Haematologica*. (2016) 101:e59–62. doi: 10.3324/haematol.2015.135590

74. Friedberg JW, Sharman J, Sweetenham J, Johnston PB, Vose JM, Lacasce A, et al. Inhibition of Syk with Fostamatinib Disodium Has Significant Clinical Activity in Non-Hodgkin Lymphoma and Chronic Lymphocytic Leukemia. *Blood*. (2010) 115:2578–85. doi: 10.1182/blood-2009-08-236471

75. Sharman J, Hawkins M, Kolibaba K, Boxer M, Klein L, Wu M, et al. An Open-Label Phase 2 Trial of Entospletinib (Gs-9973), a Selective Spleen Tyrosine Kinase Inhibitor, in Chronic Lymphocytic Leukemia. *Blood*. (2015) 125:2336–43. doi: 10.1182/blood-2014-08-595934

76. Hamlin PA, Flinn IW, Wagner-Johnston N, Burger JA, Coffey GP, Conley PB, et al. Efficacy and Safety of the Dual Syk/Jak Inhibitor Cerdulatinib in Patients with Relapsed or Refractory B-Cell Malignancies: Results of a Phase I Study. *Am J Hematol*. (2019) 94:E90– E3. doi: 10.1002/ajh.25387

77. Suljagic M, Longo PG, Bennardo S, Perlas E, Leone G, Laurenti L, et al. The Syk Inhibitor Fostamatinib Disodium (R788) Inhibits Tumor Growth in the Eμ- Tcl1 Transgenic Mouse Model of Cll by Blocking Antigen-Dependent B-Cell Receptor Signaling. *Blood*. (2010) 116:4894–905. doi: 10.1182/blood-2010-03-275180

78. Awan FT, Thirman MJ, Patel-Donnelly D, Assouline S, Rao AV, Ye W, et al. Entospletinib Monotherapy in Patients with Relapsed or Refractory Chronic Lymphocytic Leukemia Previously Treated with B-Cell Receptor Inhibitors: Results of a Phase 2 Study. *Leuk Lymphoma*. (2019) 60:1972–7. doi: 10.1080/10428194.2018.1562180

79. Danilov AV, Lam V, Thurlow B, Spurgeon SE, Park B, Orand K, et al. Final Results of a Phase 1/2 Study of Syk Inhibitor Entospletinib in Combination with Obinutuzumab in Patients with Relapsed/Refractory (R/R) Chronic Lymphocytic Leukemia (Cll). *Blood*. (2021) 138:2643.

80. Hamlin PA, Cheson BD, Farber CM, Feldman T, Fenske TS, Hess BT, et al. The Dual Syk/Jak Inhibitor Cerdulatinib Demonstrates Rapid Tumor Responses in a Phase 2 Study in Patients with Relapsed/Refractory B- and T-Cell Non-Hodgkin Lymphoma (Nhl). *J Clin Oncol*. (2018) 36:7511–. doi: 10.1200/JCO.2018.36.15_suppl.7511.80

81. Brown JR, Byrd JC, Coutre SE, Benson DM, Flinn IW, Wagner-Johnston ND, et al. Idelalisib, an Inhibitor of Phosphatidylinositol 3-Kinase P110δ, for Relapsed/Refractory Chronic Lymphocytic Leukemia. *Blood*. (2014) 123:3390–7. doi: 10.1182/blood-2013-11-535047

82. Zelenetz AD, Barrientos JC, Brown JR, Coiffier B, Delgado J, Egyed M, et al. Idelalisib or Placebo in Combination with Bendamustine and Rituximab in Patients with Relapsed or Refractory Chronic Lymphocytic Leukaemia: Interim Results from a Phase 3, Randomised, Double-Blind, Placebo-Controlled Trial. *Lancet Oncol*. (2017) 18:297–311. doi: 10.1016/S1470-2045(16)30671-4

83. Hillmen P, Cairns DA, Bloor AJCB, Allsup D, Cwynarski K, Pettitt A, et al. Abstract 631: Ibrutinib Plus Venetoclax with Mrd-Directed Duration of Treatment Is Superior to Fcr and Is a New Standard of Care for Previously Untreated Cll: Report of the Phase Iii Uk Ncri Flair Study. *65th ASH Annu Meeting Exposition; San Diego California*. (2023).

84. Byrd JC, Woyach JA, Furman RR, Martin P, O'Brien S, Brown JR, et al. Acalabrutinib in Treatment-Naive Chronic Lymphocytic Leukemia. *Blood*. (2021) 137:3327–38. doi: 10.1182/blood.2020009617

85. Byrd JC, Wierda WG, Schuh A, Devereux S, Chaves JM, Brown JR, et al. Acalabrutinib Monotherapy in Patients with Relapsed/Refractory Chronic

Lymphocytic Leukemia: Updated Phase 2 Results. *Blood*. (2020) 135:1204–13. doi: 10.1182/blood.2018884940

86. Rogers KA, Thompson PA, Allan JN, Coleman M, Sharman JP, Cheson BD, et al. Phase Ii Study of Acalabrutinib in Ibrutinib-Intolerant Patients with Relapsed/Refractory Chronic Lymphocytic Leukemia. *Haematologica*. (2021) 106:2364–73. doi: 10.3324/haematol.2020.272500

87. Tam CS, Robak T, Ghia P, Kahl BS, Walker P, Janowski W, et al. Zanubrutinib Monotherapy for Patients with Treatment Naïve Chronic Lymphocytic Leukemia and 17p Deletion. *Haematologica*. (2020) 106:2354–63. doi: 10.3324/haematol.2020.259432

88. Xu W, Yang S, Tam CS, Seymour JF, Zhou K, Opat S, et al. Zanubrutinib Monotherapy for Naïve and Relapsed/Refractory Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma: A Pooled Analysis of Three Studies. *Adv Ther*. (2022) 39:4250–65. doi: 10.1007/s12325-022-02238-7

89. Tam CS, Quach H, Nicol A, Badoux X, Rose H, Prince HM, et al. Zanubrutinib (Bgb- 3111) Plus Obinutuzumab in Patients with Chronic Lymphocytic Leukemia and Follicular Lymphoma. *Blood Adv*. (2020) 4:4802–11. doi: 10.1182/bloodadvances.2020002183

90. Walter HS, Jayne S, Rule SA, Cartron G, Morschhauser F, Macip S, et al. Long-Term Follow-up of Patients with Cll Treated with the Selective Bruton's Tyrosine Kinase Inhibitor Ono/Gs-4059. *Blood*. (2017) 129:2808–10. doi: 10.1182/blood-2017-02-765115

91. Walter HS, Rule SA, Dyer MJ, Karlin L, Jones C, Cazin B, et al. A Phase 1 Clinical Trial of the Selective Btk Inhibitor Ono/Gs-4059 in Relapsed and Refractory Mature B-Cell Malignancies. *Blood*. (2016) 127:411–9. doi: 10.1182/blood-2015-08-664086

92. Kutsch N, Pallasch C, Decker T, Hebart H, Chow KU, Graeven U, et al. Efficacy and Safety of Tirabrutinib and Idelalisib with or without Obinutuzumab in Relapsed Chronic Lymphocytic Leukemia. *Hemasphere*. (2022) 6:e729. doi: 10.1097/HS9.0000000000000729

93. Mato AR, Woyach JA, Brown JR, Ghia P, Patel K, Eyre TA, et al. Pirtobrutinib after a Covalent Btk Inhibitor in Chronic Lymphocytic Leukemia. *N Engl J Med*. (2023) 389:33–44. doi: 10.1056/NEJMoa2300696

94. Woyach JA, Stephens DM, Flinn IW, Bhat SA, Savage RE, Chai F, et al. First-in-Human Study of the Reversible Btk Inhibitor Nemtabrutinib in Patients with Relapsed/Refractory Chronic Lymphocytic Leukemia and B-Cell Non-Hodgkin Lymphoma. *Cancer Discovery*. (2024) 14:66–75. doi: 10.1158/2159-8290.CD-23-0670

95. Woyach JA, Flinn IW, Awan FT, Eradat H, Brander D, Tees M, et al. Efficacy and Safety of Nemtabrutinib, a Wild-Type and C481s-Mutated Bruton Tyrosine Kinase Inhibitor for B- Cell Malignancies: Updated Analysis of the Open-Label Phase 1/2 Dose-Expansion Bellwave- 001 Study. *Blood*. (2022) 140:7004–6. doi: 10.1182/blood-2022-163596

96. Blunt MD, Koehrer S, Dobson RC, Larrayoz M, Wilmore S, Hayman A, et al. The Dual Syk/Jak Inhibitor Cerdulatinib Antagonizes B-Cell Receptor and Microenvironmental Signaling in Chronic Lymphocytic Leukemia. *Clin Cancer Res*. (2017) 23:2313–24. doi: 10.1158/1078-0432.CCR-16-1662

97. Guo A, Lu P, Coffey G, Conley P, Pandey A, Wang YL. Dual Syk/Jak Inhibition Overcomes Ibrutinib Resistance in Chronic Lymphocytic Leukemia: Cerdulatinib, but Not Ibrutinib, Induces Apoptosis of Tumor Cells Protected by the Microenvironment. *Oncotarget*. (2017) 8:12953–67. doi: 10.18632/oncotarget.14588

98. Fasbender F, Claus M, Wingert S, Sandusky M, Watzl C. Differential Requirements for Src-Family Kinases in Syk or Zap70-Mediated Slp-76 Phosphorylation in Lymphocytes. *Front Immunol*. (2017) 8:789. doi: 10.3389/fimmu.2017.00789

99. Chen J, Moore A, Ringshausen I. Zap-70 Shapes the Immune Microenvironment in B Cell Malignancies. *Front Oncol*. (2020) 10:595832. doi: 10.3389/fonc.2020.595832

100. Gobessi S, Laurenti L, Longo PG, Sica S, Leone G, Efremov DG. Zap-70 Enhances B- Cell-Receptor Signaling Despite Absent or Inefficient Tyrosine Kinase Activation in Chronic Lymphocytic Leukemia and Lymphoma B Cells. *Blood*. (2007) 109:2032–9. doi: 10.1182/blood-2006-03-011759

101. Chen J, Sathiaseelan V, Moore A, Tan S, Chilamakuri CSR, Roamio Franklin VN, et al. Zap-70 Constitutively Regulates Gene Expression and Protein Synthesis in Chronic Lymphocytic Leukemia. *Blood*. (2021) 137:3629–40. doi: 10.1182/blood.2020009960

102. Chen J, Sathiaseelan V, Chilamakuri CS, Roamio Franklin VN, Jakwerth CA, D'Santos CS, et al. Zap-70 Augments Tonic B-Cell Receptor and Ccr7 Signaling in Ighv Unmutated Chronic Lymphocytic Leukemia. *Blood Adv*. (2023) 19:bloodadvances.2022009557. doi: 10.1182/bloodadvances.2022009557

103. Dürig J, Nückel H, Cremer M, Führer A, Halfmeyer K, Fandrey J, et al. Zap-70 Expression Is a Prognostic Factor in Chronic Lymphocytic Leukemia. *Leukemia*. (2003) 17:2426–34. doi: 10.1038/sj.leu.2403147

104. 7Packham G, Krysov S, Allen A, Savelyeva N, Steele AJ, Forconi F, et al. The Outcome of B-Cell Receptor Signaling in Chronic Lymphocytic Leukemia: Proliferation or Anergy. *Haematologica*. (2014) 99:1138–48. doi: 10.3324/haematol.2013.098384

105. Sadras T, Martin M, Kume K, Robinson ME, Saravanakumar S, Lenz G, et al. Developmental Partitioning of Syk and Zap70 Prevents Autoimmunity and Cancer. *Mol Cell*. (2021) 81:2094–111.e9. doi: 10.1016/j.molcel.2021.03.043

106. Abdelrasoul H, Werner M, Setz CS, Okkenhaug K, Jumaa H. Pi3k Induces B-Cell Development and Regulates B Cell Identity. *Sci Rep*. (2018) 8:1327. doi: 10.1038/s41598-018-19460-5

107. Ringshausen I, Schneller F, Bogner C, Hipp S, Duyster J, Peschel C, et al. Constitutively Activated Phosphatidylinositol-3 Kinase (Pi-3k) Is Involved in the Defect of Apoptosis in B-Cll: Association with Protein Kinase Cdelta. *Blood*. (2002) 100:3741–8. doi: 10.1182/blood-2002-02-0539

108. Kohlhaas V, Blakemore SJ, Al-Maarri M, Nickel N, Pal M, Roth A, et al. Active Akt Signaling Triggers Cll toward Richter Transformation *Via* Overactivation of Notch1. *Blood*. (2021) 137:646–60. doi: 10.1182/blood.2020005734

109. Leupin N, Cenni B, Novak U, Hugli B, Graber HU, Tobler A, et al. Disparate Expression of the Pten Gene: A Novel Finding in B-Cell Chronic Lymphocytic Leukaemia (B-Cll). *Br J Haematol*. (2003) 121:97–100. doi: 10.1046/j.1365-2141.2003.04227.x

110. Zou ZJ, Zhang R, Fan L, Wang L, Fang C, Zhang LN, et al. Low Expression Level of Phosphatase and Tensin Homolog Deleted on Chromosome Ten Predicts Poor Prognosis in Chronic Lymphocytic Leukemia. *Leuk Lymphoma*. (2013) 54:1159–64. doi: 10.3109/10428194.2012.733880

111. Ali AY, Wu X, Eissa N, Hou S, Ghia JE, Murooka TT, et al. Distinct Roles for Phosphoinositide 3-Kinases Γ and Δ in Malignant B Cell Migration. *Leukemia*. (2018) 32:1958–69. doi: 10.1038/s41375-018-0012-5

112. Hoellenriegel J, Meadows SA, Sivina M, Wierda WG, Kantarjian H, Keating MJ, et al. The Phosphoinositide 3'-Kinase Delta Inhibitor, Cal-101, Inhibits B-Cell Receptor Signaling and Chemokine Networks in Chronic Lymphocytic Leukemia. *Blood*. (2011) 118:3603–12. doi: 10.1182/blood-2011-05-352492

113. Fiorcari S, Brown WS, McIntyre BW, Estrov Z, Maffei R, O'Brien S, et al. The Pi3-Kinase Delta Inhibitor Idelalisib (Gs-1101) Targets Integrin-Mediated Adhesion of Chronic Lymphocytic Leukemia (Cll) Cell to Endothelial and Marrow Stromal Cells. *PloS One*. (2013) 8:e83830. doi: 10.1371/journal.pone.0083830

114. Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, et al. Idelalisib and Rituximab in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med*. (2014) 370:997– 1007. doi: 10.1056/NEJMoa1315226

115. Liu N, Rowley BR, Bull CO, Schneider C, Haegebarth A, Schatz CA, et al. Bay 80-6946 Is a Highly Selective Intravenous Pi3k Inhibitor with Potent P110α and P110δ Activities in Tumor Cell Lines and Xenograft Models. *Mol Cancer Ther*. (2013) 12:2319–30. doi: 10.1158/1535-7163.MCT-12-0993-T

116. Balakrishnan K, Peluso M, Fu M, Rosin NY, Burger JA, Wierda WG, et al. The Phosphoinositide-3-Kinase (Pi3k)-Delta and Gamma Inhibitor, Ipi-145 (Duvelisib), Overcomes Signals from the Pi3k/Akt/S6 Pathway and Promotes Apoptosis in Cll. *Leukemia*. (2015) 29:1811–22. doi: 10.1038/leu.2015.105

117. Burris HA, Flinn IW, Patel MR, Fenske TS, Deng C, Brander DM, et al. Umbralisib, a Novel Pi3kδ and Casein Kinase-1ε Inhibitor, in Relapsed or Refractory Chronic Lymphocytic Leukaemia and Lymphoma: An Open-Label, Phase 1, Dose-Escalation, First-in-Human Study. *Lancet Oncol*. (2018) 19:486–96. doi: 10.1016/S1470-2045(18)30082-2

118. Scheffold A, Jebaraj BMC, Tausch E, Bloehdorn J, Ghia P, Yahiaoui A, et al. Igf1r as Druggable Target Mediating Pi3k-Δ Inhibitor Resistance in a Murine Model of Chronic Lymphocytic Leukemia. *Blood*. (2019) 134:534–47. doi: 10.1182/blood.2018881029

119. Tausch E, Ljungström V, Agathangelidis A, Zapatka M, Scarfò L, Jebaraj BMC, et al. Secondary Resistance to Idelalisib Is Characterized by Upregulation of Igf1r Rather Than by Mapk/Erk Pathway Mutations. *Blood*. (2022) 139:3340–4. doi: 10.1182/blood.2021014550

120. von Tresckow J, Heyl N, Robrecht S, Giza A, Aldaoud A, Schlag R, et al. Treatment with Idelalisib in Patients with Chronic Lymphocytic Leukemia - Real World Data from the Registry of the German Cll Study Group. *Ann Hematol*. (2023) 102:3083–90. doi: 10.1007/s00277-023-05314-2

121. Liu TM, Ling Y, Woyach JA, Beckwith K, Yeh YY, Hertlein E, et al. Osu-T315: A Novel Targeted Therapeutic That Antagonizes Akt Membrane Localization and Activation of Chronic Lymphocytic Leukemia Cells. *Blood*. (2015) 125:284–95. doi: 10.1182/blood-2014-06-583518

122. Pal Singh S, Dammeijer F, Hendriks RW. Role of Bruton's Tyrosine Kinase in B Cells and Malignancies. *Mol Cancer*. (2018) 17:57. doi: 10.1186/s12943-018- 0779-z

123. Rawlings DJ, Scharenberg AM, Park H, Wahl MI, Lin S, Kato RM, et al. Activation of Btk by a Phosphorylation Mechanism Initiated by Src Family Kinases. *Science*. (1996) 271:822–5. doi: 10.1126/science.271.5250.822

124. Singh SP, Pillai SY, de Bruijn MJW, Stadhouders R, Corneth OBJ, van den Ham HJ, et al. Cell Lines Generated from a Chronic Lymphocytic Leukemia Mouse Model Exhibit Constitutive Btk and Akt Signaling. *Oncotarget*. (2017) 8:71981–95. doi: 10.18632/oncotarget.18234

125. Woyach JA, Bojnik E, Ruppert AS, Stefanovski MR, Goettl VM, Smucker KA, et al. Bruton's Tyrosine Kinase (Btk) Function Is Important to the Development and Expansion of Chronic Lymphocytic Leukemia (Cll). *Blood*. (2014) 123:1207–13. doi: 10.1182/blood-2013-07-515361

126. Herman SE, Gordon AL, Hertlein E, Ramanunni A, Zhang X, Jaglowski S, et al. Bruton Tyrosine Kinase Represents a Promising Therapeutic Target for Treatment of Chronic Lymphocytic Leukemia and Is Effectively Targeted by Pci-32765. *Blood*. (2011) 117:6287–96. doi: 10.1182/blood-2011-01-328484

127. de Gorter DJ, Beuling EA, Kersseboom R, Middendorp S, van Gils JM, Hendriks RW, et al. Bruton's Tyrosine Kinase and Phospholipase Cgamma2 Mediate Chemokine-Controlled B Cell Migration and Homing. *Immunity*. (2007) 26:93–104. doi: 10.1016/j.immuni.2006.11.012

128. Ramsay AD, Rodriguez-Justo M. Chronic Lymphocytic Leukaemia–the Role of the Microenvironment Pathogenesis and Therapy. *Br J Haematol*. (2013) 162:15–24. doi: 10.1111/bjh.12344

129. de Rooij MF, Kuil A, Geest CR, Eldering E, Chang BY, Buggy JJ, et al. The Clinically Active Btk Inhibitor Pci-32765 Targets B-Cell Receptor- and Chemokine-Controlled Adhesion and Migration in Chronic Lymphocytic Leukemia. *Blood*. (2012) 119:2590–4. doi: 10.1182/blood-2011-11-390989

130. Fiorcari S, Maffei R, Audrito V, Martinelli S, Ten Hacken E, Zucchini P, et al. Ibrutinib Modifies the Function of Monocyte/Macrophage Population in Chronic Lymphocytic Leukemia. *Oncotarget*. (2016) 7:65968–81. doi: 10.18632/oncotarget.11782

131. Herman SE, Mustafa RZ, Gyamfi JA, Pittaluga S, Chang S, Chang B, et al. Ibrutinib Inhibits Bcr and Nf-Kb Signaling and Reduces Tumor Proliferation in Tissue-Resident Cells of Patients with Cll. *Blood*. (2014) 123:3286–95. doi: 10.1182/blood-2014- 02-548610

132. Niemann CU, Herman SE, Maric I, Gomez-Rodriguez J, Biancotto A, Chang BY, et al. Disruption of in Vivo Chronic Lymphocytic Leukemia Tumor-Microenvironment Interactions by Ibrutinib–Findings from an Investigator-Initiated Phase Ii Study. *Clin Cancer Res*. (2016) 22:1572–82. doi: 10.1158/1078-0432.CCR-15-1965

133. Dubovsky JA, Beckwith KA, Natarajan G, Woyach JA, Jaglowski S, Zhong Y, et al. Ibrutinib Is an Irreversible Molecular Inhibitor of Itk Driving a Th1-Selective Pressure in T Lymphocytes. *Blood*. (2013) 122:2539–49. doi: 10.1182/blood-2013-06-507947

134. Ponader S, Chen SS, Buggy JJ, Balakrishnan K, Gandhi V, Wierda WG, et al. The Bruton Tyrosine Kinase Inhibitor Pci-32765 Thwarts Chronic Lymphocytic Leukemia Cell Survival and Tissue Homing in Vitro and in Vivo. *Blood*. (2012) 119:1182–9. doi: 10.1182/blood-2011-10-386417

135. Woyach JA, Smucker K, Smith LL, Lozanski A, Zhong Y, Ruppert AS, et al. Prolonged Lymphocytosis During Ibrutinib Therapy Is Associated with Distinct Molecular Characteristics and Does Not Indicate a Suboptimal Response to Therapy. *Blood*. (2014) 123:1810–7. doi: 10.1182/blood-2013-09-527853

136. Burger JA. The Cll Cell Microenvironment. *Adv Exp Med Biol*. (2013) 792:25–45. doi: 10.1007/978-1-4614-8051-8_2

137. Ten Hacken E, Burger JA. Microenvironment Interactions and B-Cell Receptor Signaling in Chronic Lymphocytic Leukemia: Implications for Disease Pathogenesis and Treatment. *Biochim Biophys Acta*. (2016) 1863:401–13. doi: 10.1016/j.bbamcr.2015.07.009

138. Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA, et al. Targeting Btk with Ibrutinib in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med*. (2013) 369:32–42. doi: 10.1056/NEJMoa1215637

139. Woyach JA, Ruppert AS, Guinn D, Lehman A, Blachly JS, Lozanski A, et al. Btk (C481s)- Mediated Resistance to Ibrutinib in Chronic Lymphocytic Leukemia. *J Clin Oncol*. (2017) 35:1437–43. doi: 10.1200/jco.2016.70.2282

140. Woyach JA, Furman RR, Liu TM, Ozer HG, Zapatka M, Ruppert AS, et al. Resistance Mechanisms for the Bruton's Tyrosine Kinase Inhibitor Ibrutinib. *N Engl J Med*. (2014) 370:2286–94. doi: 10.1056/NEJMoa1400029

141. Walliser C, Hermkes E, Schade A, Wiese S, Deinzer J, Zapatka M, et al. The Phospholipase Cγ2 Mutants R665w and L845f Identified in Ibrutinib-Resistant Chronic Lymphocytic Leukemia Patients Are Hypersensitive to the Rho Gtpase Rac2 Protein. *J Biol Chem*. (2016) 291:22136–48. doi: 10.1074/jbc.M116.746842

142. Liu TM, Woyach JA, Zhong Y, Lozanski A, Lozanski G, Dong S, et al. Hypermorphic Mutation of Phospholipase C, Γ2 Acquired in Ibrutinib-Resistant Cll Confers Btk Independency Upon B-Cell Receptor Activation. *Blood*. (2015) 126:61–8. doi: 10.1182/blood-2015-02-626846

143. Wist M, Meier L, Gutman O, Haas J, Endres S, Zhou Y, et al. Noncatalytic Bruton's Tyrosine Kinase Activates Plcγ(2) Variants Mediating Ibrutinib Resistance in Human Chronic Lymphocytic Leukemia Cells. *J Biol Chem*. (2020) 295:5717–36. doi: 10.1074/jbc.RA119.011946

144. Yosifov DY, Wolf C, Stilgenbauer S, Mertens D. From Biology to Therapy: The Cll Success Story. *Hemasphere*. (2019) 3:e175. doi: 10.1097/HS9.0000000000000175

145. Covey T, Barf T, Gulrajani M, Krantz F, van Lith B, Bibikova E, et al. Abstract 2596:Acp-196: A Novel Covalent Bruton's Tyrosine Kinase (Btk) Inhibitor with Improved Selectivity and in Vivo Target Coverage in Chronic Lymphocytic Leukemia (Cll) Patients. *Cancer Res*. (2015) 75:2596–. doi: 10.1158/1538-7445.am2015-2596

146. Byrd JC, Harrington B, O'Brien S, Jones JA, Schuh A, Devereux S, et al. Acalabrutinib (Acp-196) in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med*. (2016) 374:323–32. doi: 10.1056/NEJMoa1509981

147. Ghia P, Pluta A, Wach M, Lysak D, Kozak T, Simkovic M, et al. Ascend: Phase Iii, Randomized Trial of Acalabrutinib Versus Idelalisib Plus Rituximab or Bendamustine Plus Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. *J Clin Oncol*. (2020) 38:2849–61. doi: 10.1200/JCO.19.03355

148. Montoya S, Thompson MC. Non-Covalent Bruton's Tyrosine Kinase Inhibitors in the Treatment of Chronic Lymphocytic Leukemia. *Cancers (Basel)*. (2023) 15 (14):3648. doi: 10.3390/cancers15143648

149. Aslan B, Kismali G, Iles LR, Manyam GC, Ayres ML, Chen LS, et al. Pirtobrutinib Inhibits Wild-Type and Mutant Bruton's Tyrosine Kinase-Mediated Signaling in Chronic Lymphocytic Leukemia. . *Blood Cancer J*. (2022) 12:80. doi: 10.1038/s41408-022-00675- 9

150. Reiff SD, Mantel R, Smith LL, Greene JT, Muhowski EM, Fabian CA, et al. The Btk Inhibitor Arq 531 Targets Ibrutinib-Resistant Cll and Richter Transformation. *Cancer Discovery*. (2018) 8:1300–15. doi: 10.1158/2159-8290.CD-17-1409

151. Jebaraj BMC, Müller A, Dheenadayalan RP, Endres S, Roessner PM, Seyfried F, et al. Evaluation of Vecabrutinib as a Model for Noncovalent Btk/Itk Inhibition for Treatment of Chronic Lymphocytic Leukemia. *Blood*. (2022) 139:859–75. doi: 10.1182/blood.2021011516

152. Crawford JJ, Johnson AR, Misner DL, Belmont LD, Castanedo G, Choy R, et al. Discovery of Gdc-0853: A Potent, Selective, and Noncovalent Bruton's Tyrosine Kinase Inhibitor in Early Clinical Development. *J Med Chem*. (2018) 61:2227–45. doi: 10.1021/acs.jmedchem.7b01712

153. Jensen JL, Mato AR, Pena C, Roeker LE, Coombs CC. The Potential of Pirtobrutinib in Multiple B-Cell Malignancies. *Ther Adv Hematol*. (2022) 13:20406207221101697. doi: 10.1177/20406207221101697

154. Allan JN, Pinilla-Ibarz J, Gladstone DE, Patel K, Sharman JP, Wierda WG, et al. Phase Ib Dose-Escalation Study of the Selective, Non-Covalent, Reversible Bruton's Tyrosine Kinase Inhibitor Vecabrutinib in B-Cell Malignancies. *Haematologica*. (2022) 107:984–7. doi: 10.3324/haematol.2021.280061

155. Sun C, Mali R, Kositsky R, Tian X, Tomczak H, Nuttall B, et al. Abstract 1891: Extended Follow-up and Resistance Mutations in Cll Patients Treated with Acalabrutinib. *65th ASH Annu Meeting Exposition; San Diego California*. (2023).

156. Wang E, Mi X, Thompson MC, Montoya S, Notti RQ, Afaghani J, et al. Mechanisms of Resistance to Noncovalent Bruton's Tyrosine Kinase Inhibitors. *N Engl J Med*. (2022) 386:735–43. doi: 10.1056/NEJMoa2114110

157. Naeem A, Li L, Utro F, Cha J, Tsuji J, Fernandes SM, et al. Abstract 4623: Understanding Resistance Mechanisms and Growth Kinetics of Cll Treated with Covalent and Non-Covalent Btk Inhibitors. *65th ASH Annu Meeting Exposition; San Diego California*. (2023).

158. Blombery P, Thompson ER, Lew TE, Tiong IS, Bennett R, Cheah CY, et al. Enrichment of Btk Leu528trp Mutations in Patients with Cll On zanubrutinib: Potential for Pirtobrutinib Cross-Resistance. *Blood Adv*. (2022) 6:5589–92. doi: 10.1182/bloodadvances.2022008325

159. Qi J, Endres S, Yosifov DY, Tausch E, Dheenadayalan RP, Gao X, et al. Acquired Btk Mutations Associated with Resistance to Noncovalent Btk Inhibitors. *Blood Adv*. (2023) 7:5698–702. doi: 10.1182/bloodadvances.2022008955

160. Yasuda T. Map Kinase Cascades in Antigen Receptor Signaling and Physiology. *Curr Top Microbiol Immunol*. (2016) 393:211–31. doi: 10.1007/82_2015_481

161. Muzio M, Apollonio B, Scielzo C, Frenquelli M, Vandoni I, Boussiotis V, et al. Constitutive Activation of Distinct Bcr-Signaling Pathways in a Subset of Cll Patients: A Molecular Signature of Anergy. *Blood*. (2008) 112:188–95. doi: 10.1182/blood-2007-09-111344

162. Landau DA, Tausch E, Taylor-Weiner AN, Stewart C, Reiter JG, Bahlo J, et al. Mutations Driving Cll and Their Evolution in Progression and Relapse. *Nature*. (2015) 526:525–30. doi: 10.1038/nature15395

163. Giménez N, Martínez-Trillos A, Montraveta A, Lopez-Guerra M, Rosich L, Nadeu F, et al. Mutations in the Ras-Braf-Mapk-Erk Pathway Define a Specific Subgroup of Patients with Adverse Clinical Features and Provide New Therapeutic Options in Chronic Lymphocytic Leukemia. *Haematologica*. (2019) 104:576–86. doi: 10.3324/haematol.2018.196931

164. Vendramini E, Bomben R, Pozzo F, Benedetti D, Bittolo T, Rossi FM, et al. Kras, Nras, and Braf Mutations Are Highly Enriched in Trisomy 12 Chronic Lymphocytic Leukemia and Are Associated with Shorter Treatment-Free Survival. *Leukemia*. (2019) 33:2111–5. doi: 10.1038/s41375-019-0444-6

165. Chen Y, Germano S, Shelmani G, Kluczna D, Jayne S, Dyer MJS, et al. Paradoxical Activation of Alternative Pro-Survival Pathways Determines Resistance to Mek Inhibitors in Chronic Lymphocytic Leukaemia. *Br J Haematol*. (2018) 182:921–4. doi: 10.1111/bjh.14880

166. Ecker V, Brandmeier L, Stumpf M, Giansanti P, Moreira AV, Pfeuffer L, et al. Negative Feedback Regulation of Mapk Signaling Is an Important Driver of Chronic Lymphocytic Leukemia Progression. *Cell Rep*. (2023) 42:113017. doi: 10.1016/j.celrep.2023.113017

167. Cagnol S, Chambard JC. Erk and Cell Death: Mechanisms of Erk-Induced Cell Death– Apoptosis, Autophagy and Senescence. *FEBS J*. (2010) 277:2–21. doi: 10.1111/j.1742-4658.2009.07366.x

168. Limnander A, Depeille P, Freedman TS, Liou J, Leitges M, Kurosaki T, et al. Stim1, Pkc- Δ and Rasgrp Set a Threshold for Proapoptotic Erk Signaling During B Cell Development. *Nat Immunol*. (2011) 12:425–33. doi: 10.1038/ni.2016

169. Crassini K, Shen Y, Stevenson WS, Christopherson R, Ward C, Mulligan SP, et al. Mek1/2 Inhibition by Binimetinib Is Effective as a Single Agent and Potentiates the Actions of Venetoclax and Abt-737 under Conditions That Mimic the Chronic Lymphocytic Leukaemia (Cll) Tumour Microenvironment. *Br J Haematol*. (2018) 182:360–72. doi: 10.1111/bjh.15282

170. Dietrich S, Oleś M, Lu J, Sellner L, Anders S, Velten B, et al. Drug-Perturbation-Based Stratification of Blood Cancer. *J Clin Invest*. (2018) 128:427–45. doi: 10.1172/JCI93801

171. Bonizzi G, Karin M. The Two Nf-Kappab Activation Pathways and Their Role in Innate and Adaptive Immunity. *Trends Immunol*. (2004) 25:280–8. doi: 10.1016/j.it.2004.03.008

172. Woyach JA, Johnson AJ, Byrd JC. The B-Cell Receptor Signaling Pathway as a Therapeutic Target in Cll. *Blood*. (2012) 120:1175–84. doi: 10.1182/blood- 2012-02-362624

173. Rozovski U, Harris DM, Li P, Liu Z, Jain P, Veletic I, et al. Activation of the B-Cell Receptor Successively Activates Nf-Kb and Stat3 in Chronic Lymphocytic Leukemia Cells. *Int J Cancer*. (2017) 141:2076–81. doi: 10.1002/ijc.30892

174. Rossi D, Fangazio M, Rasi S, Vaisitti T, Monti S, Cresta S, et al. Disruption of Birc3 Associates with Fludarabine Chemorefractoriness in Tp53 Wild-Type Chronic Lymphocytic Leukemia. *Blood*. (2012) 119:2854–62. doi: 10.1182/blood-2011-12-395673

175. Puente XS, Pinyol M, Quesada V, Conde L, Ordóñez GR, Villamor N, et al. Whole- Genome Sequencing Identifies Recurrent Mutations in Chronic Lymphocytic Leukaemia. *Nature*. (2011) 475:101–5. doi: 10.1038/nature10113

176. Mansouri L, Sutton LA, Ljungström V, Bondza S, Arngården L, Bhoi S, et al. Functional Loss of Iκbε Leads to Nf-Kb Deregulation in Aggressive Chronic Lymphocytic Leukemia. *J Exp Med*. (2015) 212:833–43. doi: 10.1084/jem.20142009

177. Rodriguez S, Celay J, Goicoechea I, Jimenez C, Botta C, Garcia-Barchino MJ, et al. Preneoplastic Somatic Mutations Including. *Sci Adv*. (2022) 8:eabl4644. doi: 10.1126/sciadv.abl4644

178. Buggins AG, Pepper C, Patten PE, Hewamana S, Gohil S, Moorhead J, et al. Interaction with Vascular Endothelium Enhances Survival in Primary Chronic Lymphocytic Leukemia Cells *Via* Nf-Kappab Activation and *De Novo* Gene Transcription. *Cancer Res*. (2010) 70:7523–33. doi: 10.1158/0008-5472.CAN-10-1634

179. Smith CIE, Burger JA. Resistance Mutations to Btk Inhibitors Originate from the Nf-Kb but Not from the Pi3k-Ras-Mapk Arm of the B Cell Receptor Signaling Pathway. *Front Immunol*. (2021) 12:689472. doi: 10.3389/fimmu.2021.689472

180. Burley TA, Kennedy E, Broad G, Boyd M, Li D, Woo T, et al. Targeting the Non- Canonical Nf-Kb Pathway in Chronic Lymphocytic Leukemia and Multiple Myeloma. *Cancers (Basel)*. (2022) 14(6):1489. doi: 10.3390/cancers14061489

181. Scala S. Molecular Pathways: Targeting the Cxcr4-Cxcl12 Axis–Untapped Potential in the Tumor Microenvironment. *Clin Cancer Res*. (2015) 21:4278–85. doi: 10.1158/1078-0432.CCR-14-0914

182. Lewis R, Maurer HC, Singh N, Gonzalez-Menendez I, Wirth M, Schick M, et al. Cxcr4 Hyperactivation Cooperates with Tcl1 in Cll Development and Aggressiveness. *Leukemia*. (2021) 35:2895–905. doi: 10.1038/s41375-021-01376-1

183. Burger JA, Bürkle A. The Cxcr4 Chemokine Receptor in Acute and Chronic Leukaemia: A Marrow Homing Receptor and Potential Therapeutic Target. *Br J Haematol*. (2007) 137:288–96. doi: 10.1111/j.1365-2141.2007.06590.x

184. Ganghammer S, Gutjahr J, Hutterer E, Krenn PW, Pucher S, Zelle-Rieser C, et al. Combined Cxcr3/Cxcr4 Measurements Are of High Prognostic Value in Chronic Lymphocytic Leukemia Due to Negative Co-Operativity of the Receptors. *Haematologica*. (2016) 101:e99–102. doi: 10.3324/haematol.2015.133470

185. Chen SS, Chang BY, Chang S, Tong T, Ham S, Sherry B, et al. Btk Inhibition Results in Impaired Cxcr4 Chemokine Receptor Surface Expression, Signaling and Function in Chronic Lymphocytic Leukemia. *Leukemia*. (2016) 30:833–43. doi: 10.1038/leu.2015.316

186. Martines C, Chakraborty S, Zucchetto A, Tissino E, Guastafierro V, Vujovikj M, et al. Abstract 80: Cxcr4 Loss Reduces the Local Growth of Richter Syndrome Murine and Patient- Derived Xenograft Models and Impairs Bcr and Vla-4 Activation. *65th ASH Annu Meeting Exposition; San Diego California*. (2023).

187. Andritsos LA, Byrd JC, Cheverton P, Wu J, Sivina M, Kipps TJ, et al. A Multicenter Phase 1 Study of Plerixafor and Rituximab in Patients with Chronic Lymphocytic Leukemia. *Leuk Lymphoma*. (2019) 60:3461–9. doi: 10.1080/10428194.2019.1643463

188. Calissano C, Damle RN, Hayes G, Murphy EJ, Hellerstein MK, Moreno C, et al. *In Vivo* Intraclonal and Interclonal Kinetic Heterogeneity in B-Cell Chronic Lymphocytic Leukemia. *Blood*. (2009) 114:4832–42. doi: 10.1182/blood-2009-05-219634

189. Calissano C, Damle RN, Marsilio S, Yan XJ, Yancopoulos S, Hayes G, et al. Intraclonal Complexity in Chronic Lymphocytic Leukemia: Fractions Enriched in Recently Born/Divided and Older/Quiescent Cells. *Mol Med*. (2011) 17:1374–82. doi: 10.2119/molmed.2011.00360

190. Mazzarello AN, Fitch M, Cardillo M, Ng A, Bhuiya S, Sharma E, et al. Characterization of the Intraclonal Complexity of Chronic Lymphocytic Leukemia B Cells: Potential Influences of B-Cell Receptor Crosstalk with Other Stimuli. *Cancers (Basel)*. (2023) 15(19):4706. doi: 10.3390/cancers15194706

191. Herndon TM, Chen SS, Saba NS, Valdez J, Emson C, Gatmaitan M, et al. Direct in Vivo Evidence for Increased Proliferation of Cll Cells in Lymph Nodes Compared to Bone Marrow and Peripheral Blood. *Leukemia*. (2017) 31:1340–7. doi: 10.1038/leu.2017.11

192. Rabanal-Ruiz Y, Korolchuk VI. Mtorc1 and Nutrient Homeostasis: The Central Role of the Lysosome. *Int J Mol Sci*. (2018) 19(3):818. doi: 10.3390/ijms19030818

193. Saxton RA, Sabatini DM. Mtor Signaling in Growth, Metabolism, and Disease. *Cell*. (2017) 169:361–71. doi: 10.1016/j.cell.2017.03.035

194. Iwata TN, Ramírez-Komo JA, Park H, Iritani BM. Control of B Lymphocyte Development and Functions by the Mtor Signaling Pathways. *Cytokine Growth Factor Rev*. (2017) 35:47–62. doi: 10.1016/j.cytogfr.2017.04.005

195. Gíslason MH, Demircan GS, Prachar M, Furtwängler B, Schwaller J, Schoof EM, et al. Bloodspot 3.0: A Database of Gene and Protein Expression Data in Normal and Malignant Haematopoiesis. *Nucleic Acids Res*. (2023) 52(D1):D1138–42. doi: 10.1093/nar/gkad993

196. Lu J, Böttcher M, Walther T, Mougiakakos D, Zenz T, Huber W. Energy Metabolism Is Co-Determined by Genetic Variants in Chronic Lymphocytic Leukemia and Influences Drug Sensitivity. *Haematologica*. (2019) 104:1830–40. doi: 10.3324/haematol.2018.203067

197. Lu J, Cannizzaro E, Meier-Abt F, Scheinost S, Bruch PM, Giles HA, et al. Multi-Omics Reveals Clinically Relevant Proliferative Drive Associated with Mtor-Myc-Oxphos Activity in Chronic Lymphocytic Leukemia. *Nat Cancer*. (2021) 2:853–64. doi: 10.1038/s43018-021-00216-6

198. Bertacchini J, Heidari N, Mediani L, Capitani S, Shahjahani M, Ahmadzadeh A, et al. Targeting Pi3k/Akt/Mtor Network for Treatment of Leukemia. *Cell Mol Life Sci*. (2015) 72:2337–47. doi: 10.1007/s00018-015-1867-5

199. Chen Z, Cretenet G, Carnazzo V, Simon-Molas H, Kater AP, Windt GJWV, et al. Electron Transport Chain and Mtor Inhibition Synergistically Decrease Cd40 Signaling and Counteract Venetoclax Resistance in Chronic Lymphocytic Leukemia. *Haematologica*. (2024) 109:151–62. doi: 10.3324/haematol.2023.282760

frontiers | Frontiers in Oncology

TYPE Case Report
PUBLISHED 11 March 2024
DOI 10.3389/fonc.2024.1272209

OPEN ACCESS

EDITED BY
Michael Liew,
ARUP Laboratories, United States

REVIEWED BY
Veronika Kloboves Kloboves-Prevodnik,
Institute of Oncology Ljubljana, Slovenia
Zuheir Alshehabi,
Tishreen University, Syria

*CORRESPONDENCE
Xin Cao
cathyjiangsu@163.com

†These authors have contributed equally to this work and share the first authorship

RECEIVED 03 August 2023
ACCEPTED 27 February 2024
PUBLISHED 11 March 2024

CITATION
Gu J, Qian J and Cao X (2024) Composite diffuse large B-cell lymphoma and peripheral T-cell lymphoma: a case report with two-year follow-up and literature review.
Front. Oncol. 14:1272209.
doi: 10.3389/fonc.2024.1272209

Composite diffuse large B-cell lymphoma and peripheral T-cell lymphoma: a case report with two-year follow-up and literature review

Jiwei Gu†, Juan Qian† and Xin Cao*

Department of Hematology, Affiliated Hospital of Nantong University, Nantong, China

Composite lymphoma is an uncommon type of lymphoid malignancy, and those consisting of concurrent diffuse large B-cell lymphoma (DLBCL) and peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) in the same organ are rare. Here, we report a case of a 75-year-old male patient admitted to our emergency department with intestinal obstruction presenting with abdominal pain and vomiting. He underwent partial resection of the small intestine under general anesthesia, and subsequent histopathology confirmed the mass to be composite DLBCL and PTCL-NOS. The patient received chemotherapy with a rituximab-based regimen and achieved complete remission (CR). However, the recurrent disease presented with obstruction again ten months after treatment. He refused a second surgery, but salvage treatment was not effective. The patient survived for 20 months after diagnosis. In addition, we did a literature review to understand the clinical features, pathology, treatment, and prognosis of this type of composite lymphoma.

KEYWORDS

diffuse large B cell lymphoma, peripheral T-cell lymphoma, treatment, composite lymphoma, intestine

Introduction

Composite lymphoma (CL) is an uncommon type of lymphoid malignancy, accounting for approximately 1.0%–4.7% of all lymphomas (1). Those consisting of concurrent B- and T-cell tumors are especially rare. Diffuse large B-cell lymphoma (DLBCL) is the most common and heterogeneous B-cell neoplasm, generally expressing CD20. Peripheral T-cell lymphoma (PTCL) is a group of highly heterogeneous invasive non-Hodgkin's lymphoma (NHL) originating from mature T cells or T cells in the thymus. T cells generally do not express CD20. However, a small subpopulation of T cells also was found expressing CD20. They may be found in healthy controls, autoimmune diseases, and hematological

malignancies (2). CD20 expression in PTCL, not otherwise specified (PTCL-NOS), has rarely been reported in the literature, and its clinical significance has not been established yet (3, 4). Twelve cases of DLBCL and PTCL-NOS that occur simultaneously in the same tissue have been reported (4–13). Here, we describe a composite DLBCL and PTCL-NOS case with CD20 expression who presented to the hematology department with intestinal obstruction.

Case presentation

In October 2019, a 75-year-old man was admitted to the emergency department with worsened abdominal pain accompanied by vomiting. He had abdominal discomfort, night sweats, and loss of appetite and weight for two weeks. A computed tomography (CT) scan showed the thickened upper jejunal wall accompanied by obstructive dilatation of the proximal intestine and multiple enlarged lymph nodes. He received partial small intestine resection and was transferred to the hematology department due to the intraoperative pathology indicating malignant lymphoma. Physical examination didn't show palpable lymph nodes. He had a ten-year history of hypertension, hyperglycemia, and psoriasis with a penicillin allergy. Complete blood cell count showed mild lymphocytopenia: white blood count (WBC): 2.8×10^9/L, hemoglobulin concentration (Hb): 109g/L. A stool routine test was weakly positive for occult blood. Other results included lactate dehydrogenase (LDH) 189U/L (0-247U/L), β2-microglobulin (β2-MG) 3.00 ug/ml (1.00-3.00 ug/ml). Epstein-Barr virus (EBV) test showed EBV early antigen IgM (-), EBV viral capsid antigen (VCA) IgM (-), EBV-VCA IgG (+), EBV core antigen IgG (+), EBV-DNA (-). No apparent abnormalities were found on bone marrow biopsy, smear, or flow cytometry. Histopathology of small bowel resection (Figures 1, 2) is as follows. (1) DLBCL was found in the small intestine (1 cm, 6.5 cm, and 16 cm away from the incision), which was germinal center B-cell-like (GCB) DLBCL according to the Hans algorithm. Immunohistochemical (IHC) studies of the tumors showed that the lesion was positive for CD20, CD79a, CD21, Mum-1, Bcl-6, BCL2, CD10, and negative for CD3, CD5, CD43, and CyclinD1. The Ki67 proliferation index was 60%. EBV encodes *in situ* hybridization of small RNA (EBER) was negative. (2) The thickened area of small intestine mucosa indicated PTCL-NOS next to the DLBCL. IHC showed tumor cells were positive for CD2, CD3, CD4, CD5, CD7, CD20, PAX5, and negative for CD56, TdT, EBER with Ki67 proliferation index of 30%. (3) Three of the 30 mesenteric lymph nodes were infiltrated with PTCL-NOS. IHC was positive for CD2, CD3, CD5, CD43, CD20, Bcl2, while negative for CD10, CD79a, Mum1, Bcl6, CyclinD1, PAX5, OCT2, MPO, CD34, TdT. CD7 was lost in part of the tumor cells. The Ki67 proliferation index is about 10%. The immunoglobulin heavy chain gene (IgH) rearrangement test in DLBCL was positive, and IgH rearrangement and T cell receptor (TCR) rearrangement in the part of PTCL-NOS were negative. We arranged an ^{18}F- fluorodeoxyglucose (FDG) positron emission computed tomography (PET-CT) for him. The images showed that the operative area of the small intestine was slightly disorganized. A slight thickening of the intestinal wall at the anastomosis and its adjacent area was accompanied by a significant progressive increase of FDG uptake, suggesting the infiltration of residual lymphoma lesions. The increased FDG uptake of the multiple segments of the small intestine in the left abdomen and lymph nodes in the pelvic mesenteric indicated the involvement of lymphoma (Figure 3). Combined with clinical manifestations and laboratory findings, he was diagnosed with composite DLBCL and PTCL-NOS, and the Eastern American Cancer Collaboration (ECOG) physical condition score was 2. He received three cycles of R-CHOP (Rituximab, Cyclophosphamide, Epirubicin, Vindesine, Prednisone) and achieved partial remission (PR) (Figure 3B). We tried to add Chidamide but failed with severe gastrointestinal reaction and fatigue. The patient took rituximab monotherapy in the fourth course due to fever and neutropenia. Then, he continued three cycles of R-CHOP chemotherapy; the last chemo date was in March 2020. On August 20, 2020, a PET-CT scan showed the disease was in metabolic remission with a Duveil score of 3 (Figure 3C). There were no complaints of discomfort in the clinic and no complaints of discomfort during follow-up until March 2020. However, he presented with obstruction again at the end of January 2021, about ten months after treatment, and a CT scan confirmed recurrent disease in the small intestine. He refused a second surgery or endoscopy. Chemotherapy with R-CHOPE (etoposide) relieved his bowel obstruction, however, with increased pleura effusion. Salvage treatment R-GDP (Rituximab, gemcitabine, cis-platinum, Prednisone) was also ineffective. The patient died in May 2021 at a local hospital. He survived for 18 months after diagnosis.

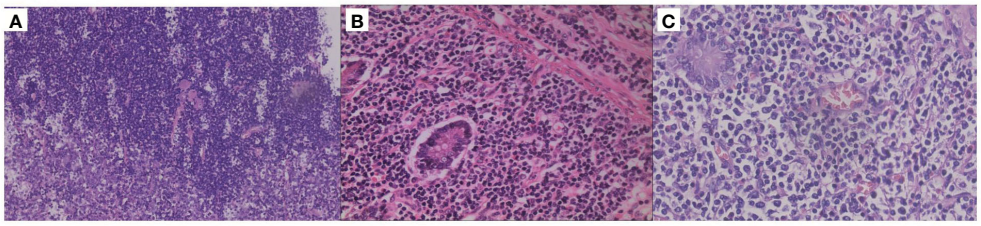

FIGURE 1
Histopathological results of different parts. **(A)** Hematoxylin-eosin (HE) staining (100X) of the junction between the diffuse large B-cell lymphoma (DLBCL) and peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) in the intestinal mucosa. **(B)** HE staining of intestinal mucosa with PTCL-NOS (×400). **(C)** HE staining of the intestinal mucosa with DLBCL (×400).

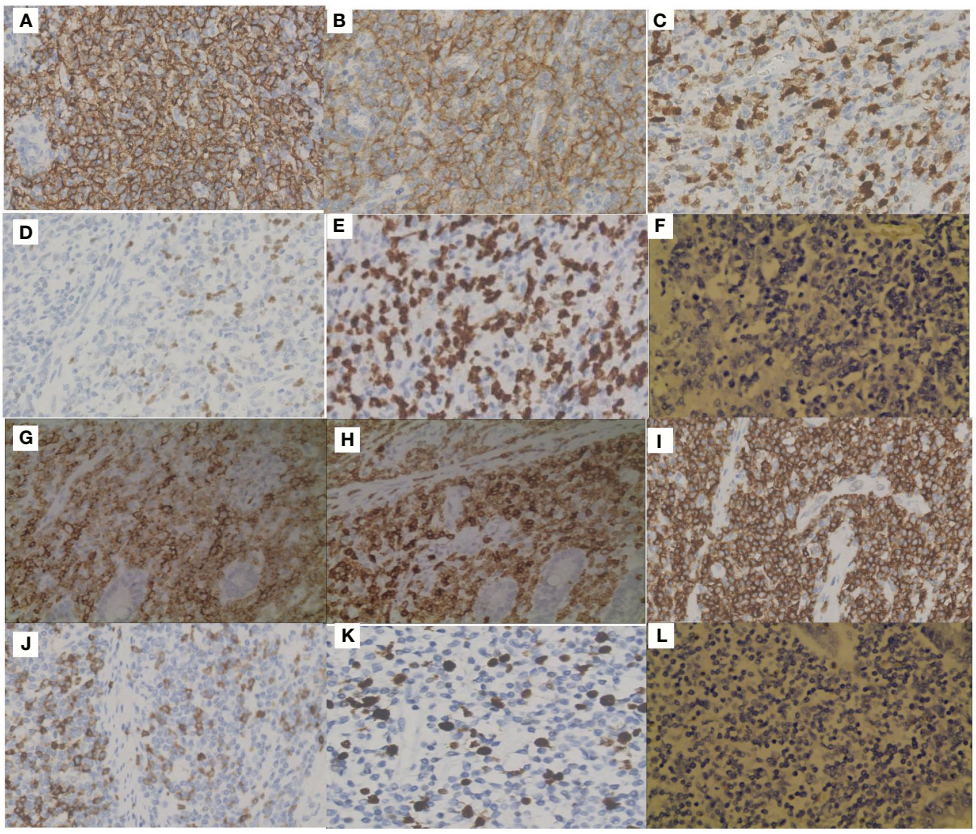

FIGURE 2
Immunohistochemical staining (IHC) of the two components. Positive IHC staining of CD20 **(A)**, CD10 **(B)**, MUM1 **(C)**, Bcl-6 **(D)**, Ki67 **(E)** and negative staining of EBER **(F)** in DLBCL. Positive IHC staining of CD20 **(G)**, CD3 **(H)**, CD5 **(I)**, CD7 **(J)**, Ki67 **(K)** and negative staining of EBER **(L)** in PTCL-NOS.

Discussion

Custer first introduced the term CL in 1954. Kim et al. further modified the concept of CL to the simultaneous occurrence of more than one histologically distinct lymphoma in the same anatomical organ in 1977 (14). Most CLs reported in the literature are classical Hodgkin's lymphoma (HL) combined with non-Hodgkin's lymphoma (NHL) or two different B-cell NHL, while the concurrence of B-NHL and T-cell lymphoma is rare (6, 15). DLBCL is the most common B-cell member, followed by hairy cell leukemia, chronic lymphocytic leukemia/small lymphocytic lymphoma, and splenic marginal zone lymphoma. The most common T-cell components are large granular lymphocytic leukemia and angioimmunoblastic T-cell lymphoma (4, 16–18). However, the co-occurrence of DLBCL and PTCL-NOS is rare, with 12 cases reported in the literature (6–13). In this case, it occurred in the small intestine with abnormal expression CD20 in PTCL-NOS.

In addition to our case, twelve cases of CL with DLBCL and PTCL-NOS have been reported. We summarized the clinicopathological features of these cases in Table 1. The male-to-female ratio was 2.25:1, ranging from 25 to 91 years (median: 67). Three were Asian, and ten were Caucasian. The occurrence site included the larynx (1 case), lymph node (3 cases), small intestine (2 cases), and bone (1 case), while the data of the remaining 6 cases were missing. Four of the ten patients had a history of hematological diseases, including polycythemia vera, Hodgkin's lymphoma, cutaneous T-cell lymphoma, and indolent B lymphoma. Of the three reported cases that provided the CD20 information, two were positive, and one was negative. Three patients were positive for EBER in DLBCL while negative for PTCL-NOS components. Six patients were negative for EBER in both DLBCL and PTCL-NOS. In another case, EBER was weakly positive in DLBCL but not in PTCL-NOS. The other three cases didn't mention the EBER result. Six of ten patients showed positive TCR gene rearrangement. Eight out of ten patients showed IgH gene rearrangement. Five patients received chemotherapy, one with chemotherapy combined with radiotherapy, one used topical therapy, one refused treatment, and five cases did not show the details. One patient achieved PR after four cycles of chemotherapy but died of surgery. One patient died of cachexia six months after topical treatment. Six patients were unknown about the prognosis. The median follow-up time for the seven cases was eleven months (1-101 months).

The pathogenesis of CL is still unclear. Scholars proposed some hypotheses for the coexistence of T-cell and B-cell tumors in the same tissue (4, 5, 8, 19, 20). One of the most commonly mentioned is the virological hypothesis. EBV infection may cause simultaneous or sequential transformation of B cell and T cell components, leading to the development of the two types of lymphoma (8, 20). On the one hand, the expression of EBV antigen in neoplastic B cells may stimulate the proliferation of T cells and eventually

transform into T-cell lymphoma via clonal selection. Alternatively, the first appearance of T-cell lymphoma may also result in a deficiency in innate immunity that renders host B cells more susceptible to EBV infection, leading to transformation into B-cell neoplasms. In such cases, the patient's T-cell and B-cell tumor components tend to be EBER positive (8, 21). Although this patient was positive for EBV-associated IgG, the pathology indicated that EBER was negative for both T and B cell components. Virological theories still can not explain our case and other instances of CL without a virological basis.

Another proposed mechanism in this era of high-throughput genome sequencing is the hypothesis of acquired oncogene mutations in lymphoid progenitor cells. Wang et al. (5) reported a case of CL composed of PTCL and mantle cell lymphoma, and both components were positive for the CCND1/IgH fusion gene and cyclin D1 overexpression. Therefore, the authors believed there were specific genetic variations in lymphoid progenitor cells. Then, other genomic modifications evolve into heterogeneous subclones, resulting in the co-development of T - and B-cell tumors. Given the clonal correlation between the two tumor components, authors assumed that the two tumors may share a co-progenitor cell or grow in the same microenvironment. The progress of genomics provides an ideal tool for studying the clonal origin and clonal evolution of similar composite lymphoid tumors (22). It is helpful to investigate the tumor lineage of CL by analyzing the genomic profiles of B-cell and T-cell tumors. However, the results of high-throughput sequencing of genes for the components of the two tumor cells were lacking in this patient. In conclusion, the pathogenesis of CL of B cell and T cell origin is complex, and there may be multiple pathophysiological pathways (4, 5, 20).

Another feature of our case is the aberrant expression of CD20 in PTCL-NOS. The incidence of CD20 expression in T cell lymphoma reported in the literature was about 5-8% (23). There are several hypotheses about its pathogenesis. First, as there are a small number of CD20 weakly expressed normal T cells in peripheral blood, bone marrow, and lymph nodes during normal hematopoiesis (24), it is speculated that CD20-positive T cell lymphoma originates from this group of malignant T cell subsets. Second, CD20 expression can be induced by T lymphocytes in the process of stimulation or proliferation and activation *in vitro* (25). Thus, CD20 expression may also be related to the activation of T-cell lymphoma cells. Third, there are progenitor cells with the potential to differentiate into B cells, T cells, and NK cells in cord blood (26), so CD20-positive PTCL may also be the product of the malignant transformation of progenitor cells at a stage of differentiation. In our case and another case reported in the literature (7), the intensity of CD20 in PTCL-NOS was weaker than that of DLBCL counterparts. Therefore, we suspect these two components may have different cellular origins, but the exact mechanism needs further investigation.

Chemotherapy therapy and radiation are common and effective treatments for lymphoma. In CL, the choice of the treatment regimen and the patient's prognosis is mainly based on the more aggressive type of lymphoma (27). In this case, DLBCL exists simultaneously with PTCL-NOS. Therefore, this patient was treated with rituximab and CHOP. Rituximab has been widely used in treating CD20-

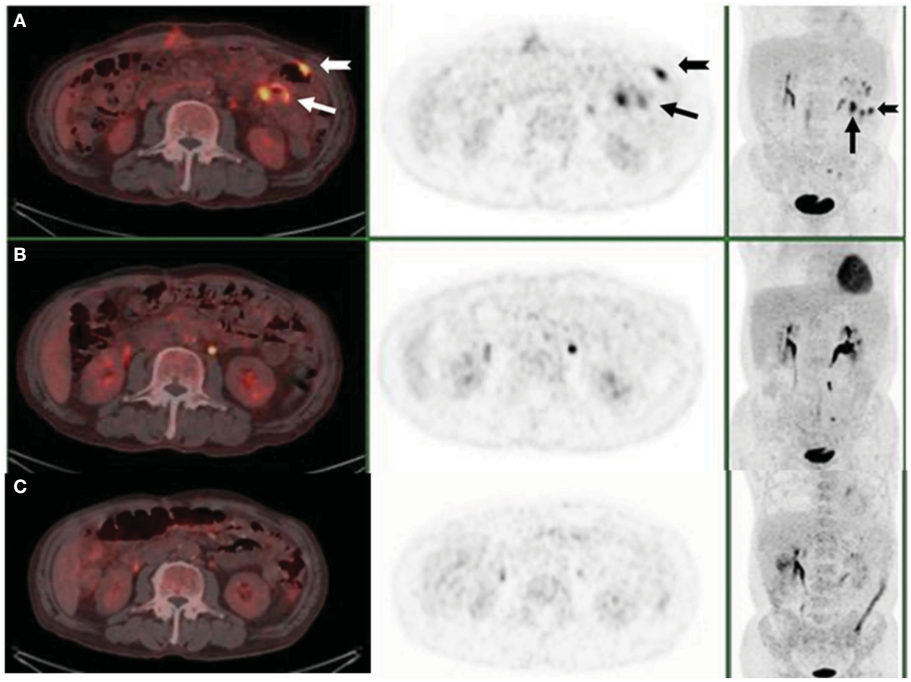

FIGURE 3
PET/CT scan after small bowel resection **(A)**, three cycles of R-CHOP **(B)**, and five months after treatment **(C)**. The arrows demonstrated the lesions where abdominal masses disappeared.

TABLE 1 Clinical and pathological features of 13 patients with composite diffuse large B-cell lymphoma (DLBCL) and peripheral T-cell lymphoma, not otherwise specified (PTCL.NOS).

Year	Country	Sex	Age	Site	Other sites involved	Past medical history	CD20 expression of PTCL.NOS	EBER		Molecular information	Treatment and follow-up	Citation
								DLBCL	PTCL.NOS			
2002	Germany	M	25	N/A	Lymph nodes, spleen, epidural space	N/A	N/A	+	-	TCR+, IgH+	Polychemotherapy, alive after 101 months	(8)
2002	Germany	F	89	N/A	Lymph nodes	N/A	N/A	+	–	TCR-, IgH-	Untreated, lost to follow-up	(8)
2002	Germany	F	91	N/A	Lymph nodes, bone marrow, skin	N/A	N/A	+	-	TCR+, IgH+	Intrathecal injection with methotrexate, follow-up for six months, died of cachexia.	(8)
2005	America	M	35	Tibia	Tibial soft tissue	None	N/A	–	–	TCR-,IgH+	N/A	(6)
2006	America	M	49	Ileum	None	Gastroesophageal reflux	-	-	-	TCR+, IgH+	Achieve CR after six cycles of R-CHOP, with no disease progression for 15 months.	(9)
2008	Italy	M	67	Lymph node	Bone marrow	Tuberculosis	N/A	–	–	TCR-, IgH+	Achieved CR after six cycles of R-CHOP and radiation, with no disease progression for 6 months.	(10)
2012	America	M	43	laryngeal	Lung, stomach, and mesenteric lymph nodes	Hodgkin lymphoma	N/A	+(weak)	N/A	TCR+, IgH+	Achieved PR after 4 cycles of R-CHOP but died of bleeding at the surgical site.	(12)
2011	Japan	F	67	cervical lymph node	Bone, epidural space	None	Positive	–	–	TCR+, IgH-	Achieve CR after six cycles of R-CHOP and radiation, with no disease progression for 8 months.	(7)
2016	America	M	82	N/A	N/A	**polycythemia vera**	N/A	+ (Unspecified)		N/A	N/A	(13)
2016	America	F	33	N/A	N/A	Cutaneous T lymphoma	N/A	-(Unspecified)		N/A	N/A	(13)
2016	America	M	70	N/A	N/A	**un-specific**	N/A	+ (Unspecified)		N/A	N/A	(12)
2019	Japan	M	73	Axillary lymph nodes	N/A	Indolent B lymphoma	N/A	–	–	TCR+, IgH+	N/A	(11)
2019	China	M	75	Small intestine	Mesenteric lymph nodes	Psoriasis	Positive	-	-	TCR-, IgH+	Achieved CR after six cycles of R-CHOP, with disease progression after 11 months, and died four months later.	This case

N/A, Not Applicable; EBER, Epstein-Barr virus encodes in situ hybridization of small RNA; TCR, T cell receptor rearrangement; IgH, Immunoglobulin gene rearrangement; R-CHOP, Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; +, positive; -, negative.

10.3389/fonc.2024.1272209

positive B-cell lymphoma, while the efficacy in CD20-positive T-cell lymphoma is still unclear. Shao et al. reported a T-cell lymphoma with CD20 expression showing excellent response to rituximab with gemcitabine, oxaliplatin, and L-asparaginase (R-pGEMOX) instead of initial chemotherapy without rituximab (28). Mangogna A. et al. provide a PTCL-NOS case with aberrant expression with CD20 and CD79a who did not benefit from rituximab-based chemotherapy (29). Kakinoki et al. considered that the effectiveness of rituximab may be associated with the intensity of CD20 expression in T cells, and patients with abundant CD20 expression will benefit the most from treatment with R-based chemotherapy (30). This patient and another case reported in the literature with CD20-positive PTCL-NOS were treated with a standard R-CHOP regimen for six courses and achieved CR. From the literature and our case data, five patients received R-CHOP therapy, four patients achieved CR, one patient achieved PR, and the median follow-up time was 11 months. However, with extended follow-up, our patient relapsed. Unfortunately, he refused to undergo another biopsy, so the type of recurrent lymphoma remains unknown. and he eventually died from the disease.

Conclusion

In conclusion, the simultaneous occurrence of DLBCL and CD20-positive PTCL-NOS in the same tissue is infrequent in clinical practice, and it is not easy to diagnose and easy to miss and misdiagnose. To correctly diagnose this rare disease, clinicians must work with pathologists carefully, combining multiple detection methods, using as many tissues as possible in the biopsy, and avoiding lymph node puncture. Due to the poor prognosis associated with the simultaneous development of numerous histological types of lymphoma and the lack of data on treatment and outcome, the exact prognosis, treatment options, molecular genetic changes, and the mechanism of the disease occurrence still need to be further studied and explored.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

Author contributions

XC: Data curation, Funding acquisition, Writing – review & editing. JG: Formal Analysis, Writing – original draft. JQ: Investigation, Resources, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by Jiangsu Province's Young Medical Talents Program (QNRC2016683).

Acknowledgments

The authors would like to thank all the reviewers who participated in the review.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Thirumala S, Esposito M, Fuchs A. An unusual variant of composite lymphoma: a short case report and review of the literature. *Arch Pathol Lab Med.* (2000) 124:1376–8. doi: 10.5858/2000-124-1376-AUVOCL

2. Lee AYS. CD20+ T cells: an emerging T cell subset in human pathology. *Inflammation Res.* (2022) 71:1181–9. doi: 10.1007/s00011-022-01622-x

3. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed.* Lyon, France: IARC (2017).

4. Mokhtar NM. Review article composite lymphoma. *J Egypt Natl Canc Inst.* (2007) 19:171–5.

5. Wang E, Papavassiliou P, Wang AR, Louissaint A Jr., Wang J, Hutchinson CB, et al. Composite lymphoid neoplasm of B-cell and T-cell origins: a pathologic study of 14 cases. *Hum Pathol.* (2014) 45:768–84. doi: 10.1016/j.humpath.2013.11.008

6. Kaleem Z, McGuire MH, Caracioni AC, Leonard RL, Pathan MH, Lessmann EA, et al. Composite B-cell and T-cell non-Hodgkin lymphoma of the tibia. *Am J Clin Pathol.* (2005) 123:215–21.

7. Yamazaki S, Fujioka Y, Nakamura F, Ota S, Shinozaki A, Yamamoto G, et al. Composite diffuse large B-cell lymphoma and CD20-positive peripheral T-cell lymphoma. *Pathol Int*. (2011) 61:662–6. doi: 10.1111/j.1440-1827.2011.02713.x

8. Zettl A, Lee SS, Rudiger T, Starostik P, Marino M, Kirchner T, et al. Epstein-Barr virus-associated B-cell lymphoproliferative disorders in angloimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma, unspecified. *Am J Clin Pathol*. (2002) 117:368–79. doi: 10.1309/6UTX-GVC0-12ND-JJEU

9. Abou-Elella AA, Nifong TP. Composite EBV negative peripheral T-cell lymphoma and diffuse large B-cell lymphoma involving the ileum: a case report and a systematic review of the literature. *Leuk Lymph*. (2006) 47:2208–17. doi: 10.1080/10428190600763348

10. Furlan A, Pietrogrande F, Marino F, Menin C, Polato G, Vianello F. Sequential development of large B cell lymphoma in a patient with peripheral T-cell lymphoma. *Haematologica*. (2008) 93:e6–8. doi: 10.3324/haematol.11269

11. Imanishi D, Hata T, Imaizumi Y, Niino D, Ohshima K, Miyazaki Y. Enteropathy-associated T-cell lymphoma coexisting with composite lymphoma composed of DLBCL and PTCL. *Rinsho Ketsueki*. (2019) 60:197–202. doi: 10.11406/rinketsu.60.197

12. Cui W, Fan F, Zhang D, Garnett D, Tilzer L. Primary composite lymphoma of the larynx, composed of diffuse large B-cell lymphoma and peripheral T-cell lymphoma, not otherwise specified, presenting as left subglottic tracheal fistula, esophageal diverticulum, and neck abscess. *Ann Clin Lab Sci*. (2012) 42:73–80.

13. Hoffmann JC, Chisholm KM, Cherry A, Chen J, Arber DA, Natkunam Y, et al. An analysis of MYC and EBV in diffuse large B-cell lymphomas associated with angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma not otherwise specified. *Hum Pathol*. (2016) 48:9–17. doi: 10.1016/j.humpath.2015.09.033

14. Kim H, Hendrickson R, Dorfman RF. Composite lymphoma. *Cancer*. (1977) 40:959–76. doi: 10.1002/1097-0142(197709)40:3<959::aid-cncr2820400302>3.0.co;2-3

15. Niino D, My Hanh LT, Miura S, Nakashima M, Iwanaga M. Incidence patterns of sequential or composite lymphoma: A population-based cancer registry study. *Tohoku J Exp Med*. (2021) 254:123–7. doi: 10.1620/tjem.254.123

16. Campidelli C, Sabattini E, Piccioli M, Rossi M, De Blasi D, Miraglia E, et al. Simultaneous occurrence of peripheral T-cell lymphoma unspecified and B-cell small lymphocytic lymphoma. Report of 2 cases. *Hum Pathol*. (2007) 38:787–92. doi: 10.1016/j.humpath.2006.10.010

17. Trimech M, Letourneau A, Missiaglia E, De Prijck B, Nagy-Hulliger M, Somja J, et al. Angioimmunoblastic T-cell lymphoma and chronic lymphocytic leukemia/small lymphocytic lymphoma: A novel form of composite lymphoma potentially mimicking richter syndrome. *Am J Surg Pathol*. (2021) 45:773–86. doi: 10.1097/PAS.0000000000001646

18. Miyagawa F, Nakajima A, Ogawa K, Takeda M, Nakamine H, Amano I, et al. Composite EBV-negative marginal zone lymphoma and angioimmunoblastic T-cell lymphoma presenting as multiple subcutaneous nodules. *Eur J Dermatol*. (2020) 30:427–9. doi: 10.1684/ejd.2020.3810

19. Demurtas A, Aliberti S, Bonello L, Di Celle PF, Cavaliere C, Barreca A, et al. Usefulness of multiparametric flow cytometry in detecting composite lymphoma: study of 17 cases in a 12-year period. *Am J Clin Pathol*. (2011) 135:541–55. doi: 10.1309/AJCPQKE25ADCFZWN

20. Suefuji N, Niino D, Arakawa F, Karube K, Kimura Y, Kiyasu J, et al. Clinicopathological analysis of a composite lymphoma containing both T- and B-cell lymphomas. *Pathol Int*. (2012) 62:690–8. doi: 10.1111/j.1440-1827.2012.02858.x

21. Kakiuchi S, Yakushijin K, Takagi I, Rikitake J, Akiyama H, Matsuba H, et al. Case report: composite angioimmunoblastic T-cell lymphoma and epstein-barr virus-positive B-cell lymphoproliferative disorder as other iatrogenic immunodeficiency-associated lymphoproliferative disorders. *Front Med (Lausanne)*. (2020) 7:625442. doi: 10.3389/fmed.2020.625442

22. Carlotti E, Wrench D, Rosignoli G, Marzec J, Sangaralingam A, Hazanov L, et al. High throughput sequencing analysis of the immunoglobulin heavy chain gene from flow-sorted B cell sub-populations define the dynamics of follicular lymphoma clonal evolution. *PloS One*. (2015) 10:e0134833. doi: 10.1371/journal.pone.0134833

23. Rahemtullah A, Longtine JA, Harris NL, Dorn M, Zembowicz A, Quintanilla-Fend L, et al. CD20+ T-cell lymphoma: clinicopathologic analysis of 9 cases and a review of the literature. *Am J Surg Pathol*. (2008) 32:1593–607. doi: 10.1097/PAS.0b013e31817d7452

24. Algino KM, Thomason RW, King DE, Montiel MM, Craig FE. CD20 (pan-B cell antigen) expression on bone marrow-derived T cells. *Am J Clin Pathol*. (1996) 106:78–81. doi: 10.1093/ajcp/106.1.78

25. Murayama Y, Mukai R, Sata T, Matsunaga S, Noguchi A, Yoshikawa Y. Transient expression of CD20 antigen (pan B cell marker) in activated lymph node T cells. *Microbiol Immunol*. (1996) 40:467–71. doi: 10.1111/j.1348-0421.1996.tb01096.x

26. Haddad R, Guardiola P, Izac B, Thibault C, Radich J, Delezoide AL, et al. Molecular characterization of early human T/NK and B-lymphoid progenitor cells in umbilical cord blood. *Blood*. (2004) 104:3918–26. doi: 10.1182/blood-2004-05-1845

27. Kuppers R, Duhrsen U, Hansmann ML. Pathogenesis, diagnosis, and treatment of composite lymphomas. *Lancet Oncol*. (2014) 15:e435–446. doi: 10.1016/S1470-2045(14)70153-6

28. Shao Y, Bai C, Sun J, Gao X. T-cell lymphoma with abundant CD20 expression showing a good response to rituximab with gemcitabine, oxiplatin, and L-asparaginase (R-pGEMOX): A case report. *Med (Baltimore)*. (2018) 97:e0199. doi: 10.1097/MD.0000000000010199

29. Mangogna A, Cox MC, Ruco L, Lopez G, Belmonte B, Di Napoli A. Rituximab plus chemotherapy provides no clinical benefit in a peripheral T-cell lymphoma not otherwise specified with aberrant expression of CD20 and CD79a: A case report and review of the literature. *Diagnostics (Basel)*. (2020) 10(6):341. doi: 10.3390/diagnostics10060341

30. Kakinoki Y, Hashiguchi J, Ishio T, Chiba K, Niino D, Ohshima K. CD20-positive primary gastric T-cell lymphoma poorly responding to initial treatment with rituximab plus CHOP, and a literature review. *Int J Hematol*. (2015) 102:702–8. doi: 10.1007/s12185-015-1841-x

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 15 March 2024
DOI 10.3389/fonc.2024.1288501

OPEN ACCESS

EDITED BY
Michael Liew,
ARUP Laboratories, United States

REVIEWED BY
Peter Wang,
Zhejiang Zhongwei Medical Research Center, China
Javier Leon,
University of Cantabria, Spain

*CORRESPONDENCE
Subhradip Karmakar
subhradip.k@aiims.edu

†PRESENT ADDRESS
Jonahunnatha Nesson George William, Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy

‡These authors have contributed equally to this work

RECEIVED 04 September 2023
ACCEPTED 15 February 2024
PUBLISHED 15 March 2024

CITATION
William JNG, Dhar R, Gundamaraju R, Sahoo OS, Pethusamy K, Raj AFPAM, Ramasamy S, Alqahtani MS, Abbas M and Karmakar S (2024) SKping cell cycle regulation: role of ubiquitin ligase SKP2 in hematological malignancies.
Front. Oncol. 14:1288501.
doi: 10.3389/fonc.2024.1288501

SKping cell cycle regulation: role of ubiquitin ligase SKP2 in hematological malignancies

Jonahunnatha Nesson George William[1†‡], Ruby Dhar[2‡], Rohit Gundamaraju[3], Om Saswat Sahoo[4], Karthikeyan Pethusamy[2], A. F. P. Allwin Mabes Raj[5], Subbiah Ramasamy[6], Mohammed S. Alqahtani[7,8], Mohamed Abbas[9] and Subhradip Karmakar[2*]

[1]Department of Medical, Oral and Biotechnological Sciences (DSMOB), Ageing Research Center and Translational Medicine-CeSI-MeT, "G. d'Annunzio" University Chieti-Pescara, Chieti, Italy, [2]Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India, [3]ER Stress and Intestinal Mucosal Biology Lab, School of Health Sciences, University of Tasmania, Launceston, TAS, Australia, [4]Department of Biotechnology, National Institute of Technology, Durgapur, India, [5]Institute of Environmental Protection and Sensors (IOS), Maribor, Slovenia, [6]Cardiac Metabolic Disease Laboratory, Department Of Biochemistry, School of Biological Sciences, Madurai Kamaraj University, Madurai, India, [7]Radiological Sciences Department, College of Applied Medical Sciences, King Khalid University, Abha, Saudi Arabia, [8]BioImaging Unit, Space Research Centre, University of Leicester, Leicester, United Kingdom, [9]Electrical Engineering Department, College of Engineering, King Khalid University, Abha, Saudi Arabia

SKP2 (S-phase kinase-associated protein 2) is a member of the F-box family of substrate-recognition subunits in the SCF ubiquitin-protein ligase complexes. It is associated with ubiquitin-mediated degradation in the mammalian cell cycle components and other target proteins involved in cell cycle progression, signal transduction, and transcription. Being an oncogene in solid tumors and hematological malignancies, it is frequently associated with drug resistance and poor disease outcomes. In the current review, we discussed the novel role of SKP2 in different hematological malignancies. Further, we performed a limited in-silico analysis to establish the involvement of SKP2 in a few publicly available cancer datasets. Interestingly, our study identified *Skp2* expression to be altered in a cancer-specific manner. While it was found to be overexpressed in several cancer types, few cancer showed a down-regulation in SKP2. Our review provides evidence for developing novel SKP2 inhibitors in hematological malignancies. We also investigated the effect of SKP2 status on survival and disease progression. In addition, the role of miRNA and its associated families in regulating *Skp2* expression was explored. Subsequently, we predicted common miRNAs against *Skp2* genes by using miRNA-predication tools. Finally, we discussed current approaches and future prospective approaches to target the *Skp2* gene by using different drugs and miRNA-based therapeutics applications in translational research.

KEYWORDS

AML, leukemia, hematological cancers, miRNA, Skp2, oncogene, *in silico*, cancer genomics

Introduction

Poly ubiquitination is the binding of numerous ubiquitin molecules into the same target protein. Generally, the polyubiquitination of proteins is induced by different signaling molecules and co-operates for protein degradation by the proteasomes. This post-translational modification process (Polyubiquitination) regulates numerous cellular events, including cell growth, proliferation, differentiation and apoptosis in mammalian cells (1). Any deregulation in the ubiquitination machinery and its components could disarrange the cellular homeostasis and initiate the process of neoplastic transformation in various cancers. The step by step action of the ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-ligating (E3) enzymes associated with the ubiquitin-proteasome system (UPS), mediate ubiquitination by which they degrade targeted substrate proteins (2).

The SKP1, CUL1, F-box protein (SCF) complex consists of three core components that remain constant: RING-box 1 (RBX1), a RING-finger protein responsible for recruiting the E2 ubiquitin-conjugating enzyme; Cullin 1 (CUL1), acting as the scaffolding protein; and S-phase kinase-associated protein 1 (SKP1), an unchanging adaptor that links the core SCF complex with a variable F-box protein and its corresponding target protein (3). The specificity of the SCF complex for particular targets is determined by F-box proteins, with each F-box protein recognizing and binding a specific set of substrates. In humans, there are a total of 69 F-box proteins, categorized into three families based on their substrate recognition domains: (1) FBXW with WD40 repeats; (2) FBXL with leucine-rich repeats (e.g., FBXL1, also known as the S-phase kinase-associated protein 2 [SKP2]); and (3) FBXO with other domains (4). To regulate the levels of specific protein targets, each F-box protein recruits one of its substrates, often phosphorylated, to the core SCF complex, facilitating polyubiquitination and subsequent degradation by the 26S proteasome (5). With a total of 69 distinct F-box genes, it suggests the existence of up to 69 unique SCF complexes, each responsible for regulating a diverse array of protein targets (4).

Few well-characterized F-box proteins regulate substrates which are involved in cell cycle regulation, signal transduction, and transcription (Table 1) (33). Among these, one of the E3 ligases called SKP2 (S-Phase Kinase Associated Protein 2 (~ 45kDa)), a member of the F box family (34), is recognized as a pro-oncogene. These F-box proteins are mostly composed of one of the four subunits of ubiquitin-protein ligases complex named SCFs but do not always recognize substrates in a phosphorylation-dependent manner. In this complex of SCF's, the F-box is referred to as a subunit, which serves as the recognition site for protein substrates. The N-terminal F-Box domain of the F-box binds to SKP1 and thereby connects with the SCF complex. After that, C-terminal Leucine-rich repeat (LRR) and WD40 repeats support substrate binding. Association of SKP1-SKP2 is found in humans (35). SKP2 assembles to SCF-type E3 ubiquitin ligase complex along with Cullin-1, Skp1, and Rbx1 (36–39). In addition, the requirement of

TABLE 1 SKP2 and its known substrates.

Substrate	Function	Reference
E2A	B/T Cell Development	(6)
p27	Cell Cycle Control	(7)
p21	Cell Cycle Control	(8)
p57	Cell Cycle Control	(9)
p130	Cell Cycle Control	(9)
Cyclin D1	Cell Cycle Control	(10)
Cyclin E	Cell Cycle Control	(11)
Cyclin A	Cell Cycle Control	(12)
RAG2	DNA Repair	(13)
BRCA2	DNA Repair	(14)
ORC1P	DNA Replication	(15)
CDT1	DNA Replication	(16)
MKP1	ERK Signaling	(17)
TAL1	Erythroid Differentiation	(18)
E2F1	Gene Transcription	(19)
MEF	Gene Transcription	(20)
TOB1	Gene Transcription	(21)
MYC	Gene Transcription	(22)
MYB	Gene Transcription	(23)
FOXO1	Gene Transcription	(24)
FOXO3A	Gene Transcription	(24)
RBL2	Gene Transcription	(25)
MLL	Gene Transcription	(26)
UBP43	Interferon Signaling	(27)
USP18	Interferon Signaling	(28)
RASSF1A	Microtubule Stabilizer	(29)
SMAD4	Signal Transduction	(30)
CDK9	Transcriptional Elongation	(31)
HPV-E7	Viral Oncogenesis	(32)

Abbreviations: SKP2, S-phase kinase-associated protein 2; CSC, Cancer Stem Cell; ALDH, Aldehyde dehydrogenase; AML, Acute myeloid leukemia; CDK, Cyclin dependent Kinase; SCF, Chemokine (C-X-C motif) ligand; DNMT, DNA methyltransferase; FAO, Fatty Acid Oxidation; GBM, Glioblastoma multiforme; HCC, Hepatocellular Carcinoma; PTEN, Phosphatase and tensin homolog; HIF, Hypoxia-inducible factor; IFN-γ, Interferon-gamma; TCGA, The Cancer Genome Atlas; BRCA1, Monoclonal antibody; MDSC, Myeloid-derived suppressor cells; BRCR-ABL, The breakpoint cluster region protein also known as renal carcinoma antigen NY-REN-26; mTORC, Mammalian Target of Rapamycin Complex; MAPK, Mitogen-activated protein Kinase; CUL1, Cullin1; JAK, Janus kinase; TAM, Tumor-associated macrophages; TAN, Tumor-associated neutrophils; TET, Ten-eleven translocation proteins; TGF β, Transforming growth factor β; TNBC, Triple-negative breast cancer; TNF α, Tumor necrosis factor α.

cell cycle regulator CDK subunit 1 [CKS1] is important for CF SKP2-mediated ubiquitinylation of p27 (7).

Skp2 gene plays a significant role in cell cycle progression and cell survival through ubiquitin-mediated degradation of many tumor suppressor proteins (p27, p21, p57, p130, FOXO1, BRCA2, RASSF1A, TOB1), cell cycle regulatory proteins (Cyclin D & E, E2F1, *etc.)* and oncogenes (*c-MYC, MYB*) (Figure 1) (40, 41). The target interruption of SKP2 leads to the accumulation of Cdk inhibitor p27, which leads to G1 phase cell cycle arrest. SKP2 mediates the degradation of p27 via ubiquitination through the 26S proteasome pathway (42, 43). Additionally, proteins like RING E3 ligases, are essential for the interaction of the E2-conjugating enzyme along with the SKP1adaptor protein (36). In addition, scaffold and ring finger proteins like Rbx1 are also required to target the substrate via its E3 ligase activity (36).

SKP2 in cancers

Higher expression of *Skp2* is associated with tumor initiation and progression (Table 2) (44). Concurrently, the level of SKP2 oscillates during the cell cycle and is controlled by both transcriptional and post-transcriptional mechanisms. During cell cycle regulation, low expression of *Skp2* is observed in both G0/G1 and late M/early G1, while a high level of SKP2 is found during G1/S transition, peaking at the S phase. Moreover, Cdk inhibitor p27 is usually stable in G0/G1 phase and unstable in the G1/S phase (8, 45). Cyclin E and E2F-1 proteolysis are essential for their rapid turnover during G1 to S phase progression, which directly increases the abundance of SKP2 during this time (46). Further, p300 acetylates SKP2 in the Nuclear Localization Signal (NLS) region, thereby mediates its localization in the cytoplasm, and enhances the stability of SKP2 (Figure 2) (43).

A high level of *Skp2* and a low level of *p27* expressions are associated with poor prognosis in solid tumors. Similarly, an inverse correlation between *Skp2* and *p27* gene expression is also frequently found in hematological malignancies (47, 48). Thus an overexpression of the *Skp2* gene concomitantly decreases the expression level of the *p27* gene in diverse cancer types (Figure 3). However, the molecular mechanisms and the cause of *p27* gene loss and elevated levels of *Skp2* gene expression are not wholly investigated in all cancer types. To further support the significance of SKP2, an *in-vivo* xenograft mice model exhibiting high expression of the *Skp2* gene was found to promote tumor growth (46). Surprisingly, following depletion of the SKP2, tumor development is dramatically reduced by inducing programmed cell death and cell senescence (49). Furthermore, another study on glioblastoma cells also demonstrated that depletion of SKP2 inhibits cancer progression via promoting cellular senescence (50). Similarly, transgenic mouse models overexpressing *Skp2* have shown tumor growth in various tissues, but the cause of how SKP2 triggers neoplastic transformation is elusive (51).

While gene amplification may result in an enhanced *Skp2* expression in cancers, oncogenic signals could also contribute to its elevated expression. Oncogenic alterations leading to higher expression of *JAK2V617F* mutation, *BCR-ABL*, and *Her2/Neu*, which further activates Jak/Stat, and PI3K/AKT signals thereby inducing *Skp2* gene expression in malignant cells (33, 52). However, in the nucleus, BCR-ABL mediated transcription of *Skp2* is associated with PI3K/AKT/SP1 pathway and mTORC2 via mTOR signaling pathways, implicating the modulation of *p27* level expression. Mainly through PI3-kinase signaling, the mTORC2 pathway elevates the *Skp2* expression, thereby reducing the *p27* expression and initiating cancer progression (53, 54). Furthermore, p300 acetylates K68 and K71 residues of SKP2 during oncogenicity, sustaining their stability and enhancing retention in the cytoplasm

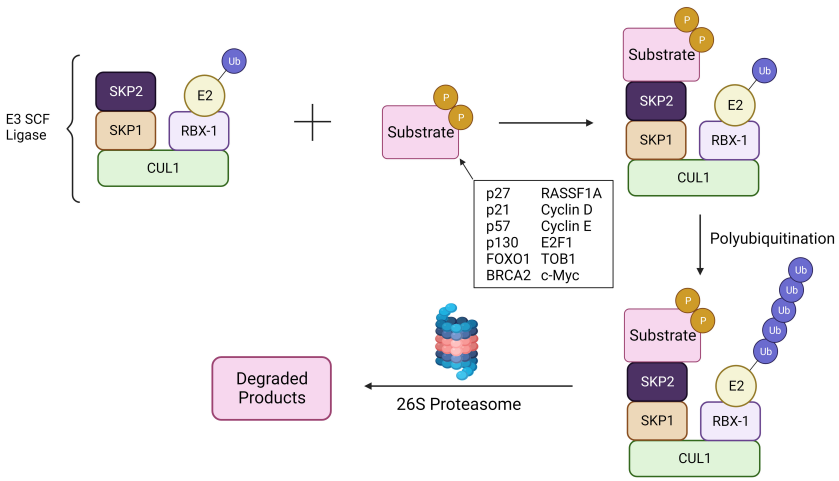

FIGURE 1
SCF^{SKP2} complex. The SCF^{SKP2} complex plays a pivotal role in regulating cell cycle progression and maintaining cellular homeostasis. SKP2, an F-box protein within the complex, acts as a substrate recognition component. It recognizes specific target substrates marking them for ubiquitination. Once ubiquitinated, the tagged proteins are targeted for degradation by the 26S proteasome. The SCF complex serves as an E3 ubiquitin ligase, facilitating the transfer of ubiquitin molecules to substrates. Ultimately, this polyubiquitination signals the proteasome to recognize and degrade the marked proteins, regulating key cellular processes and ensuring proper cell cycle dynamics.

TABLE 2 SKP2 expression profile across tumor samples (derived from gepia2.cancer-pku.cn).

SKP2 Overexpressed Cancer	SKP2 Underexpressed Cancer
ACC – Adrenocortical Carcinoma	KICH – Kidney Chromophobe
BLCA – Bladder Urothelial Carcinoma	LAML – Acute Myeloid Leukemia
BRCA - Breast invasive carcinoma	PRAD – Prostate Adenocarcinoma
CESC - Cervical squamous cell carcinoma and endocervical adenocarcinoma	THCA – Thyroid Carcinoma
CHOL – Cholangiocarcinoma	
COAD - Colon adenocarcinoma	
DLBC - Diffuse Large B-cell Lymphoma	
ESCA - Esophageal carcinoma	
GBM - Glioblastoma multiforme	
HNSC - Head and Neck squamous cell carcinoma	
KIRC - Kidney renal clear cell carcinoma	
KIRP - Kidney renal papillary cell carcinoma	
LGG - Brain Lower Grade Glioma	
LIHC - Liver hepatocellular carcinoma	
LUAD - Lung adenocarcinoma	
LUSC - Lung squamous cell carcinoma	
OV - Ovarian serous cystadenocarcinoma	
PAAD - Pancreatic adenocarcinoma	
PCPG - Pheochromocytoma and Paraganglioma	
READ - Rectum adenocarcinoma	
SARC – Sarcoma	
SKCM - Skin Cutaneous Melanoma	
STAD - Stomach adenocarcinoma	
TGCT - Testicular Germ Cell Tumors	
THYM – Thymoma	
UCES - Uterine Corpus Endometrial Carcinoma	
UCS - Uterine Carcinosarcoma	

(43). SKP2 promotes cellular invasion and migration by suppressing the tumor suppressor genes/protein expression and regulates its downstream targets, such as p21, p27Kip1, and FOXO1 (55). Aberrant regulation of the SKP2/p27 axis has also been noted in gastric cancer suppression, wherein MESP2 binds competitively to TCF4 (56). In addition, high SKP2 endorses cancer progression through the activation of various growth and survival-signaling pathways, for example, PTEN, ARF, pRB, FOXO1, and high Her2/Neu, *etc.* SKP2 acetylation and phosphorylation regulates its SCF E3 ligase activity in the cytoplasm during cancer progression, and AKT phosphorylates SKP2 at Ser72 during metastasis. The cytosolic SKP2 activates AKT and PTEN loss, implicating SKP2 translocation from the nucleus to the cytosol through Ser72 phosphorylation and induces tumor growth (57). Additionally, through neddylation, Cul-1 stabilizes the SKP2-SCF complex and negatively regulates the SKP2-SCF complex, Cul-1 dissociates from Cand1 by Cul1 neddylation and deneddylation of Cul1is mediated by Cop9-signalosome (CSN) protein complex (58). However, a complete SCF ligase activity is still largely unknown. Clinically, the elevated expression of *Skp2* is recognized as a poor prognostic marker in many solid tumor cancers and hematological malignancies (1). Concerning hematological malignancies, SKP2 being a crucial regulator of the cell cycle, plays a multifaceted role. SKP2 aberrations have been implicated in malignancies like acute myeloid leukemia, chronic lymphocytic leukemia, T-cell acute lymphoblastic leukemia, chronic myelogenous leukemia, multiple myeloma, primary effusion lymphoma, Diffuse large B-cell lymphoma, extranodal natural killer (NK)/T-cell lymphoma, myeloproliferative diseases etc. (Figure 4), disrupting hematopoietic differentiation and fostering genomic instability. The following delineates the role of SKP2 in the above mentioned malignancies.

SKP2 in acute myeloid leukemia

Skp2 expression is recognized as an independent prognostic factor in AML. High expression of *Skp2* is associated with shorter disease-free survival and overall survival. Interestingly, siRNA mediated knocking down of *Skp2* in AML cell lines HL-60/A resulted in cell cycle arrest reversing the multidrug resistance by downregulating *MRP* gene expression (59). However, further studies are required to showcase the cause of *Mrp* gene modulation in AML. The RNAi-based disruption of anti-miR-196b activity or pharmacologic inhibition of the Cks1-Skp2-containing SCF E3-ubiquitin ligase complexes significantly elevated the level of p27Kip1, which induces monocytic differentiation (60), noticeable reduction of leukemogenic potential, induced apoptosis and suppressing human AML growth (48). SKP2 and p27Kip1 are localized in the cytoplasm (61), which hints that an aberrant regulatory pathway is conducted through SKP2-mediated p27Kip1 proteolysis in most AML cases (62). On the other hand, SKP2 is positively correlated with phosphorylated PTEN, suggesting that the pPTEN-SKP2 axis might be a promising therapeutic target in AML (63).

AML is a complex heterogeneous disease with diverse pathologies. There are conflicting reports on the *Skp2* expression in AML. While results from TCGA shows a downregulation of SKP2 in AML (LAML), studies from other investigators reported an elevated SKP2 in AML (63). A possible reason for this apparent conflicting reports is due to the complex aetiology of AML. To improve the predictive value and therapeutic specificity of the *Skp2* gene in solid and hematological malignancies, we analyzed the TCGA data (data not shown). We performed different statistical analyses on diverse

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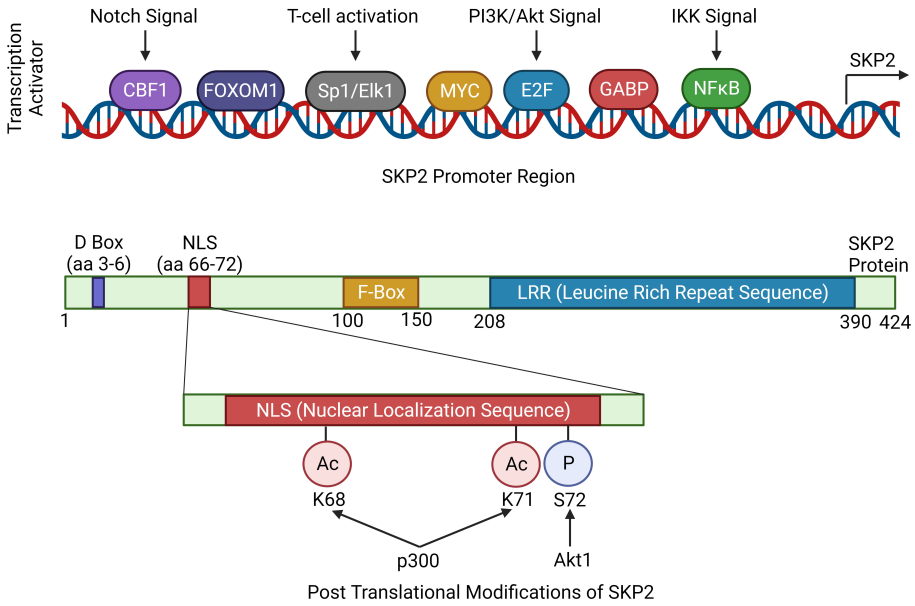

FIGURE 2
Regulation of *Skp2* gene expression. The expression of *Skp2* is intricately regulated by various signaling pathways, showcasing its significance in cellular homeostasis and proliferation. Key mitogenic signaling pathways like, Notch, PI3K/Akt and IKK, converges to SKP2 thereby modulating its expression. The coding region of *Skp2* contains functional domains essential for its function. The D-box is crucial for recognition by the anaphase-promoting complex (APC/C), marking SKP2 for degradation during cell cycle progression. The NLS (nuclear localization signal) guides SKP2 into the nucleus. The F-box domain is characteristic of SKP2's role in the SCF complex, facilitating substrate recognition. Finally, the LRR (leucine-rich repeat) domain contributes to protein-protein interactions, enabling SKP2 to engage with other components of the SCF complex and its target substrates, orchestrating precise control over cell cycle checkpoints and cellular processes.

populations. Our TCGA analysis relied on an online portal exploration, and the data was generated with an interactive web-portal (UALCAN tools (http://ualcan.path.uab.edu) through a TCGA-level setup. The three different RNA-sequence gene expression data and 31 different clinical cancer types' data were used for analysis, such as 1). Relative expression of the gene(s) across tumor and normal samples, as well as in various tumor sub-groups based on individual cancer stages over and under-expressed genes in individual cancer types, tumor grade, race, body weight, or other clinical pathologic features 2) effect of gene expression level on patient survival. Finally, we used 3) *in silico* validation studies for target genes (derived from the GENT2 database). Results depict that leukemia showed a marginally increased trend in *Skp2* expression as compared to lymphoma and Myeloma (Supplementary Table 1). Survival analysis also revealed a poor DFS (disease-free survival) with high *Skp2* expression, as also seen in leukemia vs lymphoma.

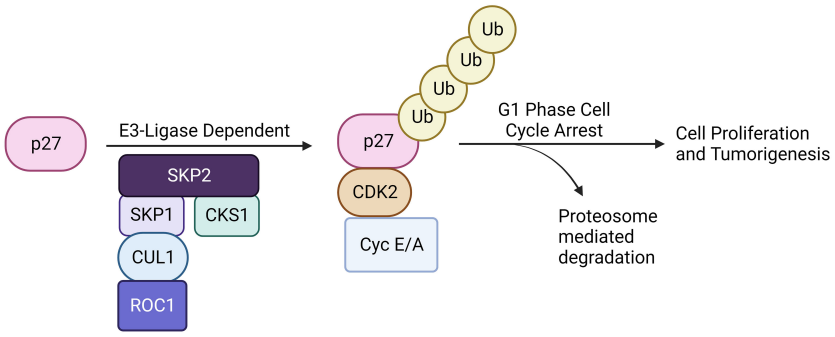

FIGURE 3
SKP2-p27 axis. The SKP2-p27 axis is a critical regulatory pathway governing cell cycle progression and proliferation. SKP2, a component of the SCF complex, targets the cyclin-dependent kinase inhibitor p27 for ubiquitination and subsequent proteasomal degradation. This ubiquitin-mediated destruction of p27 relieves its inhibitory effect on cell cycle progression, allowing cells to transition from G1 to S phase. Dysregulation of the SKP2-p27 axis is implicated in various cancers (solid and, including hematological malignancies), emphasizing its pivotal role in maintaining proper cell cycle control and highlighting its potential as a therapeutic targetin various malignancies.

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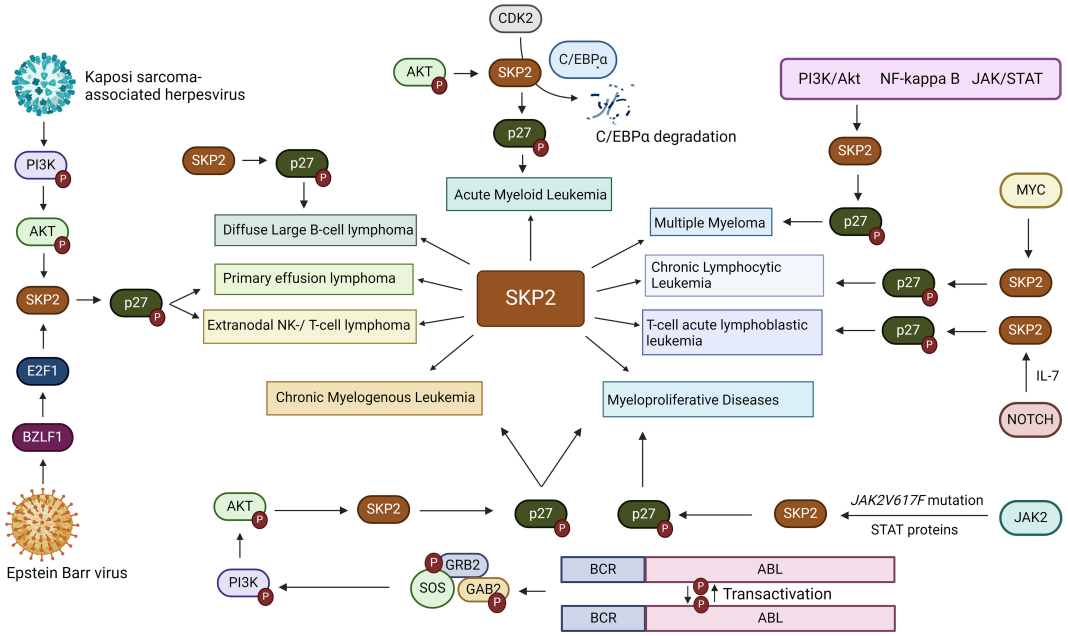

FIGURE 4
Involvement of SKP2 in hematological malignanices. SKP2, a critical player in hematological malignancies, features prominently in various cancers including acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and T-cell acute lymphoblastic leukemia (T-ALL), chronic myelogenous leukemia (CML), multiple myeloma, primary effusion lymphoma (PEL), and diffuse large B-cell lymphoma (DLBCL), Extranodal natural killer (NK)/T-cell lymphoma and myeloproliferative diseases. Mediated through diverse pathways such as PI3K/Akt, NFkB, and MYC, overexpression of SKP2 is often correlated with aggressive disease and poor outcomes, highlighting its significance in cancer biology and emphasizing the need for targeted therapeutic interventions.

Regulation of *Skp2* gene in other hematological malignancies

SKP2 in Chronic Lymphocytic Leukemia (CLL)

Chronic Lymphocytic Leukemia (CLL) is the most commonly diagnosed leukemia in the Western world. CLL, also named B cell malignancy, is characterized by indolent lymph proliferative disorder, where immature B cells expressing CD5+, CD19, CD23, and CD20 B-cells progressively accumulate in the peripheral blood, bone marrow and lymph nodes (64). During the last decades, modern therapeutic approaches significantly improved to induce CLL apoptosis at various levels, but CLL remains incurable due to its drug resistance/relapse. Interestingly, the significantly higher expression [mean of 3 fold-protein] of a cell cycle inhibitor, p27, was detected in CLL tonsil and peripheral blood B lymphocyte samples as compared to healthy B cells. Besides, the expression of *Myc* is relatively low in CLL in comparison with normal healthy B cells. The inversely correlated MYC and p27 in CLL, and the larger set of CLL in cohort patient studies clearly demonstrated that the *Skp2* gene is involved in p27 degradation. In a similar report, high *Skp2* expression correlated with high *Myc* and low *p27* expression in most of the CLL cases. On the other hand, low SKP2 samples showed high p27, and the mean MYC protein levels were significantly higher than high SKP2 levels in comparison with Tonsil and CLL. These findings demonstrated that through the MYC-SKP2-p27 axis pathway, MYC induces p27 degradation via upregulating the *Skp2* gene in CLL (45).

SKP2 in T-cell acute lymphoblastic leukemia

T-cell acute lymphoblastic leukemia malignancy is a subtype of leukemia arising from thymocytes. In fact, T-ALL constitutes around 12-15% of newly diagnosed cases of ALL in pediatric patients, notable for its distinctive clinical and biological characteristics (65). Based on the current modern combination therapy, long-term therapies are needed to be improved, especially with aged group patients. The molecular mechanism of different gene functions in T-ALL is complex, including the chromosomal translocation of *c-Myc, Hox 11, Tal1*, and *Lmo*, with the T-cell receptor locus (66, 67). High prevalence activation of mutated Notch signaling pathway emerged as an important genetic component for T-ALL pathogenesis. Interestingly, in T-ALL Notch, signaling pathways are found to regulate the *Skp2* expression and its protein target substrate p27. In T-ALL cells, the interaction of NOTCH 1 intracellular domain (ICD) with the *Skp2* promoter triggers *Skp2* expression levels and reduces p27Kip1 levels. The pharmacological agents blocking NOTCH signaling pathways reduce the expression of *SKP2*, and accumulate the p27Kip1, subsequently leading to G1 cell cycle arrest. Overall, NOTCH/SKP2/p27Kip1 axis might contribute to the pathogenesis of T-ALL (68).

SKP2 in Chronic Myelogenous Leukemia

Chronic Myelogenous Leukemia (CML) is the type of leukemia cancer subtype where dysregulation of myeloid cell growth in the bone marrow leads to the accumulation of undifferentiated white blood cells in the blood. CML is characterized by the translocation of *BCR/ABL1* genes- chromosome t(9, 22)(q34;q11. 2). Almost 95% of CML patients have BCR/ABL translocation in the chromosomes (69). This translocation elevates the transcription level of SKP2 expression. SKP2-mediated p27Kip1 dysregulation has been observed in many types of cancers, and proteasome inhibitor BTZ reduces the expression of *Skp2* in CML (70). On the other hand, the inverse relationship between SKP2 and p27Kip1 has been noticed after the gene silencing of *Skp2* in CML (69, 71).

SKP2 in multiple myeloma

Multiple myeloma (MM) malignancy develops due to uncontrolled plasma cell proliferation and relapses in most patients, which remains a challenge for modern chemotherapeutic treatments. Interestingly, Myristoylated alanine-rich C-kinase substrate (MARCKS) overexpression plays an essential role in drug resistance in MM. Activated MARCKS (p-MACKS) modulates the SKP2/p27-signaling axis. SKP2 mediates E2F1-induced cell proliferation and cell cycle progression through the reduction of p27Kip1. MARCKS activation by siRNA/drug (enzastaurin) reduces the MM resistance cell growth and induces apoptosis. The current study demonstrated that targeting MARCKS-mediated SKP2 will be a more helpful therapy against MM resistance. Furthermore, cyclin-dependent kinases regulatory subunit 1 (CKS1, encoded in humans by the *CKSB1* gene), cell cycle protein regulates p27Kip1, and p21CIP1 depends on *Skp2* expression. Similar to the above study, SKP2/p27Kip1, and CKSB1 were also found to be inversely correlated in MM cell lines (46).

SKP2 in primary effusion lymphoma

Primary effusion lymphoma (PEL) is a rare, aggressive, immune-compromised type B cell lymphoma. It is associated with human herpesvirus type-8 infection, which commonly occurs in malignant effusions of the body cavities. In PEL, the *LANA-2* gene (KSHV latent gene vIRF-3), binds to SKP2 and regulates c-MYC-dependent gene transcription by recruiting *c-Myc*onin, its promoter regulatory region (72). Since *c-MYC* is a proto-oncogene, it regulates cell proliferation and survival in cancers. High expression of vIRF-3 induces the c-MYC ubiquitylation, plays a critical role in c-MYC mediated transcription, and stabilizes the c-MYC protein, leading to c-MYC-induced KSHV combined lymphomagenesis. Numerous studies have found that targeting SKP2 by proteasome inhibitor (MEG1320) or knocking down *Skp2*, stabilizes the p27Kip1, thereby triggering the mitochondrial-induced cell death by the caspase-dependent pathway (73). Interestingly, a plant compound Apigenin, also down-regulates the SKP2, stabilizes the p27Kip1 expression, and induces apoptosis in PEL cells (74).

Diffuse large B-cell lymphoma (DLBCL)

Diffuse large B-cell lymphoma (DLBCL) is a sub-type of B-cell cancer. In adults, 30-40% of Non-Hodgkin's Lymphomas are DLBCL, thereby portraying DLBCL as the most common type of Non-Hodgkin Lymphona (75). One of the biggest challenges of DLBCL is that there is a relapse recorded in more than 50% of patients succeeding treatment with increased mortality (76). The cause of DLBCL resistance is still unclear. However, numerous studies demonstrate that dysregulation of oncogenic/tumor suppressor gene regulation and impairment of repair pathways contribute to developing DLBCL relapse. Interestingly, SKP2 is highly observed in DLBCL, which is significantly correlated with the worst clinical outcome compared to low *SKP2*-expressing patients. Further, high *Skp2* in patients displayed a poor prognosis and less survival. High *Skp2* correlated with Ki-67 but not with p27, demonstrating SKP2 as an independent prognostic marker of clinical outcome (77). Bortezomib (BTZ) treatment reduces SKP2 via escalation of p27Kip1protein, including XIAP, cIAP1, and survivin, implicating the SKP2/p27Kip1 signaling pathway in DLBCL pathogenesis (78). Unfortunately, in other studies, Rituximab via the CHOP-mediated pathway did not provide beneficial outcomes for DLBCL patients with high *Skp2* and low *p27* expression (79).

SKP2 in extranodal NK/T-cell lymphoma

Extranodal natural killer (NK)/T-cell lymphoma (ENKL) is a rare, aggressive type of malignancy in the lymph nodes besides GI tract, skin, and testis. ENKL shows poor survival among patients. The ENKT is often associated with Epstein–Barr virus (EBV) infection. The expression of *Skp2* levels is significantly increased, and an inverse correlation between SKP2 and p27Kip1 was observed with patients infected with EBV and phenotype of SKP +/p27– in ENKL (80). Overall these studies suggest that SKP2 plays a major role in the pathogenesis of ENKL carcinogenesis mediated through EBV (80, 81).

Role of SKP2 in myeloproliferative diseases (MPD)

BCR-ABL induces MPD via impaired cell cycle regulation by destabilization of p27, which inhibits cyclin-dependent kinases (CDK). In contrast, BCR-ABL inhibition induces *p27* and reduces *Skp2*, which leads to G1 arrest (33). A similar regulation pattern was also observed where leukemic cells were transformed by FLT3-ITD, JAK2V617F, and TEL-PDGFRβ, which suggests that SKP2/p27 passage may act as a common target for leukemogenic tyrosine kinases. The *in vivo* mice transplanted with BCR-ABL–infected

SKP2−/−marrow resulted in myeloproliferative syndrome with an increased survival rate compared with recipients of BCR-ABL-expressing SKP2-/- marrow (33). At the same time, in the SKP2-/−model, the nuclear *p27* expression is higher than SKP2-/- counterparts, demonstrating that leukemogenesis attenuation is regulated by high p27 levels in both MPD and CML (33, 82). The mutation of JAK2V617F commonly occurs in MPD, but in the case of its subset of polycythemia vera, homozygous JAK2V617F mutation is common. Therefore, mitotic recombination and duplication of the mutant allele are developed in MPD/CML. JAK2V617F mutation modulates the *Skp2* expression through STAT3/STAT5 transcription factors on the SKP2 promoter regulatory region (52). Therefore, inhibiting SCF-SKP2 for p27 stabilization recognition may be more beneficial for a therapeutic approach in MPD/CML and other hematological malignancies.

Role of SKP2 in hematopoietic stem cells

The hematopoiesis process is a crucial step in producing diverse blood cells. This process undergoes long-term HSCs (LT-HSCs) and short-term HSCs (ST-HSCs), compartments that are the primary sources of hematopoiesis. LT-HSCs self-renewal themselves in order to maintain HSC pool and differentiate into multipotent progenitors, and they can further differentiate into lymphoid progenitors and myeloid progenitors, which produce mature blood cells, whereas ST-HSCs have limited self-renewal ability to differentiate into multipotent progenitors (69, 82). However, the mechanism of HSCs quiescence is largely unknown, and re-entering the cell cycle by HSCs is very crucial. Interestingly, SKP2 is involved in regulating HSC quiescence, pool size, self-renewal capability, etc. (83). In HSCs, the SKP2 deletion stabilizes the CKIs p21Cip1, p27Kip2, P57Kip2, and p130, increasing proliferation and reducing the stem cell self-renewal capability (83). SKP2 targets SKI inhibitors that inhibit cell cycle progression from G1 to S phase (68, 84). Due to myelosuppression and post-transplantation occurrences, high expression of *Skp2* is associated with neoplastic transformation, including HSC and its progenitors. High expression of *Skp2* sufficiently provides hematopoietic stress.

On the other hand, depletion of SKP2 reduces HSC mitotic activity and enhances HSC quiescence, increasing pool size and maintenance (83). The depleted SKP2 results in HSC impairment during myeloablative stress because of their inability to enter the cell cycle, thereby protecting HSC regeneration. SKP2 negatively regulates cyclin D1, which might be responsible for SKP2 maintenance of HSC quiescence, pool size, and self-renewal capability (9, 85, 86). SKP2 acts as a critical regulator for HSC quiescence and self-renewal capability and gives a novel paradigm for HSCs. SKP2 maintains the HSC homing and residence in the endosteal niche. SKP2 deficiency reduces the expression of b-catenin and its target genes. Since SKP2 maintains homing of HSC succeeding the post-transplantation, SKP2 might be helpful as a predictive marker for monitoring transplantation efficiency (87). Depleted *Skp2* expression enhances the sensitivity of HSCs and CMLs to chemotherapeutic drugs and triggers the long-term HSC reconstitution ability (9). Therefore, targeting SKP2 increases BM transplantation efficiency and sensitizes the cancer cell or CSC against chemotherapy. These model studies clearly demonstrate that future SKP2 targeting-based therapy will be an efficient approach against different cancer types, including HMs.

Relationship between microRNAs and *Skp2* gene

MicroRNAs are small non-coding RNAs (10-24nts), and it regulate gene expression at the posttranscriptional level and play a critical role in cancer development (88). Mounting evidence displays the significant roles between miRNAs and *Skp2* gene expression. MicroRNA-186 regulates *Skp2* expression in pituitary tumors, induces p27Kip1-mediated cell cycle deregulation, and modulates cell proliferation. Similarly, human esophageal squamous carcinoma reduces cell proliferation and induces apoptosis (89, 90). In ovarian cancer, the expression of miR-30a-5p is low, but overexpression of miR-30a-5p reduces migration, invasion, and metastasis by posttranscriptional down-regulating *SKP2* gene expression (91). Since the miR-34a is downregulated in prostate cancer, overexpressing miR-34a downregulates RhoA and suppresses the c-Myc-SKP2 -Miz1 transcriptional assembly complex c-Myc-pTEFB complex that elongates transcription of numerous genes and affects the cellular function (92). Nevertheless, the reason for *Skp2* down-regulation through mir-34 has not been completely investigated yet in human renal carcinoma cells and prostate cancer (92). SKP2 mRNA is predicted to be a target of mir-7, but unfortunately, overexpressing miR-7 only reduces the SKP2 protein level but not at the transcriptional level. The SKP2-miR-7 mediated G1/S phase transition increases p27kip1 and reduces all G1 cell cycle indicators, such as Cks1, Cdk1/2, and CyclinD1/3, which suggests that overexpression of miR-7 arrests the CHO cell growth at G1 phase during cell undergoes stress (93, 94). miR-340 targets SKP2, inhibits non-small cell lung cancer tumor cell proliferation and induces apoptosis by targeting multiple negative regulators of p27 (95). Overexpressing miR-21-5p, miR-26-5p, and miR-30-5p in MCF-7 and tamoxifen-resistant MCF-7 cell lines showed marked reduction of SKP2 mRNA expression level (96). miR-203 targets SKP2 and regulates cell cycle and self-renewal in the hematopoietic stem cells and leukemia cells (97). Tumor suppressor miR-340 represses, the *Skp2* expression, inhibits tumor cell proliferation, migration, and invasion, and induces apoptosis in hepatocellular carcinoma (95). miR-26, miR-182, miR-340, and miR-506 share the 3'UTR of both SKP2 and PCNX and suppress their expression in non-small cell lung cancer (NSCLC) (98). Ectopic expression of miR-21 down-regulates the SKP2 in ovarian cancer cells (60). Apart from miRNAs, the long noncoding RNA meg3 and miR-3163 also coordinately repress the *Skp2* expression at the translation level and inhibit NSCLC cell growth, reducing NSCLC cell growth (99). miR-138 mimics or EZH2 inhibitor combined with a proteasome

inhibitor, bortezomib-cavalcade, significantly reduces the MM tumors in a xenograft model by targeting RBPMS (100). To identify the SKP2 targeting miRNAs, we predicted through "TargetScan", "MicroT-CDS" and "miRDB": databases, where we found the seven most common miRNAs:` hsa-miR-21-5p, hsa-miR-590-5p, hsa-miR-26a-5p, hsa-miR-1297, hsa-miR-26b-5p, hsa-miR-30d-5p, hsa-miR-30a-5p (data not shown).

Possible role of SKP2 on drug resistance in hematological malignancies–HM

In HM, the patients undergoing chemotherapy don't respond to drugs. The molecular mechanism behind cancer/tumor cell's resistance to chemotherapy is elusive. Surprisingly, overexpression of *Skp2* is associated with resistance and sensitization after pre-operative doxorubicin-based chemotherapeutically could aid in cancer cell death and successful chemotherapy in primary breast cancer patients (101, 102). SKP2 positively regulates the MAD2 via the p27-CDKs-E2F1 signaling pathway (103). Inhibition of SKP2 sensitizes paclitaxel-treated A549 and NCI-H1299 cells (103). SKP2 knockdown and/or inhibition sensitized the paclitaxel resistance prostate cancer cells, suggesting that SKP2 inhibitors might be the potential drugs against SKP2 upregulated cancers. Based on the SKP2 status in CML, USP10 inhibition significantly reduced the imatinib-sensitive and imatinib-resistant CML cell proliferation (71). Compound A (CpdA) interferes with SCF(SKP2) ligase by preventing the incorporation of SKP2 and induces G (1)/S cell-cycle arrest, SCF(SKP2)- and p27-dependent apoptosis, subsequently inducing p21 accumulation and other SCF(SKP2) substrates without affecting heat-shock protein response in MM (104). These studies indicate that SCF-SKP2 targeting agents may probably overcome the multidrug resistance mechanism and chemo-sensitize the MM cells (104). Furthermore, in breast cancer, SKP2 reactivates AKT-mediated resistance to PI3K inhibitors. Depletion of SKP2 reduces tumor growth in xenograft mice models (54). This study demonstrated that SKP2 plays a significant role in tumor progression and drug resistance. In lung cancer, small molecular inhibitors downregulated SKP2 and sensitized the lung cancer cells to paclitaxel. SKP2 has also been noted in stabilizing Mcl-1, conferring radioresistance in colorectal cancers (105).

In numerous malignancies, high SKP2 prevents apoptosis in a p53-dependent manner and promotes tumor progression and drug resistance (106). Combining SKP2 inhibitor C25 with bromocriptine sensitized the prolactinoma cells and induced apoptosis (107, 108). In multiple myeloma combinations of DT204, BTZ prevailed over drug resistance and induced apoptosis in proteasome inhibitors resistance cells. Both *in vitro* and *in vivo* model results strongly suggest that a combination of novel drug SCF-SKP2 inhibitor (DT204) and BTZ triggered synergistic anti-myeloma activity in the xenograft myeloma mouse model. Thus, targeting SCF-SKP2 by an SKP2 inhibitor combined with BTZ is a novel strategy to overcome drug resistance in MM. In addition, SKP2 inhibitor DT204 enhances the efficacy of BTZ-based therapies in multiple myeloma patients who are already BTZ-resistant (109). Proteosomal degradation of SKP2 also facilitates suppression of breast cancer growth by inducing autophagic cell death via F-box protein FBX041 (110). We previously showed that inhibition of pMARCKS potentiates BTZ-induced upregulation of p27 and p21 and downregulation of SKP2 (46). From a therapeutic perspective, it is noteworthy that targeting MARCKS can induce cell-cycle arrest and enhance apoptosis via E2F-1/SKP2/P27 axis in resistant MM cells (94). Therefore, identifying the molecular mechanism of drug resistance in HM is essential in the future. SCF-SKP2 inhibitors are the most widely used drugs to target the Ub++ proteasome system more precisely than PIs pharmacologically.

Role of epigenetic modifiers in drug resistance

SKP2 is a key regulatory protein involved in controlling cell cycle progression and the degradation of specific target proteins (111). It plays a crucial role in maintaining normal cell growth and proliferation (111). However, dysregulation of SKP2 has been implicated in various cancers, including leukemia, and is also associated with drug resistance (109). Overexpression of *Skp2* in leukemia cells can contribute to drug resistance through several mechanisms (112). SKP2-mediated degradation of pro-apoptotic proteins may decrease the ability of cells to undergo apoptosis in response to chemotherapy (112). Enhanced cell cycle progression driven by SKP2 can lead to faster tumor cell growth, making it more challenging for drugs to keep pace with cell division (111, 113). SKP2 may influence DNA repair mechanisms, potentially reducing the effectiveness of DNA-damaging chemotherapeutic agents (114, 115). It is a critical player in regulating cell cycle progression and protein degradation, and its activity is intricately linked to epigenetic processes involving heritable changes in gene expression and chromatin structure without alterations in the DNA sequence (116). SKP2 can influence epigenetic regulation in multiple ways.

Regulation of epigenetic modifiers

SKP2 can target specific proteins for ubiquitin-mediated degradation. Some of these target proteins include epigenetic modifiers such as histone deacetylases (HDACs) and histone methyltransferases (84, 117). By controlling the levels of these epigenetic modifiers, SKP2 can indirectly impact the acetylation and methylation status of histones, leading to changes in chromatin structure and gene expression (118). SKP2-mediated degradation of certain epigenetic regulators can affect chromatin remodeling complexes. Alterations in chromatin structure can lead to changes in gene accessibility, potentially impacting gene expression patterns. SKP2 can interact with various transcription factors and co-factors involved in epigenetic regulation (22, 119). These interactions can modulate the activity of transcription factors, influencing their

ability to bind to specific genomic regions and regulate gene expression (Figure 5).

Epigenetic effects on *Skp2* expression

Conversely, epigenetic modifications, such as DNA methylation and histone modifications, can also regulate the expression of *Skp2*. Aberrant epigenetic changes may result in dysregulated *Skp2* expression, contributing to altered cell cycle control and tumorigenesis. Epigenetic modifications can directly impact the expression of genes that are targets of SKP2-mediated degradation. Altered epigenetic regulation of these genes may influence their susceptibility to SKP2-dependent degradation (22). Dysregulation of *Skp2* and its interaction with epigenetic processes are associated with various diseases, including cancer (1). Aberrant *Skp2* expression and epigenetic alterations can contribute to tumorigenesis, metastasis, and drug resistance (1, 120, 121). Understanding the interplay between SKP2 and epigenetics is critical for unraveling the complexities of cancer biology and other diseases. Targeting SKP2 and its associated epigenetic processes may hold promise for developing novel therapeutic strategies, especially in the context of cancers where *Skp2* is dysregulated and contributes to disease progression. Additionally, research in this field continues to uncover the intricate mechanisms through which SKP2 and epigenetics intersect, providing insights into potential therapeutic targets and diagnostic markers. The regulation of SKP2 by epigenetic mechanisms plays a significant role in controlling its expression levels and activity. In the case of SKP2, several epigenetic mechanisms can in turn influence its expression.

The *Skp2* gene promoter is reported to be hypermethylated in some cancer types, decreasing *SKP2* expression (122). Reduced *Skp2* expression due to DNA methylation can contribute to cell cycle dysregulation and impact cancer progression (122). There is also a cancer-grade specific methylation. Results from TCGA depict that leukemia showed a marginally increased trend in *Skp2* expression compared to leukemia and myeloma. Survival analysis also revealed a poor DFS (disease-free survival) with high *Skp2* expression, as also seen in leukemia vs lymphoma (63, 123).

Histone modifications, including acetylation and methylation of histone proteins, can influence chromatin structure and gene accessibility. While histone H3 lysine 4 (H3K4) methylation is linked to gene activation, H3K9 and H3K27 methylation are associated with gene repression. Epigenetic changes in histone modifications near the *Skp2* gene are reported to modulate its transcriptional activity (124). In addition to histone modifications, specific miRNAs can target and degrade SKP2 mRNA or inhibit its translation, reducing SKP2 protein levels. Changes in miRNA expression profiles in cancer or other diseases can influence *Skp2* expression through post-transcriptional regulation as discussed before (125, 126). lncRNAs have been identified as regulators of *Skp2* expression, either by promoting its transcription or by destabilizing SKP2 mRNA (127).

Epigenetic changes can also influence the recruitment and activity of chromatin remodeling complexes that alter chromatin

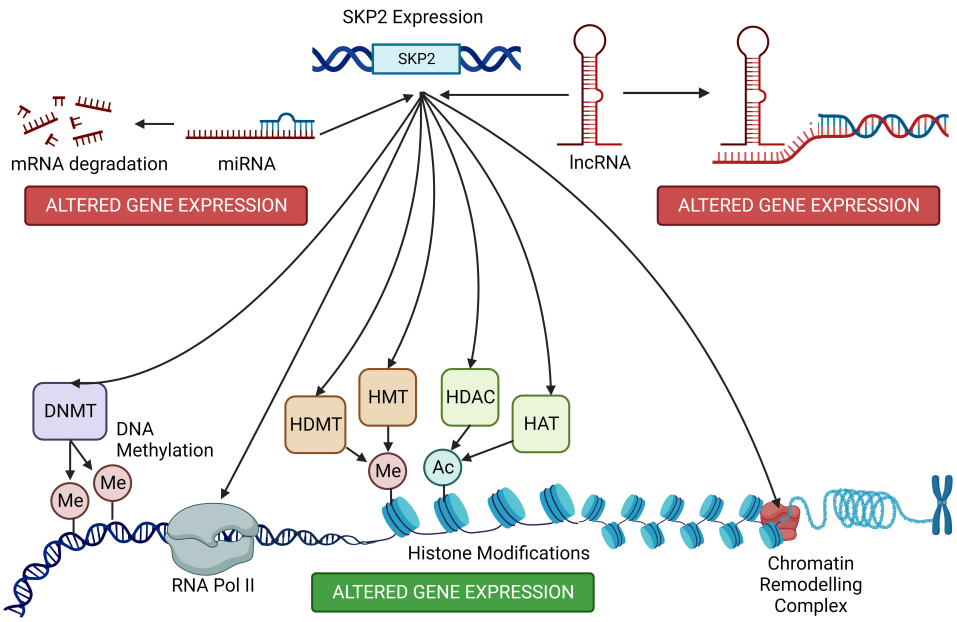

FIGURE 5
Host cell epigenetic modifications by SKP2. SKP2, beyond its canonical role in cell cycle regulation, influences host cell epigenetics through diverse mechanisms. Elevated SKP2 levels correlate with altered DNA methylation patterns and histone modifications, impacting gene expression. SKP2 promotes the degradation of key epigenetic regulators, disrupting the balance between chromatin modifications and transcriptional control. These modifications contribute to the development and progression of various diseases, including hematological malignancies. On the other hand, the expression of SKP2 is fine-tuned by a complex interplay of different miRNA and lnCNRA leading to its suppression or overexpression in different cancer types.

structure and gene accessibility. These complexes can either promote or inhibit the transcription of the *Skp2* gene by modulating the chromatin landscape around its promoter region. Epigenetic regulation of *Skp2* is particularly relevant in cancer, where dysregulated *Skp2* expression can contribute to uncontrolled cell proliferation and tumorigenesis (112). Understanding the epigenetic modifications that affect SKP2 and their functional consequences is essential for developing targeted therapies that can restore normal SKP2 regulation in cancer cells. Additionally, research in this area continues to uncover the intricate details of SKP2 epigenetic regulation and its implications in various diseases. SKP2 can also promote immune evasion in cancer by regulating immune checkpoint molecules, immune response pathways, Treg function, antigen presentation, and the overall immune microenvironment (127). Understanding the role of SKP2 in immune evasion is crucial for developing strategies to enhance immune responses against cancer cells and improve the efficacy of immunotherapies (1). Targeting SKP2 or its downstream signaling pathways may represent a potential approach to mitigate immune evasion and enhance the immune system's ability to recognize and eliminate cancer cells.

Future prospective of SKP2 inhibitors in HMs

Based on several reports, downregulation of *Skp2* induces the p27, promotes apoptosis, and sensitizes different types of cancers. However, further research is necessary to combat challenging tasks to identify the compound/inhibitors that are selectively employed for targeting the protein-protein interaction that holds the E3ligase together. Recently, the following inhibitors were developed against SKP2, named Bortezomib [FDA approved], Prodigiosin, Arsenic trioxide, Apigenin, curcumin, NSC689857, NSC681152, C1, C2, C16, C20, Compound A, Compound ZL25, and preclinical research compounds (Figure 6; Table 3) (8, 37, 74, 94, 109, 137, 162, 163).

BTZ, or pharmacological commercial name PS-341,/Valcade specifically, reversibly inhibits the 26S proteasome, an enzyme complex for regulating protein degradation under a controlled fashion. BTZ comprises of a peptide-like backbone and a boronate group, of which the latter exhibits a stronger binding affinity to the active site threonine, resulting in increased potency and selectivity toward the proteasome (164). In cancers, proteasomes' inhibition leads to the building up of the protein substrates required for the cell cycle and apoptosis. Interestingly, BTZ suppresses the expression of *Skp2* and increases the *p27Kip1* expression in many types of cancers, and it has also been proven to significantly improve xenograft cancer cells in mice models. Furthermore, when BTZ combines with cisplatin, it suppresses cell proliferation and induces apoptosis by declining SKP2 and aiding in the accumulation of *p27* expression. Recent studies denote combining a novel SKP2 inhibitor DT204 and BTZ synergistically induced anti-myeloma activity and sensitized drug resistance in MM. The antiproliferative effect of BTZ in CML implies that proteasomal inhibitors are highly potent, thus suggesting that it might be beneficial for a strategic intervention for CML (109).

Considering natural products, Apigenin (4′, 5, 7-trihydroxyflavone) is a natural plant product commonly found in dietary flavonoids found in various fruit and vegetables. Hussain et al. demonstrated that Apigenin triggers apoptosis in Primary effusion lymphoma (PEL) cells, suppressing the activation of AKT/PKB pathway via downregulating *Skp2*, hypo-phosphorylation of Rb, and accumulating *p27Kip1* expression levels, which suggest that Apigenin may possibly have future therapeutic potential in PEL (74). Curcumin induces cell death by inhibiting PI3-Kinase/AKT Pathway in B-Precursor Acute Lymphoblastic Leukemia.

In addition, the pursuit of novel anti-HM therapeutic strategies does not just restrict to SKP2 inhibition, but also remains through

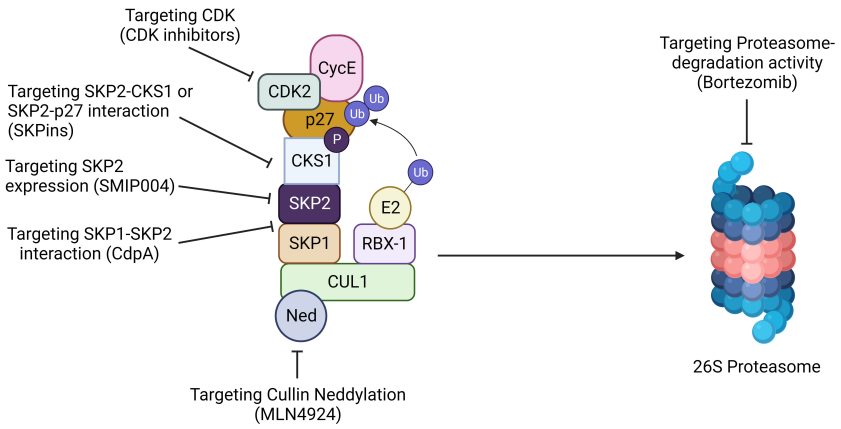

FIGURE 6
Targeting SKP2 for cancer therapy. Targeting SKP2 is emerging as a promising strategy for hematological malignancy. Inhibition of Cullin neddylation with MLN4924 disrupts SCF complex activity, leading to SKP2 degradation. CdpA hinders the SKP1-SKP2 interaction, while SMIP004 directly suppresses SKP2 expression. Compounds like SKPins disrupt SKP2-CKS1 or SKP2-p27 interactions, impeding cell cycle progression. Furthermore, targeting CDK inhibitors or using proteasome inhibitors like bortezomib prevents SKP2-mediated degradation of key proteins. These multifaceted approaches highlight the potential of SKP2 as a therapeutic target, offering diverse strategies to intervene in cancer progression and enhance treatment outcome.

TABLE 3 SKP2 inhibitors in hematological malignancies and solid tumors.

Tumor Type	Compound	Reference
Hematological Malignancies		
T cell Leukemia	SZL-P1-41	(128)
	SKPin C1	(128)
Myeloid Leukemia	Linichlorin A	(129)
	Diosmetin	(130)
	All-trans retinoic acid	(1)
Chronic Lymphocytic Leukemia	Bortezomib	(131)
Melanoma	Linichlorin A	(132)
	SKPin C1	(133)
	Betulinic Acid	(132)
	miR-590-5p	(134)
	Bortezomib	(135)
Multiple Myeloma	Neosetophomone B	(136)
	CdpA	(8)
	SKPin C1	(137)
	Bortezomib	(138)
Solid Malignancies		
Prostate Carcinoma	SZL-P1-41	(139)
	Gartanin	(140)
	Safranal	(141)
	SMIP004	(142)
	Flavokawain A	(143)
Lung Carcinoma	SZL-P1-41	(139)
	SKPin C1	(144)
	MLN4924	(144)
	Flavokawain A	(144)
	SMIP004	(103)
	Curcumin	(145)
	Tubocapsanolide A	(146)
Hepatocellular Carcinoma	Longikaurin A	(147)
	SZL-P1-41	(139)
Breast Carcinoma	Flavokawain A	(148)
	Linichlorin A	(149)
	Gentian Violet	(149)
	Diosgenin	(150)
	Rottlerin	(151)
	Curcumin	(24)
	Lycopene	(55)
	Quercetin	(55)
Osteosarcoma	SZL-P1-41	(139)
	Flavokawain A	(152)
Cervical Cancer	Linichlorin A	(149)
	Gentian Violet	(149)
Bladder Carcinoma	Flavokawain A	(143)
	ABT-751	(153)
Glioblastoma	Curcumin	(154)
Endometrial Carcinoma	SKP2E3Li C2	(155)
Colorectal Carcinoma	7-azaindoles	(156)
	Dioscin	(49)
	Sulforaphane	(157)
Pancreatic Carcinoma	Curcumin	(158)
	Rottlerin	(159)
Head and Neck Squamous Cell Carcinoma	Curcumin	(160)
Ovarian Carcinoma	Nitidine Chloride	(161)

(Continued)

selective inhibition of the ubiquitination–proteasome axis. To enhance specificity and selectivity in targeting, a promising avenue involves the focus on the E2–E3 complex (165, 166), given that the E2–E3 interaction imparts a high level of specificity and selectivity to the response by influencing specific ubiquitin bonds. Similarly, targeting the substrate binding domains of E3s offers a valuable opportunity. Inhibiting the interaction between a specific E3 and its target enhances specificity while minimizing off-target and side effects, potentially due to the limited impact on cellular events. As protein–protein interactions influence the specificity and selectivity of E3s, a deeper understanding of the three-dimensional structure of E3 enzymes through approaches like crystallography and cryo-electron microscopy will provide insights for developing novel inhibition strategies. Notably, Proteolysis Targeting Chimeras (PROTACs) (167) and molecular glues (168) represent effective approaches for promoting ubiquitination-mediated degradation of specific proteins, thereby contributing to increased substrate specificity.

Additionally, advancements in targeted drug delivery involve the use of cell membrane-coated nanoparticles (CNPs) and exosomes (169). CNPs, with their synthetic core containing anticancer drugs covered by a naturally derived cell membrane, offer a potential tool for precise delivery to disease sites. While promising, the translation of these strategies to clinical applications necessitates further technical improvements. Microenvironment-responsive drug-delivery systems based on nanoparticles and exosomes hold potential for guiding the release of SKP2/SCF component inhibitors in HM-specific microenvironments.

Moreover, the identification of synergistic combinations holds significant importance for advancing cancer treatment strategies. In the context of synthetic drug-target interaction, particularly those involving DNA damage-response genes and ubiquitination-mediated vulnerabilities, preclinical studies underscore the potential of combining these approaches with PARP inhibitors (170). CRISPR/Cas9- or RNAi- or shRNA-based whole genome screening, coupled with genomic and transcriptomic data analysis, contributes to predicting new synthetic lethal interactions for alternative anticancer therapeutic approaches. Recent discoveries, such as the identification of new substrates targeted by ubiquitination, including sugars alongside proteins, broaden the scope of this post-translational modification as a master regulator with potential implications for treating various pathologies, including HMs (171).

Discussion and concluding remarks

HMs account for a substantial number of newly diagnosed cases in most oncology settings. These malignancies are most common in the Western world. They occur due to several factors, such as abnormality of cytogenetic, epigenetic, gene mutations, and other environmental factors that influence the progression of HM (172, 173). In HM, blood cancer cells grow uncontrolled and function abnormally. Commonly, HM's are categorized into three major subtypes: such as Lymphoma, Leukemia, and Myeloma (174). Among these, AML is developed among adults. More than 80% of cases re-occurred in patients over the age of 60, and 20-30% of cases are children. However, the average survival rate among all HM types is small, especially among elderly patients due to drug-resistant drug resistance or relapse after advanced chemotherapy and transplantation treatments. The molecular mechanism of drug resistance or relapse is not entirely understood in HMs. Compared to proteasomal inhibitors BTZ, E3 ligase drugs specifically block the entire protein degradation with less toxicity.

The components of E3 ligases such as MDM2, FBW7, RBX2/ROC2, RBX1/ROC1, Cullins, and many others are referred to as oncogenes or tumor suppressors; similarly, essential proteins such as p53 and Notch are associated during cancer development. The inverse correlation between SKP2 and p27 cell cycle regulators in HM and solid cancer demonstrated the shared mechanism of neoplastic transformation (175). However, *Skp2* gene function has not been fully investigated in hematological malignancies. Hence, we analyzed in-silico RNA seq using available TCGA datasets. Our results (data not shown) demonstrated that the M7 AML within the French American classifications exhibited a high expression of *Skp2*, while there seems to be no significant difference in expression among different ethnic races. High *Skp2* gene promoter methylation among African American populations illustrates the complexity of epigenetic aberration (data not shown), and increased expression of *Skp2* across tumors demonstrates a common mechanism of SKP2 drug resistance. Poor survival rates among the African American population and FLT3 mutation suggested that common mutation patterns are linked with overexpression of the *Skp2* gene (figure not shown). Our heatmap analysis demonstrated both positively and negatively correlated genes with SKP2; identifying the relationship with complex gene network functions that may support drug resistance mechanisms in various aspects (Supplementary Table 2). In addition, our STRING analysis (data not shown) demonstrated protein networks of SKP2 interaction, which will prompt the identification of the associated factors involved in the drug resistance mechanisms. Escalated expression of *Skp2* in Basso Lymphoma results demonstrated strong evidence of a common mechanism involved in triggering the *Skp2* gene in HM and a crucial player in Hematopoietic stem cells (data not shown).

Similarly, miRNAs are also found to be a crucial player in drug resistance affecting various genes. Hence, we highlighted the *SKP2* gene function by projecting the essential role of miRNAs across various types of solid tumor malignancies. However in HM, only miR-203 was found to target SKP2 in Leukemia and hematopoietic stem cells.

Our miR target prediction and Venn diagram analysis demonstrated common miRNAs such as hsa-miR-21-5p, hsa-miR-590-5p, hsa-miR-26a-5p, hsa-miR-1297, hsa-miR-26b-5p, hsa-miR-30d-5p, hsa-miR-30a-5p *Skp2* gene (data not shown). Overexpression of mir-21-5p induces apoptosis and cell cycle arrest by down-regulating SKP2 and overcoming Bortezomib resistance in Multiple Myeloma. Similarly, some reports demonstrated that targeting the EZH2/miR-138 axis might be a potential therapeutic target against MM (100). Nevertheless, more evidence is required to validate *Skp2* gene regulation and its function in other HM, including AML (100). In the future, identifying the posttranscriptional and feedback regulatory loop mechanism of SKP2 will support the miRNA rational therapeutic approaches against relapse. Finally, we demonstrated the significance of SKP2 targeting drugs in HM, which strongly suggests a potential therapeutic strategy; however, miRNA mimics/miRNA inhibitors alongside natural products combination such as Apigenin, Dioscin, Arsenic trioxide, NSC689857, NSC681152, C1, C2 with, C16, C20, Compound A, and Compound ZL25 will open a new doorway for understanding the molecular mechanism of drug resistance or relapse in HM patients (49, 74, 160, 163, 176).

SKP2 remarkably promotes phosphorylation, ubiquitination, and degradation of PDCD4 (Programmed cell death protein 4), thereby facilitating cell proliferation and survival in breast cancer cells. SKP2 and PDCD4 displayed an inverse correlation in this cancer (177). Interestingly, its expression exhibits dynamic patterns, with some cases demonstrating overexpression in cancer samples compared to normal tissues, while others exhibit elevated levels in control samples relative to cancerous tissues. A high throughput screening identified SKP2 as a potentially novel cancer drug target (41), suggesting that pharmacologic SKP2 inactivation may limit tumor progression and overcome chemoresistance. However, in prostate cancer, SKP2 exhibits an opposite trend with high expression associated with a gain in mesenchymal and CSC-like phenotype compared with epithelial cells (178). This variability underscores the complexity of SKP2's role in cancer progression and highlights the need for personalized therapeutic approaches. Given its diverse implications, personalized therapies targeting SKP2 may offer a tailored strategy to address the specific expression patterns observed in individual patients, potentially enhancing treatment efficacy and minimizing adverse effects. As researchers unravel the intricacies of SKP2's involvement in

hematological malignancies, the exploration of targeted interventions holds promise for advancing precision medicine in cancer therapy.

Thus, we can conclude that SKP2 is critical in regulating multiple cellular functions related to cell growth, differentiation, and cell cycle. These alterations could perturb the delicate balance and contribute to different pathological states like cancer. But the in-depth and detailed exploration of these aspects of SKP2 biology will be not only helpful in understanding cancer but also in discovering a therapeutic target. It has been observed that SKP2 dysregulation is one of the fundamental driver events for oncogenesis. These observations show that SKP2 is an oncogenic modulator; hence, its expression status is vital in cancer prognosis and determining treatment response. Small molecule activators or inhibitors for SKP2 hold tremendous promise against various cancers. It was reported that an expression of *Skp2* confers drug resistance, and hence targeting SKP2 appears to be crucial for overcoming drug resistance in cancer chemotherapy. Therefore efforts have been made to develop novel inhibitors targeting SKP2 (43). However, more clinically relevant human tumor models, such as PDX and organoids and genetic mouse models should be applied to carefully evaluate the efficacy of Skp2 inhibitors. Further research is of utmost necessity to delineate the signaling pathway for SKP2 and identify its cellular target function to understand the molecular mechanism of drug resistance in different cancer types.

Author contributions

JW: Writing – original draft. RD: Data curation, Writing – review & editing. RG: Writing – review & editing. OS: Writing – review & editing. KP: Methodology, Writing – review & editing. AR: Methodology, Writing – review & editing. SR: Methodology, Writing – review & editing. MSA: Data curation, Funding acquisition, Writing – review & editing. MA: Data curation, Funding acquisition, Writing – review & editing. SK: Conceptualization, Project administration, Writing – original draft.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. SK thanks DBT (Department of Science and Technology), India for supporting the work. The authors thank the Deanship of Scientific Research at King Khalid University (KKU) for funding this research through the Research Group Program Under the Grant Number: R.G.P.2/591/44.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University (KKU) for funding this research through the Research Group Program Under the Grant Number:R.G.P.2/591/44.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1288501/full#supplementary-material

SUPPLEMENTARY TABLE 1
SKP2 expression in Lymphoma, Leukemia and Myeloma.

SUPPLEMENTARY TABLE 2
Positive and Negatively correlated genes with SKP2.

References

1. Cai Z, Moten A, Peng D, Hsu CC, Pan BS, Manne R, et al. The Skp2 pathway: A critical target for cancer therapy. *Semin Cancer Biol.* (2020) 67:16–33. doi: 10.1016/j.semcancer.2020.01.013

2. Hershko A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem.* (1998) 67:425–79. doi: 10.1146/annurev.biochem.67.1.425

3. Chandra Dantu S, Nathubhai Kachariya N, Kumar A. Molecular dynamics simulations elucidate the mode of protein recognition by Skp1 and the F-box domain in the SCF complex. *Proteins Struct Funct Bioinforma.* (2016) 84:159–71. doi: 10.1002/prot.24963

4. Thompson LL, Rutherford KA, Lepage CC, McManus KJ. The SCF complex is essential to maintain genome and chromosome stability. *Int J Mol Sci.* (2021) 22 (16):8544. doi: 10.3390/ijms22168544

5. Kulathu Y, Komander D. Atypical ubiquitylation-the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. *Nat Rev Mol Cell Biol.* (2012) 13:508–23. doi: 10.1038/nrm3394

6. Huang Z, Nie L, Xu M, Sun X-H. Notch-induced E2A degradation requires CHIP and Hsc70 as novel facilitators of ubiquitination. *Mol Cell Biol.* (2004) 24:8951–62. doi: 10.1128/mcb.24.20.8951-8962.2004

7. Ganoth D, Bornstein G, Ko TK, Larsen B, Tyers M, Pagano M, et al. The cell-cycle regulatory protein Cks1 is required for SCF (Skp2) - mediated ubiquitinylation of p27. *Nat Cell Biol.* (2001) 3:321–4. doi: 10.1038/35060126

8. Chen Q, Xie W, Kuhn DJ, Voorhees PM, Lopez-Girona A, Mendy D, et al. Targeting the p27 E3 ligase SCFSkp2 results in p27and *Skp2*-mediated cell-cycle arrest

and activation of autophagy. *Blood*. (2008) 111:4690–9. doi: 10.1182/blood-2007-09-112904

9. Tomiatti V, Istvánffy R, Pietschmann E, Kratzat S, Hoellein A, Quintanilla-Fend L, et al. Cks1 is a critical regulator of hematopoietic stem cell quiescence and cycling, operating upstream of Cdk inhibitors. *Oncogene*. (2015) 34:4347–57. doi: 10.1038/onc.2014.364

10. Yu ZK, Gervais JLM, Zhang H. Human CUL-1 associates with the SKP1/SKP2 complex and regulates p21CIP1/WAF1 and cyclin D proteins. *Proc Natl Acad Sci U.S.A*. (1998) 95:11324–9. doi: 10.1073/pnas.95.19.11324

11. Yeh KH, Kondo T, Zheng J, Tsvetkov LM, Blair J, Zhang H. The F-box protein SKP2 binds to the phosphorylated threonine 380 in cyclin E and regulates ubiquitin-dependent degradation of cyclin E. *Biochem Biophys Res Commun*. (2001) 281:884–90. doi: 10.1006/bbrc.2001.4442

12. Nakayama K, Nagahama H, Minamishima YA, Matsumoto M, Nakamichi I, Kitagawa K, et al. Targeted disruption of Skp2 results in accumulation of cyclin E and p27 (Kip1), polyploidy and centrosome overduplication. *EMBO J*. (2000) 19:2069–81. doi: 10.1093/emboj/19.9.2069

13. Jiang H, Chang FC, Ross AE, Lee J, Nakayama K, Nakayama K, et al. Ubiquitylation of RAG-2 by Skp2-SCF links destruction of the V(D)J recombinase to the cell cycle. *Mol Cell*. (2005) 18:699–709. doi: 10.1016/j.molcel.2005.05.011

14. Arbini AA, Greco M, Yao JL, Bourne P, Marra E, Hsieh JT, et al. Skp2 overexpression is associated with loss of BRCA2 protein in human prostate cancer. *Am J Pathol*. (2011) 178:2367–76. doi: 10.1016/j.ajpath.2011.01.050

15. Méndez J, Zou-Yang XH, Kim SY, Hidaka M, Tansey WP, Stillman B. Human origin recognition complex large subunit is degraded by ubiquitin-mediated proteolysis after initiation of DNA replication. *Mol Cell*. (2002) 9:481–91. doi: 10.1016/S1097-2765(02)00467-7

16. Kondo T, Kobayashi M, Tanaka J, Yokoyama A, Suzuki S, Kato N, et al. Rapid degradation of Cdt1 upon UV-induced DNA damage is mediated by SCFSkp2 complex. *J Biol Chem*. (2004) 279:27315–9. doi: 10.1074/jbc.M314023200

17. Lin YW, Yang JL. Cooperation of ERK and SCFSkp2 for MKP-1 destruction provides a positive feedback regulation of proliferating signaling. *J Biol Chem*. (2006) 281:915–26. doi: 10.1074/jbc.M508720200

18. Nie L, Wu H, Sun XH. Ubiquitination and degradation of Tal1/SCL are induced by Notch signaling and depend on Skp2 and CHIP. *J Biol Chem*. (2008) 283:684–92. doi: 10.1074/jbc.M704981200

19. Marti A, Wirbelauer C, Scheffner M, Krek W. Interaction between ubiquitin-protein ligase SCFSKP2 and E2F-1 underlies the regulation of E2F-1 degradation. *Nat Cell Biol*. (1999) 1:14–9. doi: 10.1038/8984

20. Liu Y, Hedvat CV, Mao S, Zhu X-H, Yao J, Nguyen H, et al. The ETS protein MEF is regulated by phosphorylation-dependent proteolysis *via* the protein-ubiquitin ligase SCF Skp2. *Mol Cell Biol*. (2006) 26:3114–23. doi: 10.1128/mcb.26.8.3114-3123.2006

21. Hiramatsu Y, Kitagawa K, Suzuki T, Uchida C, Hattori T, Kikuchi H, et al. Degradation of Tob1 mediated by SCFSkp2-dependent ubiquitination. *Cancer Res*. (2006) 66:8477–83. doi: 10.1158/0008-5472.CAN-06-1603

22. Von Der Lehr N, Johansson S, Wu S, Bahram F, Castell A, Cetinkaya C, et al. The F-box protein Skp2 participates in c-Myc proteosomal degradation and acts as a cofactor for c-Myc-regulated transcription. *Mol Cell*. (2003) 11:1189–200. doi: 10.1016/S1097-2765(03)00193-X

23. Charrasse S, Carena I, Brondani V, Klempnauer KH, Ferrari S. Degradation of B-Myb by ubiquitin-mediated proteolysis: Involvement of the CdC34-SCF(p45Skp2) pathway. *Oncogene*. (2000) 19:2986–95. doi: 10.1038/sj.onc.1203618

24. Jia T, Zhang L, Duan Y, Zhang M, Wang G, Zhang J, et al. The differential susceptibilities of MCF-7 and MDA-MB-231 cells to the cytotoxic effects of curcumin are associated with the PI3K/Akt-SKP2- Cip/Kips pathway. *Cancer Cell Int*. (2014) 14:1–14. doi: 10.1186/s12935-014-0126-4

25. Tedesco D, Lukas J, Reed SI. The pRb-related protein p130 is regulated by phosphorylation-dependent proteolysis *via* the protein-ubiquitin ligase SCFSkp2. *Genes Dev*. (2002) 16:2946–57. doi: 10.1101/gad.1011202

26. Liu H, Cheng EHY, Hsieh JJD. Bimodal degradation of MLL by SCFSkp2 and APCCdc20 assures cell cycle execution: A critical regulatory circuit lost in leukemogenic MLL fusions. *Genes Dev*. (2007) 21:2385–98. doi: 10.1101/gad.1574507

27. Tokarz S, Berset C, La Rue J, Friedman K, Nakayama KI, Nakayama K, et al. The ISG15 isopeptidase UBP43 is regulated by proteolysis *via* the SCF Skp2 ubiquitin ligase. *J Biol Chem*. (2004) 279:46424–30. doi: 10.1074/jbc.M403189200

28. Vuillier F, Li Z, Commere PH, Dynesen LT, Pellegrini S. USP18 and ISG15 coordinately impact on SKP2 and cell cycle progression. *Sci Rep*. (2019) 9:1–11. doi: 10.1038/s41598-019-39343-7

29. Song MS, Song SJ, Kim SJ, Nakayama K, Nakayama KI, Lim DS. Skp2 regulates the antiproliferative function of the tumor suppressor RASSF1A *via* ubiquitin-mediated degradation at the G1-S transition. *Oncogene*. (2008) 27:3176–85. doi: 10.1038/sj.onc.1210971

30. Liang M, Liang Y, Wrighton K, Ungermannova D, Wang X, Brunicardi FC, et al. Ubiquitination and proteolysis of cancer-derived Smad4 mutants by SCFSkp2. *Mol Cell Biol*. (2004) 24:7524–37. doi: 10.1128/MCB.24.17.7524

31. Kiernan RE, Emiliani S, Nakayama K, Castro A, Labbé JC, Lorca T, et al. Interaction between cyclin T1 and SCF *SKP2* targets CDK9 for ubiquitination and degradation by the proteasome. *Mol Cell Biol*. (2001) 21:7956–70. doi: 10.1128/mcb.21.23.7956-7970.2001

32. Oh K-J, Kalinina A, Wang J, Nakayama K, Nakayama KI, Bagchi S. The papillomavirus E7 oncoprotein is ubiquitinated by UbcH7 and cullin 1- and Skp2-containing E3 ligase. *J Virol*. (2004) 78:5338–46. doi: 10.1128/jvi.78.10.5338-5346.2004

33. Agarwal A, Bumm TGP, Corbin AS, O'Hare T, Loriaux M, Van Dyke J, et al. Absence of SKP2 expression attenuates BCR-ABL-induced myeloproliferative disease. *Blood*. (2008) 112:1960–70. doi: 10.1182/blood-2007-09-113860

34. Katoh M, Igarashi M, Fukuda H, Nakagama H, Katoh M. Cancer genetics and genomics of human FOX family genes. *Cancer Lett*. (2013) 328:198–206. doi: 10.1016/j.canlet.2012.09.017

35. Schulman BA, Carrano AC, Jeffrey PD, Bowen Z, Kinnucan ERE, Finnin MS, et al. Insights into SCF ubiquitin ligases from the structure of the Skp1-Skp2 complex. *Nature*. (2000) 408:381–6. doi: 10.1038/35042620

36. Spratt DE, Wu K, Kovacev J, Pan ZQ, Shaw GS. Selective recruitment of an E2~ubiquitin complex by an E3 ubiquitin ligase. *J Biol Chem*. (2012) 287:17374–85. doi: 10.1074/jbc.M112.353748

37. Wu L, Grigoryan AV, Li Y, Hao B, Pagano M, Cardozo TJ. Specific small molecule inhibitors of skp2-mediated p27 degradation. *Chem Biol*. (2012) 19:1515–24. doi: 10.1016/j.chembiol.2012.09.015

38. Xie J, Jin Y, Wang G. The role of SCF ubiquitin-ligase complex at the beginning of life. *Reprod Biol Endocrinol*. (2019) 17:1–9. doi: 10.1186/s12958-019-0547-y

39. Yao Z, Seeliger M, Itzhaki L, Robinson C. Investigation of protein interations between Cks1 and Skp2 using hydrogen exchange nano-electrospray mass spectrometry. *Protein Sci*. (2004) 13:86–6.

40. Huang Y, Zhao Q, Zhou CX, Gu ZM, Li D, Xu HZ, et al. Antileukemic roles of human phospholipid scramblase 1 gene, evidence from inducible PLSCR1-expressing leukemic cells. *Oncogene*. (2006) 25:6618–27. doi: 10.1038/sj.onc.1209677

41. Wang Z, Gao D, Fukushima H, Inuzuka H, Liu P, Wan L, et al. Skp2: A novel potential therapeutic target for prostate cancer. *Biochim Biophys Acta - Rev Cancer*. (2012) 1825:11–7. doi: 10.1016/j.bbcan.2011.09.002

42. Kimura Y, Nagao A, Fujioka Y, Satou A, Taira T, Iguchi-Ariga SMM, et al. MM-1 facilitates degradation of c-Myc by recruiting proteasome and a novel ubiquitin E3 ligase. *Int J Oncol*. (2007) 31:829–36. doi: 10.3892/ijo.31.4.829

43. Lee Y, Lim H-S. Skp2 inhibitors: novel anticancer strategies. *Curr Med Chem*. (2016) 23:2363–79. doi: 10.2174/0929867323666160510122624

44. Yang G, Ayala G, De Marzo A, Tian W, Frolov A, Wheeler TM, et al. Elevated Skp2 protein expression in human prostate cancer: Association with loss of the cyclin-dependent kinase inhibitor p27 and PTEN and with reduced recurrence-free survival. *Clin Cancer Res*. (2002) 8:3419–26.

45. Caraballo JM, Acosta JC, Cortés MA, Albajar M, Teresa Gómez-Casares M, Batlle-López A, et al. High p27 protein levels in chronic lymphocytic leukemia are associated to low Myc and Skp2 expression, confer resistance to apoptosis and antagonize Myc effects on cell cycle. *Oncotarget*. (2014) 5:4694–708. doi: 10.18632/oncotarget.2100

46. Yang Y, Chen Y, Saha MN, Chen J, Evans K, Qiu L, et al. Targeting phospho-MARCKS overcomes drug-resistance and induces antitumor activity in preclinical models of multiple myeloma. *Leukemia*. (2015) 29:715–26. doi: 10.1038/leu.2014.255

47. Bretones G, Acosta JC, Caraballo JM, Ferrándiz N, Gómez-Casares MT, Albajar M, et al. SKP2 oncogene is a direct MYC target gene and MYC down-regulates p27 KIP1 through *Skp2* in human leukemia cells. *J Biol Chem*. (2011) 286:9815–25. doi: 10.1074/jbc.M110.165977

48. Grey W, Ivey A, Milne TA, Haferlach T, Grimwade D, Uhlmann F, et al. The Cks1/Cks2 axis fine-tunes Mll1 expression and is crucial for MLL-rearranged leukaemia cell viability. *Biochim Biophys Acta - Mol Cell Res*. (2018) 1865:105–16. doi: 10.1016/j.bbamcr.2017.09.009

49. Zhou L, Yu X, Li M, Gong G, Liu W, Li T, et al. Cdh1-mediated Skp2 degradation by dioscin reprogrammes aerobic glycolysis and inhibits colorectal cancer cells growth. *EBioMedicine*. (2020) 51:1–11. doi: 10.1016/j.ebiom.2019.11.031

50. Seo SB, Lee JJ, Yun HH, Im CN, Kim YS, Ko JH, et al. 14-3-3β Depletion drives a senescence program in glioblastoma cells through the ERK/SKP2/p27 pathway. *Mol Neurobiol*. (2018) 55:1259–70. doi: 10.1007/s12035-017-0407-8

51. Hulit J, Lee RJ, Li Z, Wang C, Katiyar S, Yang J, et al. p27Kip1 repression of ErbB2-induced mammary tumor growth in transgenic mice involves Skp2 and Wnt/β-catenin signaling. *Cancer Res*. (2006) 66:8529–41. doi: 10.1158/0008-5472.CAN-06-0149

52. Furuhata A, Kimura A, Shide K, Shimoda K, Murakami M, Ito H, et al. p27 deregulation by Skp2 overexpression induced by the JAK2V617 mutation. *Biochem Biophys Res Commun*. (2009) 383:411–6. doi: 10.1016/j.bbrc.2009.04.015

53. Shapira M, Kakiashvili E, Rosenberg T, Hershko DD. The mTOR inhibitor rapamycin down-regulates the expression of the ubiquitin ligase subunit Skp2 in breast cancer cells. *Breast Cancer Res*. (2006) 8:1–9. doi: 10.1186/bcr1533

54. Clement E, Inuzuka H, Nihira NT, Wei W, Toker A. Skp2-dependent reactivation of AKT drives resistance to PI3K inhibitors. *Sci Signal*. (2018) 11:521. doi: 10.1126/scisignal.aao3810

55. Huang H-C, Lin C-L, Lin J-K. 1,2,3,4,6-penta-O-galloyl-β-D-glucose, quercetin, curcumin and lycopene induce cell-cycle arrest in MDA-MB-231 and BT474 cells

through downregulation of Skp2 protein. *J Agric Food Chem*. (2011) 59:6765–75. doi: 10.1021/jf201096v

56. Ge L, Zhao G, Lan C, Song H, Qi D, Huang P, et al. MESP2 binds competitively to TCF4 to suppress gastric cancer progression by regulating the SKP2/p27 axis. *Cell Death Discovery*. (2023) 9:1–13. doi: 10.1038/s41420-023-01367-4

57. Gao D, Inuzuka H, Tseng A, Chin RY, Toker A, Wei W. Phosphorylation by Akt1 promotes cytoplasmic localization of Skp2 and impairs APC-Cdh1-mediated Skp2 destruction. *Nat Cell Biol*. (2009) 11:397–408. doi: 10.1038/ncb1847

58. Bornstein G, Ganoth D, Hershko A. Regulation of neddylation and deneddylation of cullin1 in SCF Skp2 ubiquitin ligase by F-box protein and substrate. *Proc Natl Acad Sci U S A*. (2006) 103:11515–20. doi: 10.1073/pnas.0603921103

59. Xiao J, Yin S, Li Y, Xie S, Nie D, Ma L, et al. SKP2 siRNA inhibits the degradation of P27kip1 and down-regulates the expression of MRP in HL-60/A cells. *Acta Biochim Biophys Sin (Shanghai)*. (2009) 41:699–708. doi: 10.1093/abbs/gmp058

60. Meyer SE, Muench DE, Rogers AM, Newkold TJ, Orr E, O'Brien E, et al. miR-196b target screen reveals mechanisms maintaining leukemia stemness with therapeutic potential. *J Exp Med*. (2018) 215:2115–36. doi: 10.1084/jem.20171312

61. Sampson C, Wang Q, Otkur W, Zhao H, Lu Y, Liu X, et al. The roles of E3 ubiquitin ligases in cancer progression and targeted therapy. *Clin Transl Med*. (2023) 13 (3):e1204. doi: 10.1002/ctm2.1204

62. Winteringham LN, Kobelke S, Williams JH, Ingley E, Klinken SP. Myeloid Leukemia Factor 1 inhibits erythropoietin-induced differentiation, cell cycle exit and p27Kip1 accumulation. *Oncogene*. (2004) 23:5105–9. doi: 10.1038/sj.onc.1207661

63. Min YH, Cheong JW, Lee MH, Kim JY, Lee ST, Hahn JS, et al. Elevated S-phase kinase-associated protein 2 protein expression in acute myelogenous leukemia: Its association with constitutive phosphorylation of phosphatase and tensin homologue protein and poor prognosis. *Clin Cancer Res*. (2004) 10:5123–30. doi: 10.1158/1078-0432.CCR-04-0136

64. Darwiche W, Gubler B, Marolleau JP, Ghamlouch H. Chronic lymphocytic leukemia B-cell normal cellular counterpart: Clues from a functional perspective. *Front Immunol*. (2018) 9:683. doi: 10.3389/fimmu.2018.00683

65. Raetz EA, Teachey DT. T-cell acute lymphoblastic leukemia. *Pediatr Hematol Malig*. (2016) 2016:580–8. doi: 10.1182/asheducation-2016.1.580

66. Curtis DJ, McCormack M. The molecular basis of Lmo2-induced T-cell acute lymphoblastic leukemia. *Clin Cancer Res*. (2010) 16:5618–23. doi: 10.1158/1078-0432.CCR-10-0440

67. Van Vlierberghe P, Ferrando A. The molecular basis of T cell acute lymphoblastic leukemia. *J Clin Invest*. (2012) 122:3398–406. doi: 10.1172/JCI61269

68. Dohda T, Maljukova A, Liu L, Heyman M, Grandér D, Brodin D, et al. Notch signaling induces SKP2 expression and promotes reduction of p27Kip1 in T-cell acute lymphoblastic leukemia cell lines. *Exp Cell Res*. (2007) 313:3141–52. doi: 10.1016/j.yexcr.2007.04.027

69. Chen JY, Wang MC, Hung WC. Bcr-Abl-induced tyrosine phosphorylation of Emi1 to stabilize Skp2 protein *via* inhibition of ubiquitination in chronic myeloid leukemia cells. *J Cell Physiol*. (2011) 226:407–13. doi: 10.1002/jcp.22346

70. Iskandarani A, Bhat AA, Siveen KS, Prabhu KS, Kuttikrishnan S, Khan MA, et al. Bortezomib-mediated downregulation of S-phase kinase protein-2 (SKP2) causes apoptotic cell death in chronic myelogenous leukemia cells. *J Transl Med*. (2016) 14:1–12. doi: 10.1186/s12967-016-0823-y

71. Andreu EJ, Lledó E, Poch E, Ivorra C, Albero MP, Martínez-Climent JA, et al. BCR-ABL induces the expression of Skp2 through the PI3K pathway to promote p27Kip1 degradation and proliferation of chronic myelogenous leukemia cells. *Cancer Res*. (2005) 65:3264–72. doi: 10.1158/0008-5472.CAN-04-1357

72. Baresova P, Pitha PM, Lubyova B. Kaposi sarcoma-associated herpesvirus vIRF-3 protein binds to F-box of Skp2 protein and acts as a regulator of c-Myc protein function and stability. *J Biol Chem*. (2012) 287:16199–208. doi: 10.1074/jbc.M111.335216

73. Hussain AR, Ahmed M, Ahmed SO, Al-Thari S, Khan AS, Razack S, et al. Proteasome inhibitor MG-132 mediated expression of p27Kip1 *via* S-phase kinase protein 2 degradation induces cell cycle coupled apoptosis in primary effusion lymphoma cells. *Leuk Lymphoma*. (2009) 50:1204–13. doi: 10.1080/10428190902951799

74. Hussain AR, Khan AS, Ahmed SO, Ahmed M, Platanias LC, Al-Kuraya KS, et al. Apigenin induces apoptosis *via* downregulation of S-phase kinase-associated protein 2-mediated induction of p27Kip1 in primary effusion lymphoma cells. *Cell Prolif*. (2010) 43:170–83. doi: 10.1111/j.1365-2184.2009.00662.x

75. Susanibar-Adaniya S, Barta S. 2021 Update on Diffuse large B cell lymphoma: A review of current data and potential applications on risk stratification and management. *Am J Hematol*. (2021) 96:617–29. doi: 10.1002/ajh.26151

76. Chen B, Zou C, Wu J. Diffuse large B-cell non-Hodgkin lymphoma involving the unilateral carotid space in an elderly man: A case report. *Mol Clin Oncol*. (2017) 6:115–8. doi: 10.3892/mco.2016.1089

77. Seki R, Okamura T, Koga H, Yakushiji K, Hashiguchi M, Yoshimoto K, et al. Prognostic significance of the F-box protein Skp2 expression in diffuse large B-cell lymphoma. *Am J Hematol*. (2003) 73:230–5. doi: 10.1002/ajh.10379

78. Uddin S, Hussain A, Ahmed M, Belgaumi A, Al-Dayel F, Ajarim D, et al. S-phase kinase protein 2 is an attractive therapeutic target in a subset of diffuse large B-cell lymphoma. *J Pathol*. (2008) 216:483–94. doi: 10.1002/path.2433

79. Abdou AG, Asaad NY, Abd El-Wahed MM, Samaka RM, Gad Allah MS. The prognostic value of Skp2 expression in Egyptian diffuse large B-cell lymphoma. *Appl Immunohistochem Mol Morphol*. (2012) 20:47–55. doi: 10.1097/PAI.0b013e318219a19f

80. Xiang-Lan M, Zu-Lan S, Dan H, Bi-Hong S, Ya-Qin P, Han-Liang L. Skp2/p27 expression profile is correlated with Epstein-Barr virus status in extranodal nasal-type natural killer cell lymphoma. *Transl Res*. (2008) 151:303–8. doi: 10.1016/j.trsl.2008.04.004

81. Guo HQ, Pu XX, Guo CC, Rao HL, Li HR, Lin TY. The role of Skp2 in extranodal NK/T-cell lymphoma. *Chin J Cancer*. (2010) 29:567–71. doi: 10.5732/cjc.009.10645

82. Chen JY, Wang MC, Hung WC. Transcriptional activation of Skp2 by BCR-ABL in K562 chronic myeloid leukemia cells. *Leuk Res*. (2009) 33:1520–4. doi: 10.1016/j.leukres.2009.03.007

83. Rodriguez S, Wang L, Mumaw C, Srour EF, Lo Celso C, Nakayama KI, et al. The SKP2 E3 ligase regulates basal homeostasis and stress-induced regeneration of HSCs. *Blood*. (2011) 117:6509–19. doi: 10.1182/blood-2010-11-321521

84. Borriello A, Naviglio S, Bencivenga D, Caldarelli I, Tramontano A, Speranza MC, et al. Histone Deacetylase Inhibitors Increase p27Kip1 by Affecting Its Ubiquitin-Dependent Degradation through Skp2 Downregulation. *Oxid Med Cell Longev*. (2016) 2016:2481865. doi: 10.1155/2016/2481865

85. Wang J, Han F, Wu J, Lee SW, Chan CH, Wu CY, et al. The role of Skp2 in hematopoietic stem cell quiescence, pool size, and self-renewal. *Blood*. (2011) 118:5429–38. doi: 10.1182/blood-2010-10-312785

86. Curiel-Lewandrowski C, Yamasaki H, Si CP, Jin X, Zhang Y, Richmond J, et al. Loss of nuclear pro-IL-16 facilitates cell cycle progression in human cutaneous T cell lymphoma. *J Clin Invest*. (2011) 121:4838–49. doi: 10.1172/JCI41769

87. Wang J, Han F, Lee S-W, Wu J, Chan C-H, Zhang X, et al. E3-ligase Skp2 regulates β-catenin expression and maintains hematopoietic stem cell homing. *Biochem Biophys Res Commun*. (2014) 445:566–671. doi: 10.1016/j.bbrc.2014.02.042

88. Mudduluru G, George-William JN, Muppala S, Asangani IA, Kumarswamy R, Nelson LD, et al. Curcumin regulates miR-21 expression and inhibits invasion and metastasis in colorectal cancer. *Biosci Rep*. (2011) 31:185–97. doi: 10.1042/BSR20100065

89. He W, Feng J, Zhang Y, Wang Y, Zang W, Zhao G. MicroRNA-186 inhibits cell proliferation and induces apoptosis in human esophageal squamous cell carcinoma by targeting SKP2. *Lab Investig*. (2016) 96:317–24. doi: 10.1038/labinvest.2015.134

90. He Z, Chen L, Wang Q, Yin C, Hu J, Hu X, et al. MicroRNA-186 targets SKP2 to induce p27Kip1-mediated pituitary tumor cell cycle deregulation and modulate cell proliferation. *Korean J Physiol Pharmacol*. (2019) 23:171–9. doi: 10.4196/kjpp.2019.23.3.171

91. Qi F, He T, Jia L, Song N, Guo L, Ma X, et al. The miR-30 family inhibits pulmonary vascular hyperpermeability in the premetastatic phase by direct targeting of Skp2. *Clin Cancer Res*. (2015) 21:3071–80. doi: 10.1158/1078-0432.CCR-14-2785

92. Yamamura S, Saini S, Majid S, Hirata H, Ueno K, Deng G, et al. Microrna-34a modulates c-Myc transcriptional complexes to suppress Malignancy in human prostate cancer cells. *PLoS One*. (2012) 7:1–12. doi: 10.1371/journal.pone.0029722

93. Sanchez N, Gallagher M, Lao N, Gallagher C, Clarke C, Doolan P, et al. MiR-7 triggers cell cycle arrest at the G1/S transition by targeting multiple genes including skp2 and Psme3. *PLoS One*. (2013) 8:1–10. doi: 10.1371/journal.pone.0065671

94. Feng S, Wang Y, Zhang R, Yang G, Liang Z, Wang Z, et al. Curcumin exerts its antitumor activity through regulation of miR-7/Skp2/p21 in nasopharyngeal carcinoma cells. *Onco Targets Ther*. (2017) 10:2377–88. doi: 10.2147/OTT.S130055

95. Fernandez S, Risolino M, Mandia N, Talotta F, Soini Y, Incoronato M, et al. MiR-340 inhibits tumor cell proliferation and induces apoptosis by targeting multiple negative regulators of p27 in non-small cell lung cancer. *Oncogene*. (2015) 34:3240–50. doi: 10.1038/onc.2014.267

96. Lin YS, Lin YY, Yang YH, Lin CL, Kuan FC, Lu CN, et al. Antrodia cinnamomea extract inhibits the proliferation of tamoxifen-resistant breast cancer cells through apoptosis and skp2/microRNAs pathway. *BMC Complement Altern Med*. (2018) 18:1–11. doi: 10.1186/s12906-018-2204-y

97. Jackson SJ, Zhang Z, Feng D, Flagg M, O'Loughlin E, Wang D, et al. Rapid and widespread suppression of self-renewal by microRNA-203 during epidermal differentiation. *Dev*. (2013) 140:1882–91. doi: 10.1242/dev.089649

98. Li J, Tian H, Pan J, Jiang N, Yang J, Zhou C, et al. Pecanex functions as a competitive endogenous RNA of S-phase kinase associated protein 2 in lung cancer. *Cancer Lett*. (2017) 406:36–46. doi: 10.1016/j.canlet.2017.07.030

99. Su L, Han D, Wu J, Huo X. Skp2 regulates non-small cell lung cancer cell growth by Meg3 and miR-3163. *Tumor Biol*. (2016) 37:3925–31. doi: 10.1007/s13277-015-4151-2

100. Rastgoo N, Pourabdollah M, Abdi J, Reece D, Chang H. Dysregulation of EZH2/miR-138 axis contributes to drug resistance in multiple myeloma by downregulating RBPMS. *Leukemia*. (2018) 32:2471–82. doi: 10.1038/s41375-018-0140-y

101. Bar-On O, Shapira M, Hershko DD. Differential effects of doxorubicin treatment on cell cycle arrest and Skp2 expression in breast cancer cells. *Anticancer Drugs*. (2007) 18:1113–21. doi: 10.1097/CAD.0b013e3282ef4571

102. Davidovich S, Ben-Izhak O, Shapira M, Futerman B, Hershko DD. Over-expression of Skp2 is associated with resistance to preoperative doxorubicin-based chemotherapy in primary breast cancer. *Breast Cancer Res*. (2008) 10:1–10. doi: 10.1186/bcr2122

103. Huang T, Yang L, Wang G, Ding G, Peng B, Wen Y, et al. Inhibition of Skp2 sensitizes lung cancer cells to paclitaxel. *Onco Targets Ther*. (2017) 10:439–46. doi: 10.2147/OTT.S125789

104. Shi L, Wang S, Zangari M, Xu H, Cao TM, Xu C, et al. Over-expression of CKS1B activates both MEK/ERK and JAK/ STAT3 signaling pathways and promotes myeloma cell drugresistance. *Oncotarget*. (2010) 1:22–33. doi: 10.18632/oncotarget.105

105. Yu X, Zhou L, Liu W, Liu L, Gao F, Li W, et al. Skp2 stabilizes Mcl-1 and confers radioresistance in colorectal cancer. *Cell Death Dis*. (2022) 13:1–13. doi: 10.1038/s41419-022-04685-0

106. Kitagawa M, Lee SH, McCormick F. Skp2 Suppresses p53-Dependent Apoptosis by Inhibiting p300. *Mol Cell*. (2008) 29:217–31. doi: 10.1016/j.molcel.2007.11.036

107. Huang J, Zhang F, Jiang L, Hu G, Sun W, Zhang C, et al. Inhibition of SKP2 sensitizes bromocriptine-induced apoptosis in human prolactinoma cells. *Cancer Res Treat*. (2017) 49:358–73. doi: 10.4143/crt.2016.017

108. Lin Z, Fillmore GC, Um TH, Elenitoba-Johnson KSJ, Lim MS. Comparative microarray analysis of gene expression during activation of human peripheral blood T cells and leukemic Jurkat T cells. *Lab Investig*. (2003) 83:765–76. doi: 10.1097/01.LAB.0000073130.58435.E5

109. Malek E, Abdel-Malek MAY, Jagannathan S, Vad N, Karns R, Jegga AG, et al. Pharmacogenomics and chemical library screens reveal a novel SCF SKP2 inhibitor that overcomes Bortezomib resistance in multiple myeloma. *Leukemia*. (2017) 31:645–53. doi: 10.1038/leu.2016.258

110. Agrawal Y, Sharma T, Islam S, Nadkarni KS, Santra MK. F-box protein FBXO41 suppresses breast cancer growth by inducing autophagic cell death through facilitating proteasomal degradation of oncogene SKP2. *Int J Biochem Cell Biol*. (2022) 147:106228. doi: 10.1016/j.biocel.2022.106228

111. Frescas D, Pagano M. Deregulated proteolysis by the F-box proteins SKP2 and β-TrCP: Tipping the scales of cancer. *Nat Rev Cancer*. (2008) 8:438–49. doi: 10.1038/nrc2396

112. Wu T, Gu X, Cui H. Emerging roles of skp2 in cancer drug resistance. *Cells*. (2021) 10:1–19. doi: 10.3390/cells10051147

113. Chan CH, Lee SW, Wang J, Lin HK. Regulation of Skp2 expression and activity and its role in cancer progression. *ScientificWorldJournal*. (2010) 10:1001–15. doi: 10.1100/tsw.2010.89

114. Wu J, Zhang X, Zhang L, Wu CY, Rezaeian AH, Chan CH, et al. Skp2 E3 ligase integrates ATM activation and homologous recombination repair by ubiquitinating NBS1. *Mol Cell*. (2012) 46:351–61. doi: 10.1016/j.molcel.2012.02.018

115. Chan CH, Li CF, Yang WL, Gao Y, Lee SW, Feng Z, et al. The Skp2-SCF E3 ligase regulates akt ubiquitination, glycolysis, herceptin sensitivity, and tumorigenesis. *Cell*. (2012) 149:1098–111. doi: 10.1016/j.cell.2012.02.065

116. Benanti JA. Coordination of cell growth and division by the ubiquitin-proteasome system. *Semin Cell Dev Biol*. (2012) 23:492–8. doi: 10.1016/j.semcdb.2012.04.005

117. Lu W, Liu S, Li B, Xie Y, Adhiambo C, Yang Q, et al. SKP2 inactivation suppresses prostate tumorigenesis by mediating JARID1B ubiquitination. *Oncotarget*. (2015) 6:771–88. doi: 10.18632/oncotarget.2718

118. Inuzuka H, Gao D, Finley LWS, Yang W, Wan L, Fukushima H, et al. Acetylation-dependent regulation of Skp2 function. *Cell*. (2012) 150:179–93. doi: 10.1016/j.cell.2012.05.038

119. Zhang L, Wang C. F-box protein Skp2: A novel transcriptional target of E2F. *Oncogene*. (2006) 25:2615–27. doi: 10.1038/sj.onc.1209286

120. Wang G, Chan CH, Gao Y, Lin HK. Novel roles of Skp2 E3 ligase in cellular senescence, cancer progression, and metastasis. *Chin J Cancer*. (2012) 31:169–77. doi: 10.5732/cjc.011.10319

121. Asmamaw MD, Liu Y, Zheng YC, Shi XJ, Liu HM. Skp2 in the ubiquitin-proteasome system: A comprehensive review. *Med Res Rev*. (2020) 40:1920–49. doi: 10.1002/med.21675

122. Roychowdhury A, Pal D, Basu M, Samadder S, Mondal R, Roy A, et al. Promoter methylation and enhanced SKP2 are associated with the downregulation of CDKN1C in cervical squamous cell carcinoma. *Cell Signal*. (2023) 109:110735. doi: 10.1016/j.cellsig.2023.110735

123. Hershko DD. Oncogenic properties and prognostic implications of the ubiquitin ligase Skp2 in cancer. *Cancer*. (2008) 112:1415–24. doi: 10.1002/cncr.23317

124. Peng YT, Wu WR, Chen LR, Kuo KK, Tsai CH, Huang YT, et al. Upregulation of cyclin-dependent kinase inhibitors CDKN1B and CDKN1C in hepatocellular carcinoma-derived cells *via* goniothalamin-mediated protein stabilization and epigenetic modifications. *Toxicol Rep*. (2015) 2:322–32. doi: 10.1016/j.toxrep.2015.01.010

125. Ren H, Zhang Y, Zhu H. MiR-339 depresses cell proliferation *via* directly targeting S-phase kinase-associated protein 2 mRNA in lung cancer. *Thorac Cancer*. (2018) 9:408–14. doi: 10.1111/1759-7714.12597

126. Lin M, Xu Y, Gao Y, Pan C, Zhu X, Wang Zw. Regulation of F-box proteins by noncoding RNAs in human cancers. *Cancer Lett*. (2019) 466:61–70. doi: 10.1016/j.canlet.2019.09.008

127. Dan W, Zhong L, Yu L, Xiong L, Li J, Ye J, et al. Skp2 promotes APL progression through the stabilization of oncoprotein PML-RARα and the inhibition of JunB expression. *Life Sci*. (2022) 289:120231. doi: 10.1016/j.lfs.2021.120231

128. Rodriguez S, Abundis C, Boccalatte F, Mehrotra P, Chiang MY, Yui MA, et al. Therapeutic targeting of the E3 ubiquitin ligase SKP2 in T-ALL. *Leukemia*. (2020) 34:1241–52. doi: 10.1038/s41375-019-0653-z

129. Estévez-Sarmiento F, Saavedra E, Ruiz-Estévez M, León F, Quintana J, Brouard I, et al. Chlorinated guaiane-type sesquiterpene lactones as cytotoxic agents against human tumor cells. *Int J Mol Sci*. (2020) 21:1–14. doi: 10.3390/ijms21249767

130. Liu Y, Shao Z, Liao Y, Xia X, Huang C, He J, et al. Targeting SKP2/Bcr-Abl pathway with Diosmetin suppresses chronic myeloid leukemia proliferation. *Eur J Pharmacol*. (2020) 883:173366. doi: 10.1016/j.ejphar.2020.173366

131. Faderl S, Rai K, Gribben J, Byrd JC, Flinn IW, O'Brien S, et al. Phase II study of single-agent bortezomib for the treatment of patients with fludarabine-refractory B-cell chronic lymphocytic leukemia. *Cancer*. (2006) 107:916–24. doi: 10.1002/cncr.22097

132. Jing J, Rui L, Junyuan S, Jinfeng Y, Zhihao H, Weiguo L, et al. Small-molecule compounds inhibiting S-phase kinase-associated protein 2: A review. *Front Pharmacol*. (2023) 14:1122008. doi: 10.3389/fphar.2023.1122008

133. Zhao H, Pan H, Wang H, Chai P, Ge S, Jia R, et al. SKP2 targeted inhibition suppresses human uveal melanoma progression by blocking ubiquitylation of p27. *Onco Targets Ther*. (2019) 12:4297–308. doi: 10.2147/OTT.S203888

134. Tong Y, Jin L. MiR-590-5p targets Skp2 to inhibit the growth and invasion of Malignant melanoma cells. *Dis Markers*. (2022) 2022:8723725. doi: 10.1155/2022/8723725

135. Markovic SN, Geyer SM, Dawkins F, Sharfman W, Albertini M, Maples W, et al. A phase II study of bortezomib in the treatment of metastatic Malignant melanoma. *Cancer*. (2005) 103:2584–9. doi: 10.1002/cncr.21108

136. Kuttikrishnan S, Ahmad F, Mateo JM, Prabhu KS, El-Elimat T, Oberlies NH, et al. Neosetophomone B induces apoptosis in multiple myeloma cells *via* targeting of AKT/SKP2 signaling pathway. *Cell Biol Int*. (2023) 42(2):190–200. doi: 10.1002/cbin.12101

137. Yang Y, Yan W, Liu Z, Wei M. Skp2 inhibitor SKPin C1 decreased viability and proliferation of multiple myeloma cells and induced apoptosis. *Braz J Med Biol Res*. (2019) 52:1–10. doi: 10.1590/1414-431x20198412

138. San Miguel J, Bladé J, Boccadoro M, Cavenagh J, Glasmacher A, Jagannath S, et al. A practical update on the use of bortezomib in the management of multiple myeloma. *Oncologist*. (2006) 11:51–61. doi: 10.1634/theoncologist.11-1-51

139. Chan CH, Morrow JK, Li CF, Gao Y, Jin G, Moten A, et al. Pharmacological inactivation of Skp2 SCF ubiquitin ligase restricts cancer stem cell traits and cancer progression. *Cell*. (2013) 154:556–68. doi: 10.1016/j.cell.2013.06.048

140. Pharm V, Rendon R, Le V, Tippin M, Fu D-J, Le TH, et al. Gartanin is a novel NEDDylation inhibitor for induction of Skp2 degradation, FBXW2 expression, and autophagy. *Mol Carcinog*. (2020) 59:193–201. doi: 10.1002/mc.23140

141. Jiang X, Li Y, Feng JL, Nik Nabil WN, Wu R, Lu Y, et al. Safrana l Prevents Prostate Cancer Recurrence by Blocking the Re-activation of Quiescent Cancer Cells *via* Downregulation of S-Phase Kinase-Associated Protein 2. *Front Cell Dev Biol*. (2020) 8:598620. doi: 10.3389/fcell.2020.598620

142. Rico-Bautista E, Yang CC, Lu L, Roth GP, Wolf DA. Chemical genetics approach to restoring p27Kip1 reveals novel compounds with antiproliferative activity in prostate cancer cells. *BMC Biol*. (2010) 8:1–18. doi: 10.1186/1741-7007-8-153

143. Tang Y, Simoneau AR, Xie J, Shahandeh B, Zi X. Effects of the kava chalcone flavokawain A differ in bladder cancer cells with wild-type versus mutant p53. *Cancer Prev Res*. (2008) 1:439–51. doi: 10.1158/1940-6207.CAPR-08-0165

144. Zhao H, Iqbal NJ, Sukrithan V, Nicholas C, Xue Y, Yu C, et al. Targeted inhibition of the E3 ligase SCFSkp2/Cks1 has antitumor activity in RB1-deficient human and mouse small-cell lung cancer. *Cancer Res*. (2020) 80:2355–67. doi: 10.1158/0008-5472.CAN-19-2400

145. Chiang IT, Wang WS, Liu HC, Yang ST, Tang NY, Chung JG. Curcumin alters gene expression-associated DNA damage, cell cycle, cell survival and cell migration and invasion in NCI-H460 human lung cancer cells in *vitro*. *Oncol Rep*. (2015) 34:1853–74. doi: 10.3892/or.2015.4159

146. Chang HC, Chang FR, Wang YC, Pan MR, Hung WC, Wu YC. A bioactive withanolide Tubocapsanolide A inhibits proliferation of human lung cancer cells *via* repressing Skp2 expression. *Mol Cancer Ther*. (2007) 6:1572–8. doi: 10.1158/1535-7163.MCT-06-0812

147. Liao YJ, Bai HY, Li ZH, Zou J, Chen JW, Zheng F, et al. a natural ent-kaurane, induces G2/M phase arrest *via* downregulation of Skp2 and apoptosis induction through ROS/JNK/c-Jun pathway in hepatocellular carcinoma cells. *Cell Death Dis*. (2014) 5:1–11. doi: 10.1038/cddis.2014.66

148. Jandial DD, Krill LS, Chen L, Wu C, Ke Y, Xie J, et al. Induction of G2M arrest by flavokawain a, a kava chalcone, increases the responsiveness of HER2-overexpressing breast cancer cells to herceptin. *Molecules*. (2017) 22(3):462. doi: 10.3390/molecules22030462

149. Ooi LC, Watanabe N, Futamura Y, Sulaiman SF, Darah I, Osada H. Identification of small molecule inhibitors of p27Kip1 ubiquitination by high-throughput screening. *Cancer Sci*. (2013) 104:1461–7. doi: 10.1111/cas.12246

150. Liu Y, Zhou Z, Yan J, Wu X, Xu G. Diosgenin exerts antitumor activity *via* downregulation of Skp2 in breast cancer cells. *BioMed Res Int*. (2020) 2020:1–10. doi: 10.1155/2020/8072639

151. Yin X, Zhang Y, Su J, Hou Y, Wang L, Ye X, et al. Rottlerin exerts its anti-tumor activity through inhibition of Skp2 in breast cancer cells. *Oncotarget*. (2016) 7:66512–24. doi: 10.18632/oncotarget.11614

152. Zhang Y, Zvi YS, Batko B, Zaphiros N, O'Donnell EF, Wang J, et al. Down-regulation of Skp2 expression inhibits invasion and lung metastasis in osteosarcoma. *Sci Rep*. (2018) 8:1–13. doi: 10.1038/s41598-018-32428-9

153. Dehghanian SZ, Pan CT, Lee JM, Shiue YL. ABT-751 induces multiple anticancer effects in urinary bladder urothelial carcinoma-derived cells: Highlighting the induction of cytostasis through the inhibition of SKP2 at both transcriptional and post-translational levels. *Int J Mol Sci*. (2021) 22:1–25. doi: 10.3390/ijms22020945

154. Wang L, Ye X, Cai X, Su J, Ma R, Yin X, et al. Curcumin suppresses cell growth and invasion and induces apoptosis by down-regulation of Skp2 pathway in glioma cells. *Oncotarget*. (2015) 6:18027–37. doi: 10.18632/oncotarget.4090

155. Pavlides SC, Huang KT, Reid DA, Wu L, Blank SV, Mittal K, et al. Inhibitors of SCF-Skp2/Cks1 E3 ligase block estrogen-induced growth stimulation and degradation of nuclear p27kip1: Therapeutic potential for endometrial cancer. *Endocrinology*. (2013) 154:4030–45. doi: 10.1210/en.2013-1757

156. Altmann E, Erbel P, Renatus M, Schaefer M, Schlierf A, Druet A, et al. Azaindoles as zinc-binding small-molecule inhibitors of the JAMM protease CSN5. *Angew Chemie - Int Ed*. (2017) 56:1294–7. doi: 10.1002/anie.201608672

157. Chung YK, Chi-Hung Or R, Lu CH, Ouyang WT, Yang SY, Chang CC. Sulforaphane down-regulates SKP2 to stabilize p27KIP1 for inducing antiproliferation in human colon adenocarcinoma cells. *J Biosci Bioeng*. (2015) 119:35–42. doi: 10.1016/j.jbiosc.2014.06.009

158. Su J, Zhou X, Wang L, Yin X, Wang Z. Curcumin inhibits cell growth and invasion and induces apoptosis through down-regulation of Skp2 in pancreatic cancer cells. *Am J Cancer Res*. (2016) 6:1949–62.

159. Su J, Wang L, Yin X, Zhao Z, Hou Y, Ye X, et al. Rottlerin exhibits anti-cancer effect through inactivation of S phase kinase-associated protein 2 in pancreatic cancer cells. *Am J Cancer Res*. (2016) 6:2178–91.

160. Khan AQ, Siveen KS, Prabhu KS, Kuttikrishnan S, Akhtar S, Shaar A, et al. Curcumin-mediated degradation of S-phase kinase protein 2 induces cytotoxic effects in human papillomavirus-positive and negative squamous carcinoma cells. *Front Oncol*. (2018) 8:399. doi: 10.3389/fonc.2018.00399

161. Mou H, Guo P, Li X, Zhang C, Jiang J, Wang L, et al. Nitidine chloride inhibited the expression of S phase kinase-associated protein 2 in ovarian cancer cells. *Cell Cycle*. (2017) 16:1366–75. doi: 10.1080/15384101.2017.1327490

162. Ungermannova D, Lee J, Zhang G, Dallmann HG, McHenry CS, Liu X. High-throughput screening alphascreen assay for identification of small-molecule inhibitors of ubiquitin E3 ligase SCFSkp2-Cks1. *J Biomol Screen*. (2013) 18:910–20. doi: 10.1177/1087057113485789

163. Gao J, Wang G, Wu J, Zuo Y, Zhang J, Jin X. Skp2 Expression Is Inhibited by Arsenic Trioxide through the Upregulation of miRNA-330-5p in Pancreatic Cancer Cells. *Mol Ther - Oncolytics*. (2019) 12:214–23. doi: 10.1016/j.omto.2019.01.006

164. Fricker LD. Proteasome inhibitor drugs. *Annu Rev Pharmacol Toxicol*. (2020) 60:457–76. doi: 10.1146/annurev-pharmtox-010919-023603

165. Deng L, Meng T, Chen L, Wei W, Wang P. The role of ubiquitination in tumorigenesis and targeted drug discovery. *Signal Transduct Target Ther*. (2020) 5 (1):11. doi: 10.1038/s41392-020-0107-0

166. Celebi G, Kesim H, Ozer E, Kutlu O. The effect of dysfunctional ubiquitin enzymes in the pathogenesis of most common diseases. *Int J Mol Sci*. (2020) 21:1–24. doi: 10.3390/ijms21176335

167. Khan S, He Y, Zhang X, Yuan Y, Pu S, Kong Q, et al. PROteolysis TArgeting Chimeras (PROTACs) as emerging anticancer therapeutics. *Oncogene*. (2020) 39:4909–24. doi: 10.1038/s41388-020-1336-y

168. Sasso JM, Tenchov R, Wang DS, Johnson LS, Wang X, Zhou QA. Molecular glues: the adhesive connecting targeted protein degradation to the clinic. *Biochemistry*. (2023) 62:601–23. doi: 10.1021/acs.biochem.2c00245

169. Fang RH, Gao W, Zhang L. Targeting drugs to tumours using cell membrane-coated nanoparticles. *Nat Rev Clin Oncol*. (2023) 20:33–48. doi: 10.1038/s41571-022-00699-x

170. Spano D, Catara G. Targeting the ubiquitin–proteasome system and recent advances in cancer therapy. *Cells*. (2024) 13:29. doi: 10.3390/cells13010029

171. Kelsall IR, McCrory EH, Xu Y, Scudamore CL, Nanda SK, Mancebo-Gamella P, et al. HOIL-1 ubiquitin ligase activity targets unbranched glucosaccharides and is required to prevent polyglucosan accumulation. *EMBO J*. (2022) 41:1–17. doi: 10.15252/embj.2021109700

172. Mahlknecht U, Hoelzer D. Epigenetische Steuerelemente als neuartige therapeutische Zielstrukturen in der Hämato-/Onkologie. *Dtsch Medizinische Wochenschrift*. (2003) 128:2423–6. doi: 10.1055/s-2003-43593

173. Ross PM, Demirkan G, Birditt B, Bogatzi L, Carter W, Filanoski B, et al. An assay for simultaneous profiling of gene expression, phospho- and total protein abundance, and somatic DNA mutations for hematology-oncology research. *Blood*. (2017) 130:2481.

174. Jibu RM, Veeraraghavan VP, Gayathri R, Kavitha S. Awareness on hematological Malignancies among college students. *J Adv Pharm Technol Res*. (2022) 13:534–8. doi: 10.4103/japtr.japtr_175_22

175. Kullmann MK, Grubbauer C, Goetsch K, Jäkel H, Podmirseg SR, Trockenbacher A, et al. The p27-Skp2 axis mediates glucocorticoidinduced cell cycle arrest in T-lymphoma cells. *Cell Cycle*. (2013) 12:2625–35. doi: 10.4161/cc.25622

176. Morrow J, Lin H, Sun S, Zhang S. Targeting ubiquitination for cancer therapies. *Future Med Chem*. (2015) 7:2333–50. doi: 10.4155/fmc.15.148

177. Li C, Du L, Ren Y, Liu X, Jiao Q, Cui D, et al. SKP2 promotes breast cancer tumorigenesis and radiation tolerance through PDCD4 ubiquitination. *J Exp Clin Cancer Res*. (2019) 38:1–15. doi: 10.1186/s13046-019-1069-3

178. Šimečková Š, Kahounová Z, Fedr R, Remšík J, Slabáková E, Suchánková T, et al. High Skp2 expression is associated with a mesenchymal phenotype and increased tumorigenic potential of prostate cancer cells. *Sci Rep*. (2019) 9:1–10. doi: 10.1038/s41598-019-42131-y

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