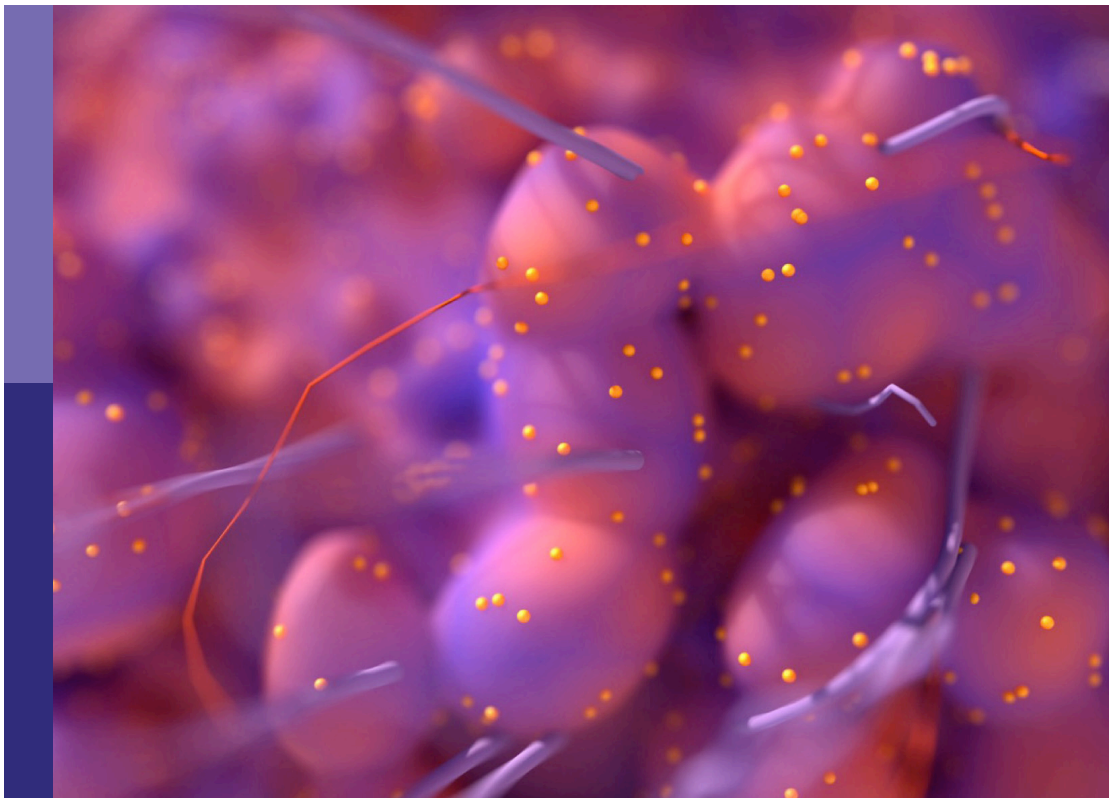
frontiers | Research Topics

Hybrid or mixed myelodyplastic/ myeloproliferative disorders: Current trends in diagnosis and treatment

Edited by
Argiris Symeonidis and Ulrich Germing

Published in
Frontiers in Oncology



ISSN 1664-8714
ISBN 978-2-83251-986-8
DOI 10.3389/978-2-83251-986-8

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Hybrid or mixed myelodyplastic/ myeloproliferative disorders: Current trends in diagnosis and treatment

Topic editors

Argiris Symeonidis — University of Patras, Greece

Ulrich Germing — Heinrich Heine University of Düsseldorf, Germany

Citation

Symeonidis, A., Germing, U., eds. (2023). *Hybrid or mixed myelodyplastic/myeloproliferative disorders: Current trends in diagnosis and treatment*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83251-986-8

Table of contents

frontiers | Frontiers in Oncology

TYPE Editorial
PUBLISHED 08 March 2023
DOI 10.3389/fonc.2023.1166163

OPEN ACCESS

EDITED AND REVIEWED BY
Alessandro Isidori,
AORMN Hospital, Italy

*CORRESPONDENCE
Argiris Symeonidis
argiris.symeonidis@yahoo.gr

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

RECEIVED 14 February 2023
ACCEPTED 22 February 2023
PUBLISHED 08 March 2023

CITATION
Symeonidis A and Germing U (2023) Editorial: Hybrid or mixed myelodyplastic/myeloproliferative disorders: Current trends in diagnosis and treatment. *Front. Oncol.* 13:1166163. doi: 10.3389/fonc.2023.1166163

Editorial: Hybrid or mixed myelodyplastic/ myeloproliferative disorders: Current trends in diagnosis and treatment

Argiris Symeonidis[1]* and Ulrich Germing[2]

[1]Hematology Division, Department of Internal Medicine, University of Patras, Patras, Greece, [2]Department of Hematology and Oncology, Heinrich Heine University of Düsseldorf, Düsseldorf, Germany

KEYWORDS

mixed MDS/MPN, chronic myelomonocytic leukemia (CCML), atypical chronic myeloid leukemia (aCML), myelodyslastic/myeloproliferative neoplasms (MDS/MPN), WHO classification - myeloid neoplasms - diagnostic criteria

Editorial on the Research Topic

Hybrid or mixed myelodyplastic/myeloproliferative disorders: Current trends in diagnosis and treatment

This Research Topic in *Frontiers in Oncology* is dedicated to the current trends in the diagnosis and treatment of hybrid or mixed myelodysplastic/myeloproliferative disorders. This group of myeloid neoplasms has not yet received enough attention in the various classifications proposed for these diseases, for several decades, been placed in the past under the umbrella of myelodysplastic syndromes (MDS) or that of myeloproliferative neoplasms (MPN).

However, it has ultimately become clear that hybrid or mixed MDS/MPN have a profile of clinical, hematologic, cytomorphologic, histomorphologic, pathogenetic, cytogenetic, and molecular characteristics, indicating that enlisting or classifying them among other disease entities, such as the typical and clearly identifiable MPN or MDS, may not be appropriate or even useful and practical. It should be emphasized that all the articles included in this Research Topic have been submitted, revised, and published before the recently published fifth edition of the World Health Organization (WHO) Classification of Myeloid Neoplasms. Thus, the authors have followed the previous fourth edition of WHO Hybrid or Mixed MDS/MPN, published in 2016, according to which five disease entities were included, namely, atypical BCR-ABL negative chronic myelogenous leukemia (aCML), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), MDS/MPN unclassifiable (MDS/MPN-U), and MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RST) as a provisional entity (1).

In the fifth edition of the WHO classification, the provisional entity MDS/MPN-RST has been confirmed as a fully, clearly, and/or molecularly identified disease, associated (although not mandatorily) with SF3B1 mutations. A new entity, namely myeloproliferative neoplasm with PCM1-JAK2 rearrangement, has been proposed, whereas JMML has been removed

from this category (2). These changes have restricted the content of the so-called "Mixed MDS/MPN unclassifiable" group. Finally, another change was the recognition that a subgroup of patients with neutrophilic leukemia exhibits morphologic features of trilineage dysplasia, and, despite neutrophilia, have ineffective erythropoiesis with or without transfusion dependence. This subgroup is proposed to be classified among MDS/MPN rather than among typical MPN as it is classified as another subgroup of this condition (3). This new proposal justifies our decision to include a review article on chronic neutrophilic leukemia in the contents of this Research Topic.

In contrast to the true MPN, which can develop following the emergence of a single gene mutation, the majority of cases have a normal cytogenetic profile, are primarily characterized by an increase in peripheral blood cell counts, and are associated with thromboembolic complications and constitutional symptoms. In contrast to MDS, which usually exhibits more than one driver mutation, commonly has an abnormal cytogenetic profile, is generally associated with ineffective hematopoiesis with peripheral blood cytopenias, and shares a high risk of progression to acute leukemia, hybrid or mixed MDS/MPN may exhibit features of both hematopoietic insufficiencies with morphological aberrations and also feature the proliferation of at least one cell lineage, either of megakaryocytes or of monocytes and/or of different myeloid progenitors. In addition, these diseases can sometimes be presented with constitutional symptoms and even with thromboembolic complications. However, none of these entities included within the group of hybrid or mixed MDS/MPN is a monogenetic entity, but all of them (although to a different extent) are characterized by a variety of chromosomal and, particularly, of molecular alterations, such as somatic mutations. Moreover, none of these somatic mutations, or any combination of them, nor any chromosomal aberration is pathognomonic for the entire group of mixed MDS/MPN or even for a single member-entity of the group. An additional logistic problem is that these are very rare and inhomogeneous diseases, and therefore, large databases and biobanks, which could potentially allow us to better analyze the disease biology, are not easily available (4, 5).

This Research Topic in *Frontiers in Oncology* has therefore asked experienced hematologists, hematopathologists, and geneticists to report on the epidemiology, pathogenesis, diagnosis, classification, prognosis, and therapeutic approaches of mixed MDS/MPN. In this Research Topic, Barone et al. report that in patients with polycythemia vera (PV), the circulating megakaryocyte-derived extracellular vesicles (EV) were significantly decreased, whereas platelet-derived EVs were increased and that PV patients also had an abnormal microbial DNA signature, which can generate an abnormal inflammatory network, potentially favoring the pathogenesis of the disease. In a review, Diamantopoulos and Viniou summarize the state-of-the-art diagnostic procedures, the recently-described molecular characterization of the disease, i.e., the recurrent pathogenetic mutations, and the current treatment approaches for patients with aCML. A comprehensive review of the complex and heterogeneous molecular pathogenesis and pathophysiology of CMML, with the application of the currently available research technologies, as well as the development of potential molecular targets for the design of novel therapies is presented in an excellent article by Geissler. The rare entity of myeloproliferative neoplasm with PCM1-JAK2 rearrangement is reviewed by Sun et al., following the description of two new cases. The authors discuss the poor prognosis and the lack of treatment guidelines for this entity, as well as the unpredictable and heterogeneous response pattern of treatment with ruxolitinib. They suggest that the combination of ruxolitinib and pegylated interferon might be more effective. Fontana et al. add molecular pathogenetic considerations on BCR/ABL-negative aCML, and discuss the influence of the molecular profile of the disease on prognosis and on the potential treatment options, including the novel and emerging targeted treatments. Küendgen et al. compile the epidemiological characteristics of hybrid or mixed MDS/MPN and emphasize that with the exception of the most common disease, namely CMML, there is a lack of reliable and convincing information, mainly attributed to the rarity of these syndromes, but also to the changing classification systems and the blurry and vague limits of each of them. Liapis et al. discuss the various options for first-line treatment available to patients with advanced CMML and analyze the existing data of the two main treatment approaches, namely, cytotoxic treatment and hypomethylated agents, and how this kind of treatment can be used as a bridge for allogeneic stem cell transplantation in transplant-eligible patients. Symeonidis et al. present all the published data from a few prospective studies but mainly from retrospective studies on allogeneic stem cell transplantation for mixed MDS/MPN and analyze the prognostic factors for the various transplantation outcomes. Finally, Thomopoulos et al. complete the round of articles with an overview of the clinical characteristics, molecular genetics, and the existing and emerging therapeutic approaches for patients with chronic neutrophilic leukemia, a very rare disease with several unmet needs for appropriate diagnosis and effective treatment.

In summary, this Research Topic consists of a collection of excellent articles, aiming to help interested readers to navigate through the classifications, understand the complex pathogenesis a little bit more, and get inspiration with regard to therapeutic considerations. We believe that the readers will enjoy reading this Research Topic.

Author contributions

AS and UG have equally contributed in the design and scientific supervision of the content of this Research Topic, and they also have both contributed to the writing of this Editorial. 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. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the world health organization classification of myeloid neoplasms and acute leukemia. *Blood* (2016) 127(20):2391–405. doi: 10.1182/blood-2016-03-643544

2. Khoury JD, Solary E, Abla O, Akkari Y, Allagiy R, Apperly JF, et al. The 5th edition of the world health organization classification of haematolymphoid tumours: Myeloid and Histiocytic/Dendritic neoplasms. *Leukemia* (2022) 36(7):1703–19. doi: 10.1038/s41375-022-01613-1

3. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International consensus classification of myeloid neoplasms and acute leukemias: Integrating morphologic, clinical, and genomic data. *Blood* (2022) 140(11):1200–28. doi: 10.1182/blood.2022015850

4. Zheng G, Li P, Zhang X, Pan Z. The fifth edition of the world health organization classification and the international consensus classification of myeloid neoplasms: Evolving guidelines in the molecular era with practical implications. *Curr Opin Hematol* (2023) 30(2):53–63. doi: 10.1097/MOH.0000000000000748

5. Prakash S, Arber DA, Bueso-Ramos C, Hasserjian RP, Orazi A. Advances in myelodysplastic/ myeloproliferative neoplasms. *Virchows Arch* (2023) 482(1):69–83. doi: 10.1007/s00428-022-03465-7

frontiers in Oncology

SYSTEMATIC REVIEW
published: 30 September 2021
doi: 10.3389/fonc.2021.751668

Molecular Pathogenesis of Chronic Myelomonocytic Leukemia and Potential Molecular Targets for Treatment Approaches

Klaus Geissler[1,2]*

[1] Medical School, Sigmund Freud University, Vienna, Austria, [2] Department of Internal Medicine V with Hematology, Oncology and Palliative Care, Hospital Hietzing, Vienna, Austria

OPEN ACCESS

Edited by:
Argiris Symeonidis, University of Patras, Greece

Reviewed by:
Haifa Al-Ali, University Hospital in Halle, Germany
Marta Sonia González Pérez, University Clinical Hospital of Santiago, Spain

***Correspondence:**
Klaus Geissler
geissler.klaus@gmx.at

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

Received: *01 August 2021*
Accepted: *26 August 2021*
Published: *30 September 2021*

Citation:
Geissler K (2021) Molecular Pathogenesis of Chronic Myelomonocytic Leukemia and Potential Molecular Targets for Treatment Approaches. Front. Oncol. 11:751668. doi: 10.3389/fonc.2021.751668

Numerous examples in oncology have shown that better understanding the pathophysiology of a malignancy may be followed by the development of targeted treatment concepts with higher efficacy and lower toxicity as compared to unspecific treatment. The pathophysiology of chronic myelomonocytic leukemia (CMML) is heterogenous and complex but applying different research technologies have yielded a better and more comprehensive understanding of this disease. At the moment treatment for CMML is largely restricted to the unspecific use of cytotoxic drugs and hypomethylating agents (HMA). Numerous potential molecular targets have been recently detected by preclinical research which may ultimately lead to treatment concepts that will provide meaningful benefits for certain subgroups of patients.

Keywords: CMML, chronic myelomonocytic leukemia, molecular, pathogenesis, targets, treatment

HIGHLIGHTS

- CMML is a clinically, molecularly and biologically heterogenous disease
- The combination of molecular data, functional *in vitro* findings, and *in vivo* preclinical models provide a comprehensive view of CMML pathogenesis
- Mutations in *TET2* are common initial clonal driver abnormalities in CMML
- *ASXL1* mutations play a major role in the transformation process into AML
- There is a close correlation between growth factor-independent myeloid colony-formation and the presence of RAS-pathway mutations
- RAS-pathway activation is a crucial pathophysiologic process for GM-CSF hypersensitivity, myeloproliferation, progressive disease and transformation into AML
- Numerous molecular targets provide the rationale for individualized treatment concepts in patients with CMML

INTRODUCTION

Although the term chronic myelomonocytic leukemia (CMML) has been used previously, CMML has been officially, based on morphological criteria/phenotype, acknowledged as a specific entity in the FAB classification 1976 (1, 2). It is characterized by leukocytosis with monocytes and granulocytic cells in all stages of development, marked dysmyelopoiesis, a variable course, unresponsiveness to aggressive chemotherapy and an inherent risk of transformation to acute myeloid leukemia (AML) (3). With regard to the presence of myeloproliferation CMML was originally subdivided into myeloproliferative disorder MP-CMML (WBC count >13 x 10^9/L) versus myelodysplastic syndrome MD-CMML (WBC count ≤13 x 10^9/L MD-CMML) by the FAB criteria (4). Since CMML is characterized by features of both a MDS and a MPN the World Health Organization (WHO) classification of 2002 assigned CMML to the mixed category MDS/MPN (5). CMML is further subclassified by WHO into three groups based on blast equivalent (blasts plus promonocytes) in peripheral blood (PB) and bone marrow (BM) as follows: CMML-0 if PB <2% and BM <5% blast equivalent, CMML-1 if PB 2-4% or BM 5-9% blast equivalent, and CMML-2 if PB 5-19% or BM 10-19% blast equivalent, and/or Auer rods are present (6). The median survival of reported series is highly variable indicating a significant clinical heterogeneity of the disease (7–12).

PATHOGENESIS OF CMML

Cancer is a biologically complex disease with characteristics acquired during the course of a multistep development process. In the past many research tools have been applied to better characterize the phenotypic, genotypic and functional features of cancer and to deeper understand the pathophysiology of malignancy with the ultimate goal to identify prognostic and predictive biomarkers, to render diagnosis more precisely and to develop targeted therapeutics for personalized medicine. No single technology is sufficient to consider all aspects of tumor complexity and information from different technologies are required to provide a comprehensive picture of cancer.

Structural Analysis by Sequencing Studies

In 1987 a mutation within codon 12 of the *NRAS* gene was reported for the first time by Janssen et al. in a patient with CMML in a study investigating molecular alterations of *RAS* genes in a variety of preleukemic disorders and leukemias of myeloid origin (13). Subsequently is has been shown that *RAS* mutations are rare events in BCR/ABL negative chronic myeloid leukemia (CML) but are prevalent in CMML (14). In this study mutations in the *RAS* oncogene were found in 17 of 30 (57%) CMML patients. In the last years the molecular landscape in patients with CMML has been described by several groups. Molecular abnormalities can be seen in >90% of patients with CMML (15) with a marked heterogeneity among CMML patients. A large number of gene mutations in genes encoding epigenetic regulators (*TET2*, *ASXL1*, *EZH2*, *UTX*, *IDH1*, *IDH2*, *DNMT3A*) (9, 16–22) splicing factors (*SF3B1*, *SRSF2*, *ZRSF2*, *U2AF1*) (23, 24), and cytokine signaling molecules (*NRAS*, *KRAS*, *CBL*, *JAK2*, *FLT3*) have been reported (9, 25–29). Mutations in the transcription regulators *RUNX1*, *NPM1*, and *TP53* have also been found in CMML (9, 30, 31). **Table 1** shows the frequencies of gene mutations in 3 different CMML cohorts in which comprehensive molecular analyses has been reported (9, 11, 32). Considering all molecular data reported mutations in *TET2* (~60%), *SRSF2* (~50%), *ASXL1* (~40%) and RAS pathway (~30%) are most common (15) but no molecular aberration is specific of this entity, as they can be detected with different frequencies in other myeloid neoplasms (33).

Functional Analysis by *In Vitro* Studies

In 1988 Geissler et al. have originally reported extensive *in vitro* formation of myelomonocytic colony forming units (CFU-GM) without addition of exogenous growth factors in a subset of patients with CMML (**Table 2**) (34). This spontaneous CFU-GM colony formation in CMML was markedly reduced by addition of anti-granulocyte/macrophage colony-stimulating factor (GM-CSF) antibodies, but not by antibodies against other growth factors, suggesting that this is a GM-CSF-dependent *in vitro* phenomenon (35) **Figure 1**. The biologic basis for this observation was later provided by Padron when he reported hypersensitivity of CMML progenitors using phospho-STAT5 flow cytometry (36). Moreover, the group in Vienna could show in a small retrospective study that CMML patients with high spontaneous CFU-GM growth (>100/10^5 PB mononuclear cells) have an inferior prognosis as compared to patients with low

TABLE 1 | Frequencies of molecular aberrations in different CMML cohorts.

Category	Gene	French n = 312	Mayo Clinic n = 175	Austrian n = 222
Epigenetic regulation	*TET2*	58%	46%	67%
	ASXL1	40%	47%	24%
	EZH2	5%	1%	16%
	IDH1	<1%	2%	NA
	IDH2	6%	5%	NA
	DNMT3A	2%	5%	8%
RNA splicing	*SF3B1*	6%	6%	5%
	SRSF2	46%	45%	20%
	ZRSF2	8%	5%	7%
	U2AF1	5%	8%	6%
Cytokine signaling	*NRAS*	11%	12%	15%
	KRAS	8%	NA	9%
	CBL	10%	14%	10%
	PTPN11	NA	5%	5%
	JAK2	8%	4%	13%
	FLT3	3%	1%	NA
Others	*RUNX1*	15%	14%	9%
	NPM1	1%	3%	NA
	TP53	1%	5%	3%
	SETBP1	NA	19%	21%
	CEBPA	NA	6%	NA

NA, not available.

TABLE 2 | Myeloid colony formation in patients with CMML.

	Source	CFU-C/2.5 x 10^4 MNC
With CSA	P1 1 BM MNC	910
	6 Controls BM MNC	19.8 ± 8.5
	Pt 2 PB MNC	23.0
	6 Controls	0.36 ± 0.15
Without CSA	P1 1 BM MNC	815
	6 Controls BM MNC	0.0 ± 0.0
	Pt 2 PB MNC	27.0
	6 Controls	0.0 ± 0.0

In vitro cultures from patients with CMML using the CFU-C assay. Mononuclear cells from patients and normal individuals were cultivated in semisolid cultures with or without colony-stimulating activity (CSA). Data show in both CMML patients massively increased myeloid colony (CFU-C) growth as compared to controls and also the formation of CFU-C without the addition of exogenous CSA [adapted from Geissler, K., et al., Leuk Res 1988 (34)].

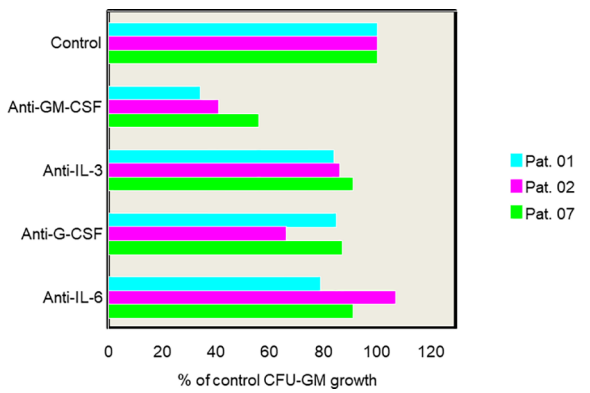


FIGURE 1 | Effect of anticytokine antibodies on spontaneous growth of CMML cells in 3 patients. PB MNC were cultured with medium alone or with antibodies against GM-CSF, G-CSF, IL-3, or IL-6, respectively. Data show a marked inhibition of spontaneous CFU-GM growth in the presence of anti-GM-CSF antibodies in all 3 patients indicating that autonomous colony formation is a GM-CSF dependent *in vitro* phenomenon [adapted from Geissler, K., et al., J Exp Med 1996 (35)].

myeloid colony formation suggesting a clinical significance of the original observation (37). These results have been recently extended in a much larger CMML patient cohort indicating that spontaneous myeloid colony-formation was, compared to other single established prognostic factors, the strongest predictor regarding overall survival (OS) (38). This may indicate that *in vitro* cultures using unmanipulated mononuclear cells (MNC) may be a more global test that covers different aspects of malignancy better than any of the single parameters that are currently used to characterize the behavior of a tumor.

There is also another *in vitro* phenomenon that seems to be characteristic for CMML patients. Semisolid *in vitro* cultures from PBMNC of normal individuals usually contain a higher concentration of erythroid colonies (BFU-E) as compared to myeloid colonies (CFU-GM). Skewed differentiation toward the myelomonocytic over erythroid commitment in patients, as indicated by an inverse BFU-E/CFU-GM ratio, is a common finding in CMML patients (39). Interestingly, the lack of myelomonocytic skewing separated patients with a particularly favorable prognosis and a minimal risk of transformation.

In Vivo Analysis by Preclinical Mouse Models

Myelomonocytic leukemias can be recapitulated by transplantation of mouse BM cells carrying an oncogenic mutation in the *Nras* locus (40–42). Interestingly, alterations of the other RASopathy genes including *Kras, Cbl, Ptpn11* and *Nf1* may also result in a similar phenotype in preclinical mouse models (**Table 3**) (44–47). In all these *in vivo*-models animals develop a myeloproliferative disorder with clonal expansion of the granulomonopoiesis.

The effects of molecular aberrations in genes of the epigenetig machinery have been also studied in preclinical animal models (48–50). In a mouse model with complete functional deletion of *Tet2* resulted in a progressive enlargement of the hematopoietic stem cell compartment and eventual myeloproliferation *in vivo*. *Tet2* +/- mice displayed increased stem cell self-renewal and extramedullary hematopoiesis, indicating that Tet2 haploinsufficiency contributes to hematopoietic transformation *in vivo* (48). Importantly, one third of *Tet2* -/- and 8% of *Tet2* +/- mice died within 1 year of age because of the development of myeloid malignancies reminiscent of CMML indicating that Tet2 loss may represent a predisposition for the development of this malignancy. Moreover, it was shown that transplantation of *Tet2* -/-, but not wild-type (WT) or *Tet2* +/- BM cells, was associated with elevated white blood cell (WBC) counts, monocytosis and splenomegaly in WT recipient mice (49).

Comprehensive View of Pathogenesis

Recent evidence suggests that considering cancer only as a consequence of genetic aberrations is too simple (77). There is growing evidence that the complex nature of transformation from a normal to a cancer cell within different tissues is a result of the interplay among genetic and epigenetic events, tissue structure, exposure and the tissue microenvironment. Thus, molecular analysis of a tumor by NGS alone may be not sufficient to cover the biology of a tumor and emphasize the need for more comprehensive methods to characterize the biology of a tumour. By combining structural data, functional *in vitro* findings, an *in vivo* preclinical models a comprehensive view of pathogenesis of CMML is possible.

Similar to the *in vitro* phenomenon of spontaneous erythroid colony (78) and megakaryocyte colony formation (79) due to hypersensitivity to growth factors in patients with BCR/ABL negative MPN spontaneous myeloid colony formation seems to be an *in vitro* feature in a subset of patients with CMML. Molecular aberrations of RASopathy genes in murine hematopoietic cells induce growth-factor-independent CFU-GM formation *in vitro* due to hypersensitivity of granulomonocytic precursors to GM-CSF (40, 41, 43, 44, 46, 47). Moreover, in juvenile myelomonocytic leukemia (JMML) in which molecular aberrations are mainly restricted to RASopathy genes autonomous myeloid colony formation due to GM-CSF-specific hypersensitivity is a hallmark feature of disease, which has been included in the diagnostic criteria (80). In a small series of CMML patients who had *in vitro* cultures and molecular analyses Geissler et al. observed a close correlation between high spontaneous myeloid colony growth and the presence

TABLE 3 | Mouse models with CMML-like phenotype.

Genotype	Strain	Activation	Phenotype	Reference
Nras G12D	C57BL/6	Conditional activation	Monocytosis, granulocytosis, splenomegaly spontaneous CFU-GM growth	Wang (40)
Nras G12D	C57Bl/6	Conditional activation	Leukocytosis, splenomegaly spontaneous CFU-GM growth	Li (41)
Nras G12D	BALB/c	Transgenic activation	Granulocytosis, monocytosis, mastocytosis splenomegaly	Parikh (42)
Kras G12D	C57BL/6	Conditional activation	Leukocytosis, myeloid hyperplasi a in BM splenomegaly spontaneous CFU-GM growth	Chan (43)
Kras G12D	C57BL/6	Conditional activation	Expansion of progenitor cells in spleen spontaneous CFU-GM growth	VanMeter (44)
c-CBL -/-	C57BL/6	Transgenic inactivation	Splenomegaly, thrombocytosis lymphoid hyperplasia altered T-cell receptor expression	Murphy (45)
NF1 -/-	C57Bl/6	Conditional inactivation	Leukocytosis, splenomegaly spontaneous CFU-GM growth	Le (46)
PTPN11 D61Y	C57Bl/6	Conditional activation	Leukocytosis, anemia, hepatosplenomegaly spontaneous CFU-GM growth	Chan (47)
TET2 -/-	C57BL/6	Conditional inactivation	monocytosis splenomegaly	Moran-Crusio (48)
TET2 -/-	C57BL/6	Conditional inactivation	Monocytosis, splenomegaly skewed differentiation toward G/M lineage	Li (49)
ASXL1 +/-	B6.SJL	Conditional inactivation	Dyshematopoiesis, leukocytes heterogenous, anemia, thrombocytopenia skewed differentiation toward G/M lineage	Wang (50)

of RAS pathway mutations as shown in **Figure 2** (81). This initial observation was later confirmed in a larger patient cohort including 100 CMML patients (82). The median number of spontaneously formed CFU-GM/10^5 MNC was 147.5 in RAS-mutated patients as compared with 2 in RAS-wildtype patients ($p<0.00001$). Unstimulated myeloid colony formation in RAS-mutated patients was also much higher than spontaneous formation of CFU-GM in normal individuals (median 4.8/10^5 PBMNC) which has been reported by this group previously (83). There was no significant difference regarding spontaneous CFU-GM formation in CMML patients with molecular aberrations in genes of epigenetic regulation and RNA-splicing, respectively. High spontaneous myeloid colony formation was also never observed in CMML patients with the *JAK2 V617F* mutation as the only molecular aberration in signaling pathways [Geissler et al., unpublished data]. All these findings, in mouse and human, indicate that hypersensitivity to GM-CSF, as manifested by growth factor-independent CFU-GM growth *in vitro*, is caused by molecular aberrations of the RAS-pathway which may be a major driver in CMML pathogenesis, in particular in MP-CMML. Moreover, it reveals high autonomous CFU-GM growth as a functional surrogate parameter of RAS pathway hyperactivation in CMML.

Myelomonocytic skewing has been proposed as a key phenomenon in the pathophysiology of CMML. In a seminal paper using mutation-specific discrimination analysis of single-cell-derived colonies in 28 patients with CMML, Itzykson et al. could show that the main features of this disease are early clonal dominance, arising at the CD34+/CD34- stage of hematopoiesis, and granulomonocytic differentiation skewing of multipotent and common myeloid progenitors (84). Geissler et al. could demonstrate that myelomonocytic skewing as determined by semisolid cultures can separate subgroups of CMML patients with a different phenotype, a different genotype and a different prognosis (39). The definitive link of this phenomenon to the pathophysiology of CMML comes from animal studies in which hematopoietic cells are genetically manipulated with molecular aberrations that are commonly found in CMML patients. Functional inactivation of *TET2* in cord blood CD34+ cells skews progenitor differentiation toward the granulomonocytic lineage at the expense of lymphoid and erythroid lineages (85). In mice, deletion of *Tet2* results in an increased hematopoietic repopulating capacity with an altered differentiation skewing towards the granulomonocytic lineage (49). Other epigenetic modifiers such as ASXL1 have also been shown to impact skewing of hematopoiesis. *Asxl1 -/-* mice had a reduced hematopoietic stem cell (HSC) pool, and Asxl1 -/- HSCs exhibited decreased hematopoietic repopulating capacity, with skewed cell differentiation favoring granulocytic lineage (50). Furthermore the splicing factors SRSF2 and U2AF1 seem to affect skewing. Mutations in both *SRSF2* and *U2AF1* are associated with abnormal differentiation by skewing granulomonocytic differentiation towards monocytes (86). Collectively, many molecular aberrations that can be found in CMML, induce myelomonocytic skewing in the preclinical mouse model providing the genetic basis for this key finding in patients.

Age Related Mutations in CMML

Recent molecular analyses of large populations have indicated that somatic mutations in hematopoietic cells leading to clonal expansion are commonly acquired during human aging (87). Clonally restricted hematopiesis is associated with an increased risk

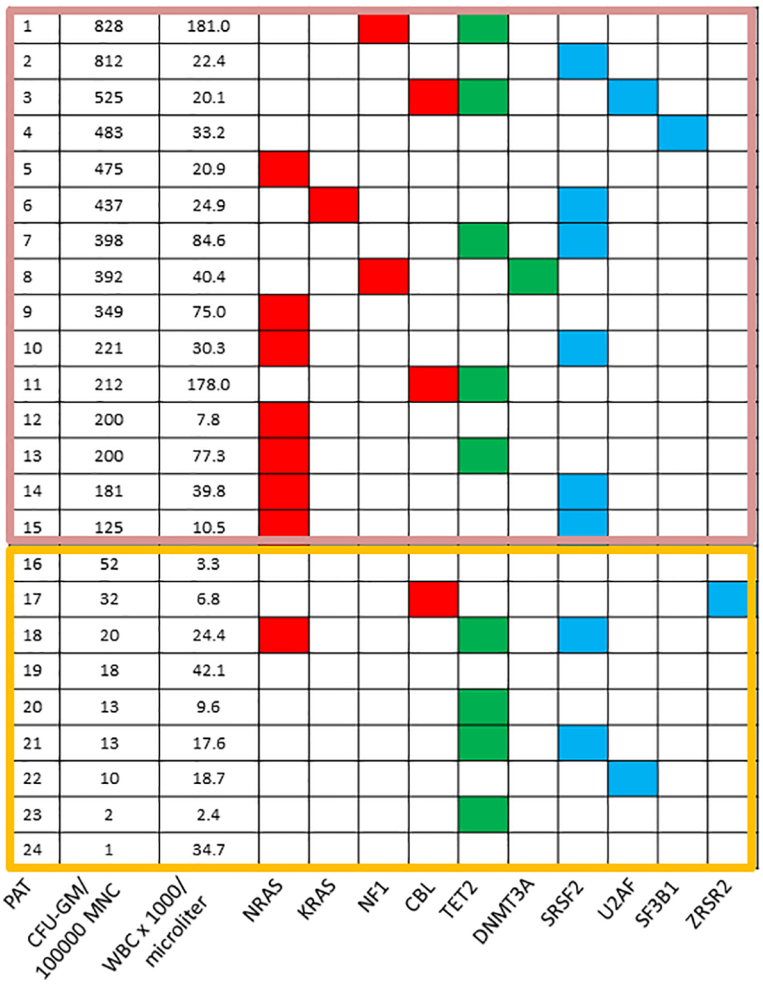


FIGURE 2 | Mutation profiles in CMML according to spontaneous CFU-GM growth. Each row corresponds to one patient. The first column indicates the patient number, the second the number of CFU-GM per 10^5 peripheral blood mononuclear cells (PBMNC), the third the white blood cell (WBC) count and all other columns represent the status of genes. Colored squares indicate mutated, white squares wild-type genes. The clores of mutant genes indicate the most affected functional categories. Red, green, and blue indicate RAS-pathway, epigenetic factors, and splicing factors, respectively. Mutations in the components of the RAS-pathway were found in 12/15 (80%) CMML patients with high colony growth (≥100 CFU-GM/10^5 PBMNC) and in 2/9 (22%) patients with low spontaneous colony formation (<100 CFU-GM/10^5 PBMNC). [adapted from Geissler, K. MEMO 2016 (81)].

of subsequent diagnosis of myeloid neoplasia. As some of the genes frequently mutated in age-related clonal hematopoesis such as *TET2* and *ASXL1* are also commonly mutated in CMML and aged hematopoiesis is characterized by a myelomonocytic differentiation bias it was recently hypothesized that CMML and aged hematopoiesis may be closely related (88). Analyses of the somatic mutation landscape of CMML by whole exome sequencing followed by gene-targeted validation indicated that most CMML patients (71%) had mutations in >2 age-related clonal hematopoiesis (ARCH) genes and 52% had >7 mutations overall (89). A higher mutation burden was associated with inferior survival. Considering age-adjusted population incidence and ARCH mutation rates one may speculate that CMML represents the leukemic conversion of the myelomonocytic-lineage-biased aged hematopoietic system.

There is now increasing evidence that mutations in *TET2* are in fact an initial clonal driver in CMML (88, 90). This view is based on the high frequency (60%) of these mutations in CMML patients (9, 19, 21, 22), the fact that *TET2* mutated clones can be detected in a small fraction of older subjects with clonal, but non-leukemic hematopoiesis (90–93), the competitive advantage of murine and human HSC invalidated for *TET2* (48, 85) and the results of single-cell clonal tracking experiments indicating that a *TET2* mutation, when present, is often the earliest recurrent genetic event in CMML (84). According to data from this study the preferred order of mutational accumulation is *TET2* (or *IDH1/2*) or *ASXL1 (EZH2)* first, followed by molecular aberrations in spliceosome components *(SRSF2, SF3B1, U2AF1, or ZRSR2)*. Mutations in the RAS-signaling pathway seem to be rather late events which induce GM-CSF hypersensitivity and myeloproliferation.

Progression of CMML and Transformation to AML

Around 20% of CMML patients progress and transform to AML. Although the mechanisms behind are not known in detail, available data suggest that molecular aberrations in chromatin modelling as well in cell signaling may contribute to this process. Among genes of the epigenetic machinery, ASXL1 may have the most important impact on transformation. The ASXL1 gene regulates chromatin by interacting with the polycob-group repressive complex proteins (PRC1 and PRC2). Abdel-Wahab et al. reported that *ASXL1* mutations resulted in loss of PRC2-mediated H3K27 trimethylation (94). In a study by Itzykson et al. in which the prognostic impact of different molecular aberrations in CMML patients was studied, only *ASXL1* mutations retained their significant impact on AML-free survival in the multivariate analysis indicating the major role of this molecular aberration in the transformation process (9). Of these, only nonsense and frameshift ASXL1 mutations have been shown to negatively impact OS. The impact of mutations of RAS-pathway components on progression/transformation of CMML is more complex. The first indication of a potential role of *NRAS* aberrations in CMML evolution has been reported, at the molecular level, by Ricci et al. (95). In this study molecular analyses have been performed in 22 MD-CMML patients and in 18 MP-CMML patients. MP-CMML patients had a higher frequency of *RAS* mutations compared with MD-CMML. In two patients who progressed from MDS-CMML to MP-CMML, allele specific PCR showed low levels of the *RAS* mutations at the time of myelodysplastic disease which became predominant in the myeloproliferative phase, documenting for the first time the expansion of a *RAS* mutated clone in concomitance with CMML evolution. Other studies have confirmed that the MPN phenotye of CMML is a disease phase significantly associated with hyperactivation of the RAS-pathway. In a study reported by the Austrian study group MP-CMML as compared to MD-CMML patients had higher circulating blasts, LDH, RAS-pathway mutations, more often splenomegaly and higher growth-factor-independent myeloid colony growth *in vitro* (12). Recently, genetic differences were assessed between subtypes in 973 molecularly annotated Mayo Clinic-GFM-Austrian CMML patients. In this analysis *NRAS* mutations alone did not reach statistical significance as an independent factor impacting AML-free survival, however, the combined oncogenic RAS-pathway category including *NRAS, KRAS* and *CBL* was statistically significant in a model that only included genetic factors (74). Considering the fact, that spontaneous colony formation in CMML functionally covers the most frequent RASopathy gene mutations (38) these data are in line with findings in a small study which have been previously reported. In this study patients with CMML growth-factor-independent colony formation after transformation was significantly increased as compared to CFU-GM growth before transformation (37). Furthermore, a correlation of RAS-pathway mutations and spontaneous myeloid colony growth with progression and transformation could be demonstrated in a retrospective analysis of 337 CMML patients (96). Moreover, recent preclinical models also suggest that activating *Nras* mutations and somatic loss-of-function mutations in *Tet2* exert cooperating effects and accelerate disease progression (97, 98). Altogether, these findings suggest that oncogenic RAS-pathway activation is a phenomenon associated with the MP-CMML phenotype, progressive disease and with transformation to AML.

RISK ASSESSMENT OF CMML

The management of patients with CMML should be based on risk assessment. Several studies have shown that the percentage of PB and BM blasts is the most important factor determining survival (7, 99–104). Genetic alterations including gene mutations (7, 9, 10, 32) and chromosomal aberrations (105–107) further refine prognosis and have been included in different prognostic scoring systems. In the EHA guideline from 2018 five risk stratification systems are recommended (7, 9, 10, 106, 108, 109). Mutations in *ASXL1* are included in all 3 molecularly based scoring systems whereas the molecular CMML-specific prognostic scoring system (CPSS-mol) also includes *NRAS, SETBP1* and *RUNX1* (10). A recent study validating different prognostic models demonstrated comparable performance with significant heterogeneity in predicting outcomes (110).

TREATMENT OF CMML

Traditionally many cancers have been treated with more or less unspecific treatments such as cytotoxic drugs in the past. In a molecular heterogenous malignancy this may have the advantage that many subclones may be impacted by one drug with the potential to improve symptoms associated with a high tumor mass and potentially improve survival. Unfortunately, these drugs often cause significant side effects due to the fact, that also normal cells from tissues with a high proliferation rate may be affected. Targeted drugs on the other hand may be of interest if they are able to specifically hit a cellular component which is critical for the pathophysiology of disease. Many examples from other cancers have shown that with targeted treatment we can expect higher efficacy and lower toxicity as compared to conventional therapy. Unfortunately, CMML is a clinically and molecularly heterogenous disease with sometimes multiple clones that may be pathophysiologically relevant. Theoretically, targeted treatment might offer clinical benefit only if these subclones contribute to inferior prognosis and/or symptoms in patients. Symptoms in patients with CMML are often the clinical consequence of a high tumor mass. Myeloproliferation in CMML is commonly associated with molecular aberrations in cytokine signaling. In particular, as mentioned before, molecular aberrations in components of the RAS signaling pathway are frequently found in these patients. On the other hand there are molecular markers that predict inferior survival. Targeting these components may have the potential to modify the biology of disease and to delay transformation to AML. For such concepts it would be important to know if targeted treatment, at all, will be beneficial in a complex disease such as CMML. Although this

question cannot be answered for patients with CMML at the moment, there is some indication from other malignancies that treatment of subclones could be beneficial in patients. Patel et al. published a small series of patients with BCR/ABL negative MPN and a IDH2 mutation who were treated with the IDH2 inhibitor enasidenib which is approved for the treatment of patients with AML harboring this molecular aberration. Although IDH2 is often subclonal in this disease treatment with this IDH2 inhibitor resulted in clinically meaningful responses in these patients (111).

Unspecific Targeting of DNA Replication by Cytotoxic Molecules

Etoposide (VP16) is a DNA-damaging molecule by inhibition of topoisomerase. Preliminary reports suggested that etposide could give good results in CMML, with true complete responses in some cases and in improvement rather than worsening of cytopenias (51) (**Table 4**). Hydroxyurea (HU), a potent ribonucleotide reductase inhibitor, acts as an S-phase-specific agent with inhibition of DNA synthesis. In a randomized phase III trial in patients with proliferative CMML, HU was more effective and achieved faster response than cytotoxic chemotherapy with VP16 (52). Interestingly, this study remains up to now the only randomized trial in a pure CMML patient population which demonstrated a survival benefit. Allogeneic stem cell transplantation which is the only curative therapy is rarely feasible because of age and/or comorbidities. While unresponsiveness to aggressive chemotherapy is a characteristic for most CMML patients, there may be subgroups that might benefit from more intensive chemotherapy. Although the presence of an *NPM1* mutation, in contrast to AML patients, is an inferior prognostic parameter in CMML, CMML patients with this molecular aberration have shown relatively high response rates in a retrospective analysis (112).

Specific Targeting of DNA Replication by Antibody-Drug Conjugates

More targeted treatment with cytotoxic drugs can be expected by more detailed immunophenotypical characterization of surface proteins on CMML stem cells which could be used as potential target structures for antibody-drug conjugates (113). One example is the use of the IL-3 receptor as target structure for tagraxofusp, a CD123-directed cytotoxin consisting of human IL-3 fused to truncated diphtheria toxin. This antibody-drug conjugate has shown impressive activity in blastic plasmacytoid dendritic-cell neoplasm (BPDCN) that overexpresses CD123 (114). In an early clinical trial in patients with relapsed/refractory CMML 80% (8/10) of the patients receiving tagraxofusp showed ≥50% reduction in splenomegaly and three patients achieved bone marrow CR (53).

Unspecific Targeting of the Epigenetic Machinery by Hypomethylating Agents

It is important to note that the approval of the hypomethylating agents (HMA) azacitidine and decitabine (DEC), respectively, was originally based on MDS studies which included only few patients with CMML. In a phase III clinical multicenter trial of 358 MDS patients including 11 patients with dysplastic CMML the median overall survival was 24.5 months in the azacitidine (AZA) group as compared to 15.0 months in the conventional care group leading to the FDA approval of AZA for this subtype of CMML (54). The approval of decitabine (DEC) for CMML was also based on a phase III clinical trial of 170 patients with MDS, 14 of them with CMML (55). The ORR was significantly higher in the DEC group versus supportive care (17% *vs.* 0%, $p < 0.001$), but the median OS was not significantly different between the two arms. Additional phase II studies confirmed the efficacy of hypomethylating agents in all subtypes of CMML and, therefore, these agents are considered commonly as standard of care for higher risk CMML (**Table 3**) (56–63). In the largest retrospective study with a pure CMML cohort patients were treated with AZA (n = 56) and DEC (n = 65) (115). The ORRs were 41% by the IWG MDS/MPN response criteria (AZA-56%, DEC-58%), with CR rates of <20% for both agents. No significant differences in response rates were seen between MP-CMML and MD-CMML. Similar findings were reported in a smaller, prospective phase II Italian study, with 43 CMML patients receiving DEC (63). The ORR after 6 cycles was 47.6%, with seven CRs (16.6%), eight marrow responses (19%), one partial response (2.4%) and four hematological improvements (9.5%). After a median follow-up of 51.5 months, median OS was 17 months, with responders having a significantly longer survival than non-responders. Despite some efficacy of HMA in CMML patients one has to keep in mind that this treatment does not alter mutational allele burden and disease biology (116).

Proof of efficacy but greatly variable response with HMA provide the rationale for searching biomarkers that predict response. Differentially methylated regions of DNA have been shown to separate DEC responders from non-responders by Meldi (117). Other predictors for response to HMA treatment were reported by Duchmann et al. (118). In a retrospective analysis of 174 CMML patients treated with HMA multivariate analysis mutations in *ASXL1* predicted lower ORR, and *RUNX1* mutations and *CBL* mutations predicted inferior OS. The combination of *TET2* mutation and *ASXL1* wildtype predicted higher CR and better OS. A multicenter retrospective study including 949 non-selected, consecutive CMML patients investigated whether HMA provide a benefit in subgroups of CMML patients (119). Adjusted median OS for patients treated with HU versus HMA was 15.6 months as compared to 20.7 months; ($p=0.0002$). In patients with MP-CMML, median OS was 12.6 months as compared to 17.6 months; ($p=0.0027$) for patients treated with HU versus HMA. HMA were not associated with an OS advantage for patients classified as having lower-risk disease (ie, MD-CMML with <10% blasts, CMML-0, or lower-risk CPSS). Considering all the caveats of a retrospective nonrandomized trial these data suggest HMA as the preferred treatment for patients with higher-risk CMML and those with MP-CMML. A recent European multicenter randomized phase III trial evaluating DEC +/- HU *versus* HU in advanced MP-CMML, however, did not show significant differences in outcome. Although HMA definitively play an important role in the management of CMML patients the need for newer, rationally derived therapies is apparent (120).

TABLE 4 | Potential molecular targets in CMML.

Target	Preclinical information	Clinical study	Reference
DNA-replication unspecific		Phase I/II, n=10; etoposide (VP16) oral 50 mg 2 x weekly – 100 mg 1 x daily ORR: 70%	Oscier (51)
		Phase III trial, n=105; HU arm: n=53; 1 g/d up to 4 g/d ORR: 60%, md OS 20 mo VP16 arm: n=52; 150 mg/wk up to 600 mg/wk ORR: 36%, md OS 9 mo	Wattel (52)
DNA-replication CD123-targeted		Phase II, n=10; tagraxofusp relapsed/refractory 80% (8/10) spleen response (≥50% reduction in splenomegaly) 3 patients achieved bone marrow CR	Patnaik (53)
Epigenetic machinery unspecific		Phase III, n=358, MDS including CMML AZA 525mg/m^2 per course ORR 27%, md OS 24.5 mo Conventional care group ORR 5%, OS 15.0 mo	Fenaux (54)
		Phase III, n=170, MDS including CMML DEC 135 mg/m^2 per course ORR 17%, md OS 12.1 mo Best supportive care ORR 0%, md OS 7.8 mo	Kantarjian (55)
		Phase II, n=19; DEC 100mg/m^2 per course, ORR: 69%, md OS 19 mo	Aribi (56)
		Phase II, n=31; DEC 135mg/m^2 per course, ORR: 36%, md OS 15 mo	Wijermans (57)
		Phase II, n=38; AZA 500-525mg/m^2 per course ORR. 39%, md OS 12 mo	Costa (58)
		Phase II, n=39; DEC 100mg/m^2 per course, ORR: 38%, md OS 18 mo	Braun (59)
		Phase II, n=10; AZA 500-525mg/m^2 per course, ORR. 60%, md OS 29 mo	Thorpe (60)
		Phase II, n=76; AZA 375-525mg/m^2 per course, ORR. 43%, md OS 29 mo	Ades (61)
		Phase II, n=48; AZA 500-525mg/m^2 per course, ORR. 70%, md OS 27.7 mo	Pleyer (62)
		Phase II, n=43; DEC 100mg/m^2 per course, ORR: 47.6%, md OS 17 mo	Santini (63)
Epigenetic machinery TET2-, IDH1-, IDH2-targeted	Treatment with vitamin C mimicked TET2 restoration in a reversible transgenic RNAi mouse model	Phase II, Ascorbic acid + AZA AML, MDS, MDS/MPN with TET2 mutations NCT03397173	Preclinical Cimmino (64)
GM-CSF signaling	growth factor independent *in vitro* myeloid colony formation by CMML cells was inhibited by the addition of Anti-GM-CSF antibodies Demonstration of GM-CSF hypersensitivity of CMML progenitors using phospho-STAT5 flow cytometry	Phase II, n=5, rhIL-10 4-8 mcg/kg/day sc no meaningful effects on the WBC counts, 1/3 patients with skin infiltration markedly improved during IL-10 therapy. Phase I, n= of 15, lenzilumab (anti-GM-CSF) 200-600 mg iv days 1, 15 and day 1 in subsequent cycles ORR of 33.3% 3/5 responders were NRAS mutated 1/10 nonresponders was NRAS mutated	Preclinical Geissler (35) Padron (36) Clinical Pöchlauer (65) Patnaik (66)
FLT3 signaling	Increased FLT3-signaling in an MPN model of mice carrying a mutation in the RING finger domain of c-CBL	Phase I/II, quizartinib (FLT3i) + AZA, MDS, MDS/MPN with FLT3 or CBL mutations NCT04493138	Preclinical Rathinam (67)
RAS pathway signaling	The MEKi PD0325901 induced a rapid and sustained reduction in leukocyte counts, enhanced erythropiesis, prolonged survival, corrected aberrant proliferation and differentiation of BM progenitor cells in a *Kras G12D* mouse model	Phase II, n=11 (RAS mutated CMML cohort); trametinib (MEKi), 2 mg/day orally ORR 27%, md OS 14.5 mo	Preclinical Lyubynska (68) Chang (69)
	The MEKi PD0325901 induced a durable drop in leukocyte counts, enhanced erythropoietic function and markedly reduced spleen sizes in a *Nf1 -/-* mouse model		Clinical Borthakur (70)

(Continued)

TABLE 4 | Continued

Target	Preclinical information	Clinical study	Reference
JAK signaling	The specific JAK2 inhibitor TG101209 inhibited spontaneous CFU-GM growth *in vitro* in all 10 CMML patients tested	Phase I/II, n=20, ruxolitinib in 5-20 mg twice daily ORR 35% 5/9 spleen response 10/11 symptom response	Preclinical Geissler (71) Clinical Padron (72) Preclinical
PI3K signaling	Inhibition of PI3K signaling was effective in *Kras+* and *NF1-* mouse models that show many characteristics of CMML including leukocytosis, anemia and splenomegaly		Akutagawa (73)
Cell cycle machinery	Pharmacologic inhibition of PLK1 was effective in *RAS* mutant patient-derived xenografts	Phase II, CFI-400945 (PLK4 inhibitor) + HMA AML, MDS, CMML NCT04730258	Preclinical Carr (74)
Inflammasome	Kras driven myeloproliferation and cytopenia was reversed by functional inactivation of NLRP3 as well as by therapeutic IL-1-receptor blockade.	Phase II, canakinumab (anti-IL-1ß) LR-MDS, CMML NCT04239157	Preclinical Hammershe (75)
Multiple signaling pathway	Combined inhibition of the MEK and JAK/STAT signaling greatly inhibited human and mouse CMML cell growth *in vitro*, rescued mutant *NrasG12D* expressing HSC function *in vivo*, and promoted long-term survival without evident disease manifestation in animals with RAS-pathway driven MP-CMML		Preclinical Kong (76)

ORR, Overall response rates; include CR, complete remission; PR, partial remission; HI, hematologic improvement.

Specific Targeting of the Epigenetic Machinery by IDH Inhibitors and TET2 Modifiers

TET2 enzymes have been shown to provide a homeostatic link between intracellular metabolism and epigenetic gene regulation (121). These evolutionary conserved dioxygenases play a key role in the conversion of 5-methyl-cytosine (5-mC) to 5-hydoxymethyl-cytosine (5-hmC). TET dioxygenases require alpha-ketoglutarate, oxygen, Fe(II), and ascorbate for optimal activity (122). Isocitrate dehydrogenase (IDH) is a key enzyme for cellular respiration in the tricarboxylic acid (TCA) cycle. *IDH* mutations found in malignancies block normal cellular differentiation and promote tumorigenesis *via* the abnormal production of the oncometabolite 2-hydroxy-glutarate (2-hG). Recently, two inhibitors targeting *IDH2* and *IDH1* gene mutations, have become important components in AML management since molecular aberrations of *IDH* genes can be found in 20% of patients AML (123, 124). Although mutations involving *IDH1* and *IDH2* are uncommon in CMML (1% and 5-10%, respectively) IDH1/2 inhibitors are likely to present therapeutic options for these patients.

Loss-of-function mutations in *TET2* occur in around 60% of CMML patients and are considered mutually exclusive with *IDH1/2* mutations. Recently there has been accumulated significant preclinical evidence suggesting that ascorbate can restore dysfunctional TET2 activity. Agatocleous et al. generated mice lacking Gulo, the enzyme responsible for ascorbate synthesis. The resulting phenotype resembled mice carrying a homozygous *Tet2* deletion (48). Indeed, ascorbate-depleted stem and progenitor cells showed decreased levels of 5-hmC, predominantly mediated by reduction of Tet2 function (125). On the other hand treatment with vitamin C mimicked Tet2 restoration in a reversible transgenic RNAi mouse model as described by Cimmino (64). Low ascorbate levels have been demonstrated in a subgroup of patients with hematologic malignancies (126). Although no beneficial effects of vitamin C intake regarding leukemia development have been seen in previous reports, these new preclinical data show that the possible impact of supra-physiological concentrations of vitamin C on leukemogenesis remains an interesting treatment concept, particular in CMML-patients harboring a partial or complete loss of TET2 function. In fact the is a current phase II trial which studies the effect of ascorbate in combination with AZA in patients with newly diagnosed AML, MDS, MDS/MPN with *TET2* mutations (NCT03397173).

Targeting of GM-CSF Associated Signaling

Geissler et al. have shown that growth factor-independent *in vitro* myeloid colony formation by CMML cells can be inhibited by the addition of anti-GM-CSF antibodies, but not by addition of antibodies against IL-6, Il-3, or G-CSF indicating that GM-CSF signaling may play an important role in the pathophysiology of CMML (35). Because of its cytokine synthesis-inhibiting effects IL-10 was studied on CMML cell growth *in vitro*. The addition of IL-10 revealed a profound and dose dependent inhibitory effect on spontaneous *in vitro* growth of CMML cells (35). It was shown that IL-10 induced suppression of CMML cell proliferation was associated with reduced GM-CSF production by leukemic cells, both at the mRNA and protein level. Therefore it was concluded that the inhibitory effect of IL-10 *in vitro* is most likely through suppression of endogenous GM-CSF release. Based on these findings a small pilot trial was initiated in which five patients with CMML were treated with 4µg/kg/day recombinant human IL-10 sc for 1 month and with 8 µg/kg/day for another month (65). Although no meaningful effects of IL-10 treatment was seen on the WBC counts in any of the five patients, one out of three

patients with histologically confirmed skin infiltration markedly improved during IL-10 therapy. IL-10 administration was associated with a decline in lysozyme serum levels, a biomarker of the monocytic cell lineage, and downregulation of CD86 which has been shown to be upregulated by GM-CSF and downregulated by IL-10 *in vitro*. Interestingly, the clinical impact of IL-10 war recently supported by a study in which cytokine profiles were analyzed using cryopreserved PB plasma samples from 215 CMML patients (127). CMML patients with decreased IL-10 expression were found to have a poor OS when compared to CMML patients with increased IL-10 expression (P = 0.017), even when adjusted for other prognostic features including ASXL1.

Lenzilumab is a monoclonal antibody with high affinity for human GM-CSF. Based on data showing that anti-GM-CSF antibodies significantly inhibited the growth factor independent myeloid *in vitro* colony formation from primary CMML patient samples (35) and a study reporting that 90% of primary CMML samples demonstrated GM-CSF-dependent STAT5 hypersensitivity (36) lenzilumab was studied in CMML patients. In this early clinical trial of 15 CMML patients the antibody was well tolerated and effective with a durable ORR of 33.3% (66).

Targeting of FLT3 Associated Signaling

The clinical management of *FLT3*-mutated AML has been changed by the development of FLT3 inhibitors such as midostaurin and gilteritinib which are now in use in the frontline and relapsed/refractory settings in patients with a *FLT3* mutation (128, 129). *FLT3* aberrations have been reported in 1-3% of CMML patients (9, 32). Although these aberrations are uncommon in CMML FLT3 signaling may also occur in wildtype *FLT3* malignancies. Thus, mice carrying a mutation in the RING finger domain of *c-CBL* develop a myeloproliferative disease involving hematopoietic progenitors that show increased FLT3 signaling (67). The incidence of molecular aberrations of the *CBL* gene has been reported from 10-14% (9, 11, 32) and thus is more common than that of the *FLT3* gene. Therefore, CMML patients with mutations in the *CBL* gene could be potential candidates for studies with FLT3 inhibitors. In an ongoing phase I/II trial the FLT3 inhibitor quizartinib in combination with AZA is investigated in patients with untreated or HMA-refractory MDS, MDS/MPN with *FLT3* or *CBL* mutations (NCT04493138).

Targeting of RAS-Pathway Signaling

Mutated RAS proteins have been deemed "undruggable" for a long time due to their high affinity for GTP and lack of accessible binding pockets. However, the discovery by Ostrem et al. of compounds that covalently bind to the switch II pocket of *KRAS G12C* provided the rationale for the development of inhibitors suitable for clinical testing (130). At the moment this concept does not play an important role in the treatment concepts for CMML, since the *KRAS G12C* mutation is extremely rare in CMML.

RAS proteins require post-translational farnesylation by the enzyme farnesyltransferase to become functionally active. Therefore, inhibitors of this enzyme have been considered as potential candidates for RAS-pathway inhibition. In a clinical phase III trial 85 patients with newly diagnosed JMML, a RAS pathway driven disease, were enrolled between 2001 and 2006 (131). 47 patients received the FTI tipifarnib alone in a phase II window before proceeding to HSCT. Tipifarnib as a single agent was safe and achieved a response rate of 51%, but failed to reduce relapse rates or improve long-term overall survival in the phase III trial. In a preliminary report of a phase II trial in CMML patients tipifarnib was well tolerated, however, had only limited efficacy (132).

The elucidation of the RAS/MEK/ERK signaling pathway in regulating cell proliferation has stimulated the development of selective MEK inhibitors (MEKi). These molecules represent promising therapies for RAS-driven neoplasias and RASopathies associated with hyperactivated RAS signaling. Preclinically, the MEKi PD0325901 was highly effective in reversing the CMML-like phenotype in a *Kras G12D* and in a *NF1 -/-* mouse model (68, 69). In a phase II study in patients with in Neurofibromatosis 1 (NF1) which is a prototypic RASopathy the MEKi selumetinib resulted in at least 20% reduction in the size of plexiform neurofibromas (pNF) from baseline in 71% of patients and was associated with clinically meaningful improvements (133). On the basis of this clinical benefit, selumetinib received FDA approval for children 2 years of age and older with inoperable, symptomatic pNF. In another phase II trial trametinib, another MEKi, was studied in patients with relapsed/refractory leukemias (70). Cohort 1 included patients with relapsed/refractory AML or high-risk MDS with *NRAS* or *KRAS* mutations, cohort 2 patients with AML, MDS, or CMML with a *RAS* wild-type mutation or an unknown mutation status, and cohort 3 patients with CMML with an *NRAS* or *KRAS* mutation. The recommended dose for trametinib was 2 mg orally daily. The overall response rates for cohorts 1, 2, and 3 were 20%, 3%, and 27%, respectively, with a preferential activity among myeloid malignancies with *RAS* mutations. Repeated cycles of trametinib were well tolerated with manageable or reversible toxicities. Thus, some therapeutic potential of trametinib was demonstrated in myeloid malignancies, particularly in RAS-pathway mutated CMML.

Targeting of JAK-Stat Signaling

There is some evidence of activity or JAK inhibitors in CMML patients. In a study by Geissler the specific JAK2 inhibitor TG101209 was found to either block or strongly inhibit spontaneous CFU-GM growth *in vitro* in all 10 CMML patients tested (71). Among these 10 patients 6 were tested by NGS and, in 5 of them, RAS-pathway hyperactivation was documented due to mutations in NRAS (n=3) or PTPN11 (n=2), respectively. In a *NRAS*-mutant CMML patient who was treated with the JAK1/2 inhibitor ruxolitinib off label, spleen response and the disappearance of constitutional symptoms was associated with a decrease of autonomous CFU-GM formation *ex vivo*. Thus, therapeutic potential of inhibition of the JAK2/STAT5 pathway by ruxolitinib in CMML is suggested. In a phase I/II clinical trial of ruxolitinib in 20 CMML patients the recommended dose of ruxolitinib was 20 mg twice daily and the ORR of 35%, 5/9 spleen responses, and 10/11 symptom responses were seen (72). Correlative analysis demonstrated a downregulation in

inflammatory cytokines and GM-CSF-dependent STAT5 phosphorylation in responders. Further studies are required to demonstrate a potential disease modifying effect of ruxolitinib in CMML.

Targeting PI3 Kinase Signaling

Biological crosstalk is a phenomenon in which one component of a signal transduction pathway can affects another pathway. Thus, the PI3 Kinase-pathway may be aberrantly activated in CMML without molecular aberration in it. Treatment with inhibitors of this aberrantly activated signaling could have the potential to impact malignant cell growth. Using the class I PIK3 inhibitor pictilisib this approach has been successfully applied in a *Kras G12D* and in a *NF1* -/- mouse model with a CMML-like phenotype (73). In this models, pictilisib attenuated activation of both PI3K/AKT and RAS/MEK/ERK pathways in primary hematopoietic cells. Several PI3K inhibitors have now received regulatory approval for the treatment of breast cancer and B-cell malignancies suggesting that the treatment concept of PI3K-pathway inhibition comes into the clinic (134, 135). Thus, based on some crosstalk between the RAS-signaling and the PI3K/AKT-pathway PI3K inhibitors could be important molecules for the design of future therapeutic strategies for patients with CMML.

Targeting the Cell Cycle Machinery

In MP-CMML RAS-pathway mutations are associated with a unique gene expression profile enriched in mitotic kinases including polo-like kinase 1 (*PLK1)* (74) as shown in a study using a multiomics platform and biochemical and molecular analyses. In this study unmutated MLL regulated *PLK1* transcript levels *via* promoter monomethylation of lysine 4 of histone 3. In the preclinical mouse model pharmacologic inhibition of PLK1 was effective in *RAS*-mutant patient-derived xenografts providing the rationale for a new biomarker-driven therapeutic approach in patients with proliferative CMML. Currently the administration of the PLK4 inhibitor CFI-400945 with or without HMA is tested in a phase II trial in patients with relapsed/refractory or untreated AML, MDS, or CMML (NCT04730258).

Targeting of the Inflammasome

The inflammasome is a multimeric protein complex including NLRP3, NLRC4, AIM2 and NLRP6 that initiates an inflammatory form of cell death (pyroptosis) and triggers the release of proinflammatory cytokines (136). Recently, a functional link between oncogenic *Kras G12D* and inflammasome activation was reported in a preclinical model (75). In this mouse model Kras driven myeloproliferation and cytopenia was reversed by functional inactivation of NLRP3. A similar phenotypic improvement was seen with therapeutic IL-1-receptor blockade. Importantly, Kras activation induced the production of reactive oxygen species suggesting that KRAS not only has an ongogenic driver function but also activates the proinflammatory machinery. These findings open a new therapeutic opportunity for Kras mediated malignancies including CMML. Interestingly, there is a current phase II study, in which the anti-IL1ß inhibitor canakinumab is studied in ESA or HMA-refractory low risk-MDS or CMML (NCT04239157).

Targeting More Than One Pathway

Given the complexity of CMML one can expect, that combinations of molecules impacting different pathways may yield better efficacy. At least in preclinical models this seems to be true (76). In Nras hyperactive mouse models mimicking MP-CMML inhibition of the MEK-pathway alone was only partially effective to improve disease associated features. Despite MEK inhibitor treatment 60% of *Nras G12D* expressing mice died within 20 weeks and surviving animals continued to retain their MP-CMML phenotype. Combined inhibition of the MEK and JAK/STAT signaling, however, greatly inhibited human and mouse CMML cell growth *in vitro*, rescued mutant *Nras G12D*-expressing HSC function *in vivo*, and promoted long-term survival without evident disease manifestation in animals with RAS-pathway driven MP-CMML. Still much work has to be done to address optimal ways to target these pathways in patients with CMML to improve clinical outcome.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

This article was supported by the "Gesellschaft zur Erforschung der Biologie und Therapie von Tumorkrankheiten" – ABCMML-112015".

REFERENCES

1. Bennett JM, Catovsky D, Daniel M-T, Flandrin G, Galton DAG, Gralnick HR, et al. Proposals for the Classification of the Acute Leukaemias French-American-British (FAB) Co-Operative Group. *Br J Haematol* (1976) 33 (4):451–8. doi: 10.1111/j.1365-2141.1976.tb03563.x
2. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the Classification of the Myelodysplastic Syndromes. *Br J Haematol* (1982) 51(2):189–99. doi: 10.1111/j.1365-2141.1982.tb08475.x
3. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 Revision of the World Health Organization (WHO) Classification of Myeloid Neoplasms and Acute Leukemia: Rationale and Important Changes. *Blood* (2009) 114(5):937–51. doi: 10.1182/blood-2009-03-209262
4. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick H, et al. The Chronic Myeloid Leukaemias: Guidelines for Distinguishing Chronic Granulocytic, Atypical Chronic Myeloid, and Chronic Myelomonocytic Leukaemia: Proposals by the French - American -

British Cooperative Leukaemia Group. *Br J Haematol* (1994) 87(4):746–54. doi: 10.1111/j.1365-2141.1994.tb06734.x
5. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) Classification of the Myeloid Neoplasms. *Blood* (2002) 100 (7):2292–302. doi: 10.1182/blood-2002-04-1199
6. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Beau L, et al. The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. *Blood* (2016) 127(20):2391–405. doi: 10.1182/blood-2016-03-643544
7. Onida F, Kantarjian HM, Smith TL, Ball G, Keating MJ, Estey EH, et al. Prognostic Factors and Scoring Systems in Chronic Myelomonocytic Leukemia: A Retrospective Analysis of 213 Patients. *Blood* (2002) 99 (3):840–9. doi: 10.1182/blood.v99.3.840
8. Patnaik MM, Padron E, LaBorde RR, Lasho TL, Finke CM, Hanson CA, et al. Mayo Prognostic Model for WHO-Defined Chronic Myelomonocytic Leukemia: ASXL1 and Spliceosome Component Mutations and Outcomes. *Leukemia* (2013) 27(7):1504–10. doi: 10.1038/leu.2013.88
9. Itzykson R, Kosmider O, Renneville A, Gelsi-Boyer V, Meggendorfer M, Morabito M, et al. Prognostic Score Including Gene Mutations in Chronic Myelomonocytic Leukemia. *J Clin Oncol* (2013) 31(19):2428–36. doi: 10.1200/JCO.2012.47.3314
10. Elena C, Gallì A, Such E, Meggendorfer M, Germing U, Rizzo E, et al. Integrating Clinical Features and Genetic Lesions in the Risk Assessment of Patients With Chronic Myelomonocytic Leukemia. *Blood* (2016) 128 (10):1408–17. doi: 10.1182/blood-2016-05-714030
11. Machherndl-Spandl S, Jäger E, Barna A, Gurbisz M, Marschon R, Graf T, et al. Impact of Age on the Cumulative Risk of Transformation in Patients With Chronic Myelomonocytic Leukaemia. *Eur J Haematol* (2021) 107 (2):265–74. doi: 10.1111/ejh.13647
12. Geissler K, Jäger E, Barna A, Gurbisz M, Marschon R, Graf T, et al. The Austrian Biodatabase for Chronic Myelomonocytic Leukemia (ABCMML): A Representative and Useful Real-Life Data Source for Further Biomedical Research. *Wien Klin Wochenschr* (2019) 131(17–18):410–8. doi: 10.1007/s00508-019-1526-1
13. Janssen JW, Steenvoorden AC, Lyons J, Anger B, Bohlke JU, Bos JL, et al. RAS Gene Mutations in Acute and Chronic Myelocytic Leukemias, Chronic Myeloproliferative Disorders, and Myelodysplastic Syndromes. *Proc Natl Acad Sci* (1987) 84(24):9228–32. doi: 10.1073/pnas.84.24.9228
14. Hirsch-Ginsberg C, LeMaistre AC, Kantarjian H, Talpaz M, Cork A, Freireich EJ, et al. RAS Mutations are Rare Events in Philadelphia Chromosome-Negative/Bcr Gene Rearrangement-Negative Chronic Myelogenous Leukemia, But Are Prevalent in Chronic Myelomonocytic Leukemia. *Blood* (1990) 76(6):1214–9. doi: 10.1182/blood.V76.6.1214.1214
15. Patnaik MM, Tefferi A. Chronic Myelomonocytic Leukemia: 2020 Update on Diagnosis, Risk Stratification and Management. *Am J Hematol* (2020) 95 (1):97–115. doi: 10.1002/ajh.25684
16. Abdel-Wahab O, Pardanani A, Patel J, Wadleigh M, Lasho T, Heguy A, et al. Concomitant Analysis of EZH2 and ASXL1 Mutations in Myelofibrosis, Chronic Myelomonocytic Leukemia and Blast-Phase Myeloproliferative Neoplasms. *Leukemia* (2011) 25(7):1200–2. doi: 10.1038/leu.2011.58
17. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, et al. Inactivating Mutations of the Histone Methyltransferase Gene EZH2 in Myeloid Disorders. *Nat Genet* (2010) 42(8):722–6. doi: 10.1038/ng.621
18. Gelsi-Boyer V, Trouplin V, Adélaïde J, Bonansea J, Cervera N, Carbuccia N, et al. Mutations of Polycomb-Associated Gene ASXL1 in Myelodysplastic Syndromes and Chronic Myelomonocytic Leukaemia. *Br J Haematol* (2009) 145(6):788–800. doi: 10.1111/j.1365-2141.2009.07697.x
19. Smith AE, Mohamedali AM, Kulasekararaj A, Lim Z, Gaken J, Lea NC, et al. Next-Generation Sequencing of the TET2 Gene in 355 MDS and CMML Patients Reveals Low-Abundance Mutant Clones With Early Origins, But Indicates No Definite Prognostic Value. *Blood* (2010) 116(19):3923–32. doi: 10.1182/blood-2010-03-274704
20. Jankowska AM, Makishima H, Tiu RV, Szpurka H, Huang Y, Traina F, et al. Mutational Spectrum Analysis of Chronic Myelomonocytic Leukemia Includes Genes Associated With Epigenetic Regulation: UTX, EZH2, and DNMT3A. *Blood* (2011) 118(14):3932–41. doi: 10.1182/blood-2010-10-311019
21. Grossmann V, Kohlmann A, Eder C, Haferlach C, Kern W, Cross NCP, et al. Molecular Profiling of Chronic Myelomonocytic Leukemia Reveals Diverse Mutations in <80% of Patients With TET2 and EZH2 Being of High Prognostic Relevance. *Leukemia* (2011) 25(5):877–9. doi: 10.1038/leu.2011.10
22. Tefferi A, Lim K-H, Abdel-Wahab O, Lasho TL, Patel J, Patnaik MM, et al. Detection of Mutant TET2 in Myeloid Malignancies Other Than Myeloproliferative Neoplasms: CMML, MDS, MDS/MPN and AML. *Leukemia* (2009) 23(7):1343–5. doi: 10.1038/leu.2009.59
23. Meggendorfer M, Roller A, Haferlach T, Eder C, Dicker F, Grossmann V, et al. SRSF2 Mutations in 275 Cases With Chronic Myelomonocytic Leukemia (CMML). *Blood* (2012) 120(15):3080–8. doi: 10.1182/blood-2012-01-404863
24. Patnaik MM, Lasho TL, Finke CM, Hanson CA, Hodnefield JM, Knudson RA, et al. Spliceosome Mutations Involving SRSF2, *SF3B1*, and *U2AF35* in Chronic Myelomonocytic Leukemia: Prevalence, Clinical Correlates, and Prognostic Relevance. *Am J Hematol* (2013) 88(3):201–6. doi: 10.1002/ajh.23373
25. Gelsi-Boyer V, Trouplin V, Adélaïde J, Aceto N, Remy V, Pinson S, et al. Genome Profiling of Chronic Myelomonocytic Leukemia: Frequent Alterations of RAS and RUNX1 Genes. *BMC Cancer* (2008) 8:299. doi: 10.1186/1471-2407-8-299
26. Kohlmann A, Grossmann V, Klein H-U, Schindela S, Weiss T, Kazak B, et al. Next-Generation Sequencing Technology Reveals a Characteristic Pattern of Molecular Mutations in 72.8% of Chronic Myelomonocytic Leukemia by Detecting Frequent Alterations in TET2, CBL, RAS, and RUNX1. *J Clin Oncol* (2010) 28(24):3858–65. doi: 10.1200/JCO.2009.27.1361
27. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJP, et al. Activating Mutation in the Tyrosine Kinase JAK2 in Polycythemia Vera, Essential Thrombocythemia, and Myeloid Metaplasia With Myelofibrosis. *Cancer Cell* (2005) 7(4):387–97. doi: 10.1016/j.ccr.2005.03.023
28. Makishima H, Cazzolli H, Szpurka H, Dunbar A, Tiu R, Huh J, et al. Mutations of E3 Ubiquitin Ligase Cbl Family Members Constitute a Novel Common Pathogenic Lesion in Myeloid Malignancies. *J Clin Oncol* (2009) 27(36):6109–16. doi: 10.1200/JCO.2009.23.7503
29. Daver N, Strati P, Jabbour E, Kadia T, Luthra R, Wang S, et al. FLT3 Mutations in Myelodysplastic Syndrome and Chronic Myelomonocytic Leukemia. *Am J Hematol* (2013) 88(1):56–9. doi: 10.1002/ajh.23345
30. Laborde RR, Patnaik MM, Lasho TL, Finke CM, Hanson CA, Knudson RA, et al. SETBP1 Mutations in 415 Patients With Primary Myelofibrosis or Chronic Myelomonocytic Leukemia: Independent Prognostic Impact in CMML. *Leukemia* (2013) 27(10):2100–2. doi: 10.1038/leu.2013.97
31. Damm F, Itzykson R, Kosmider O, Droin N, Renneville A, Chesnais V, et al. SETBP1 Mutations in 658 Patients With Myelodysplastic Syndromes, Chronic Myelomonocytic Leukemia and Secondary Acute Myeloid Leukemias. *Leukemia* (2013) 27(6):1401–3. doi: 10.1038/leu.2013.35
32. Patnaik MM, Lasho TL, Vijayvargiya P, Finke CM, Hanson CA, Ketterling RP, et al. Prognostic Interaction Between ASXL1 and TET2 Mutations in Chronic Myelomonocytic Leukemia. *Blood Cancer J* (2016) 6(1):e385–5. doi: 10.1038/bcj.2015.113
33. Padron E. Surveying the Landscape of MDS/MPN Research: Overlap Among the Overlap Syndromes? *Hematology* (2015) 2015(1):349–54. doi: 10.1182/asheducation-2015.1.349
34. Geissler K, Hinterberger W, Bettelheim P, Haas O, Lechner K. Colony Growth Characteristics in Chronic Myelomonocytic Leukemia. *Leuk Res* (1988) 12(5):373–7. doi: 10.1016/0145-2126(88)90055-0
35. Geissler K, Ohler L, Födinger M, Virgolini I, Leimer M, Kabrna E, et al. Interleukin 10 Inhibits Growth and Granulocyte/Macrophage Colony-Stimulating Factor Production in Chronic Myelomonocytic Leukemia Cells. *J Exp Med* (1996) 184(4):1377–84. doi: 10.1084/jem.184.4.1377
36. Padron E, Painter JS, Kunigal S, Mailloux AW, McGraw K, McDaniel JM, et al. GM-CSF-Dependent Pstat5 Sensitivity Is a Feature With Therapeutic Potential in Chronic Myelomonocytic Leukemia. *Blood* (2013) 121 (25):5068–77. doi: 10.1182/blood-2012-10-460170
37. Sagaster V, Ohler L, Berer A, Kabrna E, Ofner P, Lechner K, et al. High Spontaneous Colony Growth in Chronic Myelomonocytic Leukemia Correlates With Increased Disease Activity and Is a Novel Prognostic Factor for Predicting Short Survival. *Ann Hematol* (2004) 83(1):9–13. doi: 10.1007/s00277-003-0743-9
38. Geissler K, Jäger E, Barna A, Gurbisz M, Graf T, Graf E, et al. Molecular Basis and Clinical Application of Growth-Factor-Independent *In Vitro* Myeloid

Colony Formation in Chronic Myelomonocytic Leukemia. *Int J Mol Sci* (2020) 21(17):6057. doi: 10.3390/ijms21176057
39. Geissler K, Jäger E, Barna A, Graf T, Graf E, Öhler L, et al. Myelomonocytic Skewing in Chronic Myelomonocytic Leukemia: Phenotypic, Molecular and Biologic Features and Impact on Survival. *Eur J Haematol* (2021) 106 (5):627–33. doi: 10.1111/ejh.13577
40. Wang J, Liu Y, Li Z, Du J, Ryu M-J, Taylor PR, et al. Endogenous Oncogenic Nras Mutation Promotes Aberrant GM-CSF Signaling in Granulocytic/Monocytic Precursors in a Murine Model of Chronic Myelomonocytic Leukemia. *Blood* (2010) 116(26):5991–6002. doi: 10.1182/blood-2010-04-281527
41. Li Q, Haigis KM, McDaniel A, Harding-Theobald E, Kogan SC, Akagi K, et al. Hematopoiesis and Leukemogenesis in Mice Expressing Oncogenic NrasG12D From the Endogenous Locus. *Blood* (2011) 117(6):2022–32. doi: 10.1182/blood-2010-04-280750
42. Parikh C. Oncogenic NRAS Rapidly and Efficiently Induces CMML- and AML-Like Diseases in Mice. *Blood* (2006) 108(7):2349–57. doi: 10.1182/blood-2004-08-009498
43. Chan IT, Kutok JL, Williams IR, Cohen S, Kelly L, Shigematsu H, et al. Conditional Expression of Oncogenic K-Ras From Its Endogenous Promoter Induces a Myeloproliferative Disease. *J Clin Invest* (2004) 113(4):528–38. doi: 10.1172/JCI20476
44. Van Meter MEM, Diaz-Flores E, Archard JA, Passegue E, Irish JM, Kotecha N, et al. K-RasG12D Expression Induces Hyperproliferation and Aberrant Signaling in Primary Hematopoietic Stem/Progenitor Cells. *Blood* (2007) 109(9):3945–52. doi: 10.1182/blood-2006-09-047530
45. Murphy MA, Schnall RG, Venter DJ, Barnett L, Bertoncello I, Thien CB, et al. Tissue Hyperplasia and Enhanced T-Cell Signalling *via* ZAP-70 in C-Cbl-Deficient Mice. *Mol Cell Biol* (1998) 18(8):4872–82. doi: 10.1128/MCB.18.8.4872
46. Le DT. Somatic Inactivation of Nf1 in Hematopoietic Cells Results in a Progressive Myeloproliferative Disorder. *Blood* (2004) 103(11):4243–50. doi: 10.1182/blood-2003-08-2650
47. Chan G, Kalaitzidis D, Usenko T, Kutok JL, Yang W, Mohi MG, et al. Leukemogenic Ptpn11 Causes Fatal Myeloproliferative Disorder *via* Cell-Autonomous Effects on Multiple Stages of Hematopoiesis. *Blood* (2009) 113 (18):4414–24. doi: 10.1182/blood-2008-10-182626
48. Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Lobry D, Lobry C, et al. Tet2 Loss Leads to Increased Hematopoietic Stem Cell Self-Renewal and Myeloid Transformation. *Cancer Cell* (2011) 20(1):11–24. doi: 10.1016/j.ccr.2011.06.001
49. Li Z, Cai X, Cai C-L, Wang J, Zhang W, Petersen BE, et al. Deletion of Tet2 in Mice Leads to Dysregulated Hematopoietic Stem Cells and Subsequent Development of Myeloid Malignancies. *Blood* (2011) 118(17):4509–18. doi: 10.1182/blood-2010-12-325241
50. Wang J, Li Z, He Y, Pan F, Chen S, Rhodes S, et al. Loss of Asxl1 Leads to Myelodysplastic Syndrome–Like Disease in Mice. *Blood* (2014) 123(4):541–53. doi: 10.1182/blood-2013-05-500272
51. Oscier DG, Worsley A, Hamblin TJ, Mufti GJ. Treatment of Chronic Myelomonocytic Leukaemia with Low Dose Etoposide. *Br J Haematol* (1989) 72(3):468–71. doi: 10.1111/j.1365-2141.1989.tb07735.x
52. Wattel E, Guerci A, Hecquet B, Economopoulos T, Copplestone A, Mahé B, et al. A Randomized Trial of Hydroxyurea Versus VP16 in Adult Chronic Myelomonocytic Leukemia. Groupe Français Des Myélodysplasies and European CMML Group. *Blood* (1996) 88(7):2480–7.
53. Patnaik MM, Ali H, Gupta V, Schiller GJ, Lee S, Yacoub A, et al. Results From Ongoing Phase 1/2 Clinical Trial of Tagraxofusp (SL-401) in Patients With Relapsed/Refractory Chronic Myelomonocytic Leukemia (CMML). *Blood* (2018) 132:1821. doi: 10.1182/blood-2018-99-119302
54. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, et al. Efficacy of Azacitidine Compared With That of Conventional Care Regimens in the Treatment of Higher-Risk Myelodysplastic Syndromes: A Randomised, Open-Label, Phase III Study. *Lancet Oncol* (2009) 10(3):223–32. doi: 10.1016/S1470-2045(09)70003-8
55. Kantarjian H, Oki Y, Garcia-Manero G, Huang X, O'Brien S, Cortes J, et al. Results of a Randomized Study of 3 Schedules of Low-Dose Decitabine in Higher-Risk Myelodysplastic Syndrome and Chronic Myelomonocytic Leukemia. *Blood* (2007) 109(1):52–7. doi: 10.1182/blood-2006-05-021162
56. Aribi A, Borthakur G, Ravandi F, Shan J, Davisson J, Cortes J, et al. Activity of Decitabine, a Hypomethylating Agent, in Chronic Myelomonocytic Leukemia. *Cancer* (2007) 109(4):713–7. doi: 10.1002/cncr.22457
57. Wijermans PW, Rüter B, Baer MR, Slack JL, Saba HI, Lübbert M. Efficacy of Decitabine in the Treatment of Patients With Chronic Myelomonocytic Leukemia (CMML). *Leuk Res* (2008) 32(4):587–91. doi: 10.1016/j.leukres.2007.08.004
58. Costa R, Abdulhaq H, Haq B, Shadduck RK, Latsko J, Zenati M, et al. Activity of Azacitidine in Chronic Myelomonocytic Leukemia. *Cancer* (2011) 117(12):2690–6. doi: 10.1002/cncr.25759
59. Braun T, Itzykson R, Renneville A, de Renzis B, Dreyfus F, Laribi K, et al. Molecular Predictors of Response to Decitabine in Advanced Chronic Myelomonocytic Leukemia: A Phase 2 Trial. *Blood* (2011) 118(14):3824–31. doi: 10.1182/blood-2011-05-352039
60. Thorpe M, Montalvão A, Pierdomenico F, Moita F, Almeida A. Treatment of Chronic Myelomonocytic Leukemia With 5-Azacitidine: A Case Series and Literature Review. *Leuk Res* (2012) 36(8):1071–3. doi: 10.1016/j.leukres.2012.04.024
61. Adès L, Sekeres MA, Wolfromm A, Teichman ML, Tiu RV, Itzykson R, et al. Predictive Factors of Response and Survival Among Chronic Myelomonocytic Leukemia Patients Treated With Azacitidine. *Leuk Res* (2013) 37(6):609–13. doi: 10.1016/j.leukres.2013.01.004
62. Pleyer L, Germing U, Sperr WR, Linkesch W, Burgstaller S, Stauder R, et al. Azacitidine in CMML: Matched-Pair Analyses of Daily-Life Patients Reveal Modest Effects on Clinical Course and Survival. *Leuk Res* (2014) 38(4):475–83. doi: 10.1016/j.leukres.2014.01.006
63. Santini V, Allione B, Zini G, Gioia D, Lunghi M, Poloni A, et al. Multicentre Trial of Decitabine in Higher-Risk Chronic Myelomonocytic Leukemia. *Leukemia* (2018) 32(2):413–8. doi: 10.1038/leu.2017.186
64. Cimmino L, Dolgalev I, Wang Y, Yoshimi A, Martin GH, Wang J, et al. Restoration of TET2 Function Blocks Aberrant Self-Renewal and Leukemia Progression. *Cell* (2017) 170(6):1079–95.e20. doi: 10.1016/j.cell.2017.07.032
65. Pöchlauer S, Jäger E, Jäger U, Geissler K. Recombinant Human Interleukin-10 in Patients With Chronic Myelomonocytic Leukemia. *Ann Hematol* (2014) 93(10):1775–6. doi: 10.1007/s00277-014-2012-5
66. Patnaik MM, Sallman DA, Mangaonkar AA, Heuer R, Hirvela J, Zblewski D, et al. A Phase 1 Study of Lenzilumab, a Humaneered Recombinant Anti-Human Granulocyte-Macrophage Colony- Stimulating Factor (Anti-hGM-CSF) Antibody, for Chronic Myelomonocytic Leukemia (CMML). *Blood* (2019) 134(Suppl_1):4234–4. doi: 10.1182/blood-2019-131181
67. Rathinam C, Thien CBF, Flavell RA, Langdon WY. Myeloid Leukemia Development in C-Cbl RING Finger Mutant Mice Is Dependent on FLT3 Signaling. *Cancer Cell* (2010) 18(4):341–52. doi: 10.1016/j.ccr.2010.09.008
68. Lyubynska N, Gorman MF, Lauchle JO, Hong WX, Akutagawa JK, Shannon K, et al. A MEK Inhibitor Abrogates Myeloproliferative Disease in Kras Mutant Mice. *Sci Trans Med* (2011) 3(76):76ra27. doi: 10.1126/scitranslmed.3001069
69. Chang T, Krisman K, Theobald EH, Xu J, Akutagawa J, Lauchle JO, et al. Sustained MEK Inhibition Abrogates Myeloproliferative Disease in Nf1 Mutant Mice. *J Clin Invest* (2013) 123(1):335–9. doi: 10.1172/JCI63193
70. Borthakur G, Popplewell L, Boyiadzis M, Foran J, Platzbecker U, Vey N, et al. Activity of the Oral Mitogen-Activated Protein Kinase Kinase Inhibitor Trametinib in RAS-Mutant Relapsed or Refractory Myeloid Malignancies. *Cancer* (2016) 122(12):1871–9. doi: 10.1002/cncr.29986
71. Geissler K, Jäger E, Barna A, Sliwa T, Knöbl P, Schwarzinger I, et al. *In Vitro* and *In Vivo* Effects of JAK2 Inhibition in Chronic Myelomonocytic Leukemia. *Eur J Haematol* (2016) 97(6):562–7. doi: 10.1111/ejh.12773
72. Padron E, Dezern A, Andrade-Campos M, Vaddi K, Scherle P, Zhang Q, et al. A Multi-Institution Phase I Trial of Ruxolitinib in Patients With Chronic Myelomonocytic Leukemia (CMML). *Clin Cancer Res* (2016) 22 (15):3746–54. doi: 10.1158/1078-0432.CCR-15-2781
73. Akutagawa J, Huang TQ, Epstein I, Chang T, Quirindongo-Crespo M, Cottonham CL, et al. Targeting the PI3K/Akt Pathway in Murine MDS/MPN Driven by Hyperactive Ras. *Leukemia* (2016) 30(6):1335–43. doi: 10.1038/leu.2016.14
74. Carr RM, Vorobyev D, Lasho T, Marks DL, Tolosa EJ, Vedder A, et al. RAS Mutations Drive Proliferative Chronic Myelomonocytic Leukemia via a KMT2A-PLK1 Axis. *Nat Commun* (2021) 12(1):2901. doi: 10.1038/s41467-021-23186-w

75. Hamarsheh S, Osswald L, Saller BS, Unger S, De Feo D, Vinnakota JM, et al. Oncogenic KrasG12D Causes Myeloproliferation *via* NLRP3 Inflammasome Activation. *Nat Commun* (2020) 11(1):1659. doi: 10.1038/s41467-020-15497-1
76. Kong G, Wunderlich M, Yang D, Ranheim EA, Young KH, Wang J, et al. Combined MEK and JAK Inhibition Abrogates Murine Myeloproliferative Neoplasm. *J Clin Invest* (2014) 124(6):2762–73. doi: 10.1172/JCI74182
77. Yizhak K, Aguet F, Kim J, Hess JM, Kübler K, Grimsby J, et al. RNA Sequence Analysis Reveals Macroscopic Somatic Clonal Expansion Across Normal Tissues. *Science* (2019) 364(6444):eaaw0726. doi: 10.1126/science.aaw0726
78. Prchal JF, Adamson JW, Murphy S, Steinmann L, Fialkow PJ. Polycythemia Vera. The *In Vitro* Response of Normal and Abnormal Stem Cell Lines to Erythropoietin. *J Clin Invest* (1978) 61(4):1044–7. doi: 10.1172/JCI109003
79. Juvonen E, Partanen S, Ruutu T. Colony Formation by Megakaryocytic Progenitors in Essential Thrombocythaemia. *Br J Haematol* (1987) 66 (2):161–4. doi: 10.1111/j.1365-2141.1987.tb01293.x
80. Estrov Z, Grunberger T, Chan HS, Freedman MH. Juvenile Chronic Myelogenous Leukemia: Characterization of the Disease Using Cell Cultures. *Blood* (1986) 67(5):1382–7. doi: 10.1182/blood.V67.5.1382.1382
81. Geissler K. Molecular Pathogenesis of Chronic Myelomonocytic Leukemia. *Memo - Mag Eur Med Oncol* (2016) 9(4):172–7. doi: 10.1007/s12254-016-0295-z
82. Geissler K, Jäger E, Barna A, Alendar T, Ljubuncic E, Sliwa T, et al. Chronic Myelomonocytic Leukemia Patients With RAS Pathway Mutations Show High *In Vitro* Myeloid Colony Formation in the Absence of Exogenous Growth Factors. *Leukemia* (2016) 30(11):2280–1. doi: 10.1038/leu.2016.235
83. Oehler L, Foedinger M, Koeller M, Kollars M, Reiter E, Bohle B, et al. Interleukin-10 Inhibits Spontaneous Colony-Forming Unit-Granulocyte-Macrophage Growth From Human Peripheral Blood Mononuclear Cells by Suppression of Endogenous Granulocyte-Macrophage Colony-Stimulating Factor Release. *Blood* (1997) 89(4):1147–53. doi: 10.1182/blood.V89.4.1147
84. Itzykson R, Kosmider O, Renneville A, Morabito M, Preudhomme C, Berthon C, et al. Clonal Architecture of Chronic Myelomonocytic Leukemias. *Blood* (2013) 121(12):2186–98. doi: 10.1182/blood-2012-06-440347
85. Pronier E, Almire C, Mokrani H, Vasanthakumar A, Simon A, da Costa Reis Monte Mor B, et al. Inhibition of TET2-Mediated Conversion of 5-Methylcytosine to 5-Hydroxymethylcytosine Disturbs Erythroid and Granulomonocytic Differentiation of Human Hematopoietic Progenitors. *Blood* (2011) 118(9):2551–5. doi: 10.1182/blood-2010-12-324707
86. Bapat A, Keita N, Martelly W, Kang P, Seet C, Jacobsen JR, et al. Myeloid Disease Mutations of Splicing Factor SRSF2 Cause G2-M Arrest and Skewed Differentiation of Human Hematopoietic Stem and Progenitor Cells. *Stem Cells (Dayton Ohio)* (2018) 36(11):1663–75. doi: 10.1002/stem.2885
87. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal Hematopoiesis of Indeterminate Potential and Its Distinction From Myelodysplastic Syndromes. *Blood* (2015) 126(1):9–16. doi: 10.1182/blood-2015-03-631747
88. Itzykson R, Solary E. An Evolutionary Perspective on Chronic Myelomonocytic Leukemia. *Leukemia* (2013) 27(7):1441–50. doi: 10.1038/leu.2013.100
89. Mason CC, Khorashad JS, Tantravahi SK, Kelley TW, Zabriskie MS, Yan D, et al. Age-Related Mutations and Chronic Myelomonocytic Leukemia. *Leukemia* (2016) 30(4):906–13. doi: 10.1038/leu.2015.337
90. Busque L, Patel JP, Figueroa ME, Vasanthakumar A, Provost S, Hamilou Z, et al. Recurrent Somatic TET2 Mutations in Normal Elderly Individuals With Clonal Hematopoiesis. *Nat Genet* (2012) 44(11):1179–81. doi: 10.1038/ng.2413
91. Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, et al. Age-Related Mutations Associated With Clonal Hematopoietic Expansion and Malignancies. *Nat Med* (2014) 20(12):1472–8. doi: 10.1038/nm.3733
92. Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal Hematopoiesis and Blood-Cancer Risk Inferred From Blood DNA Sequence. *N Engl J Med* (2014) 371(26):2477–87. doi: 10.1056/NEJMoa1409405
93. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-Related Clonal Hematopoiesis Associated With Adverse Outcomes. *N Engl J Med* (2014) 371(26):2488–98. doi: 10.1056/NEJMoa1408617
94. Abdel-Wahab O, Adli M, LaFave LM, Gao J, Hricik T, Shih AH, et al. ASXL1 Mutations Promote Myeloid Transformation Through Loss of PRC2-Mediated Gene Repression. *Cancer Cell* (2012) 22(2):180–93. doi: 10.1016/j.ccr.2012.06.032
95. Ricci C, Fermo E, Corti S, Molteni M, Faricciotti A, Cortelezzi A, et al. RAS Mutations Contribute to Evolution of Chronic Myelomonocytic Leukemia to the Proliferative Variant. *Clin Cancer Res* (2010) 16(8):2246–56. doi: 10.1158/1078-0432.CCR-09-2112
96. Geissler K, Jäger E, Barna A, Gurbisz M, Graf T, Graf E, et al. Correlation of RAS-Pathway Mutations and Spontaneous Myeloid Colony Growth With Progression and Transformation in Chronic Myelomonocytic Leukemia—A Retrospective Analysis in 337 Patients. *Int J Mol Sci* (2020) 21(8):3025. doi: 10.3390/ijms21083025
97. Kunimoto H, Meydan C, Nazir A, Whitfield J, Shank K, Rapaport F, et al. Cooperative Epigenetic Remodeling by TET2 Loss and NRAS Mutation Drives Myeloid Transformation and MEK Inhibitor Sensitivity. *Cancer Cell* (2018) 33(1):44–59.e8. doi: 10.1016/j.ccell.2017.11.012
98. Jin X, Qin T, Zhao M, Bailey N, Liu L, Yang K, et al. Oncogenic N-Ras and Tet2 Haploinsufficiency Collaborate to Dysregulate Hematopoietic Stem and Progenitor Cells. *Blood Adv* (2018) 2(11):1259–71. doi: 10.1182/bloodadvances.2018017400
99. Fenaux P, Beuscart R, Lai JL, Jouet JP, Bauters F. Prognostic Factors in Adult Chronic Myelomonocytic Leukemia: An Analysis of 107 Cases. *J Clin Oncol: Off J Am Soc Clin Oncol* (1988) 6(9):1417–24. doi: 10.1200/JCO.1988.6.9.1417
100. Germing U, Strupp C, Aivado M, Gattermann N. New Prognostic Parameters for Chronic Myelomonocytic Leukemia. *Blood* (2002) 100 (2):731–2; author reply 732-733. doi: 10.1182/blood-2002-01-0330
101. Storniolo AM, Moloney WC, Rosenthal DS, Cox C, Bennett JM. Chronic Myelomonocytic Leukemia. *Leukemia* (1990) 4(11):766–70.
102. Schuler E, Schroeder M, Neukirchen J, Strupp C, Xicoy B, Kündgen A, et al. Refined Medullary Blast and White Blood Cell Count Based Classification of Chronic Myelomonocytic Leukemias. *Leuk Res* (2014) 38(12):1413–9. doi: 10.1016/j.leukres.2014.09.003
103. Tefferi A, Hoagland HC, Therneau TM, Pierre RV. Chronic Myelomonocytic Leukemia: Natural History and Prognostic Determinants. *Mayo Clin Proc* (1989) 64(10):1246–54. doi: 10.1016/s0025-6196(12)61287-7
104. Worsley A, Oscier DG, Stevens J, Darlow S, Figes A, Mufti GJ, et al. Prognostic Features of Chronic Myelomonocytic Leukaemia: A Modified Bournemouth Score Gives the Best Prediction of Survival. *Br J Haematol* (1988) 68(1):17–21. doi: 10.1111/j.1365-2141.1988.tb04173.x
105. Such E, Cervera J, Costa D, Solé F, Vallespí T, Luño E, et al. Cytogenetic Risk Stratification in Chronic Myelomonocytic Leukemia. *Haematologica* (2011) 96(3):375–83. doi: 10.3324/haematol.2010.030957
106. Such E, Germing U, Malcovati L, Cervera J, Kuendgen A, Della Porta MG, et al. Development and Validation of a Prognostic Scoring System for Patients With Chronic Myelomonocytic Leukemia. *Blood* (2013) 121 (15):3005–15. doi: 10.1182/blood-2012-08-452938
107. Wassie EA, Itzykson R, Lasho TL, Kosmider O, Finke CM, Hanson CA, et al. Molecular and Prognostic Correlates of Cytogenetic Abnormalities in Chronic Myelomonocytic Leukemia: A Mayo Clinic-French Consortium Study. *Am J Hematol* (2014) 89(12):1111–5. doi: 10.1002/ajh.23846
108. Itzykson R, Fenaux P, Bowen D, Cross NCP, Cortes J, De Witte T, et al. Diagnosis and Treatment of Chronic Myelomonocytic Leukemias in Adults: Recommendations From the European Hematology Association and the European LeukemiaNet. *HemaSphere* (2018) 1. doi: 10.1097/HS9.0000000000000150
109. Patnaik MM, Itzykson R, Lasho TL, Kosmider O, Finke CM, Hanson CA, et al. ASXL1 and SETBP1 Mutations and Their Prognostic Contribution in Chronic Myelomonocytic Leukemia: A Two-Center Study of 466 Patients. *Leukemia* (2014) 28(11):2206–12. doi: 10.1038/leu.2014.125
110. Padron E, Garcia-Manero G, Patnaik MM, Itzykson R, Lasho T, Nazha A, et al. An International Data Set for CMML Validates Prognostic Scoring Systems and Demonstrates a Need for Novel Prognostication Strategies. *Blood Cancer J* (2015) 5(7):e333. doi: 10.1038/bcj.2015.53
111. Patel AA, Cahill K, Charnot-Katsikas A, Liu H, Gurbuxani S, Thirman M, et al. Clinical Outcomes of *IDH2* -Mutated Advanced-Phase Ph-Negative

Myeloproliferative Neoplasms Treated With Enasidenib. *Br J Haematol* (2020) 190(1):e48–51. doi: 10.1111/bjh.16709
112. Montalban-Bravo G, Kanagal-Shamanna R, Sasaki K, Patel K, Ganan-Gomez I, Jabbour E, et al. NPM1 Mutations Define a Specific Subgroup of MDS and MDS/MPN Patients With Favorable Outcomes With Intensive Chemotherapy. *Blood Adv* (2019) 3(6):922–33. doi: 10.1182/bloodadvances.2018026989
113. Eisenwort G, Sadovnik I, Keller A, Ivanov D, Peter B, Berger D, et al. Phenotypic Characterization of Leukemia-Initiating Stem Cells in Chronic Myelomonocytic Leukemia. *Leukemia* (2021). doi: 10.1038/s41375-021-01227-z
114. Pemmaraju N, Lane AA, Sweet KL, Stein AS, Vasu S, Blum W, et al. Tagraxofusp in Blastic Plasmacytoid Dendritic-Cell Neoplasm. *N Engl J Med* (2019) 380(17):1628–37. doi: 10.1056/NEJMoa1815105
115. Coston T, Pophali P, Vallapureddy R, Lasho TL, Finke CM, Ketterling RP, et al. Suboptimal Response Rates to Hypomethylating Agent Therapy in Chronic Myelomonocytic Leukemia; a Single Institutional Study of 121. *Am J Hematol* (2019). doi: 10.1002/ajh.25488
116. Merlevede J, Droin N, Qin T, Meldi K, Yoshida K, Morabito M, et al. Mutation Allele Burden Remains Unchanged in Chronic Myelomonocytic Leukaemia Responding to Hypomethylating Agents. *Nat Commun* (2016) 7 (1):10767. doi: 10.1038/ncomms10767
117. Meldi K, Qin T, Buchi F, Droin N, Sotzen J, Micol J-B, et al. Specific Molecular Signatures Predict Decitabine Response in Chronic Myelomonocytic Leukemia. *J Clin Invest* (2015) 125(5):1857–72. doi: 10.1172/JCI78752
118. Duchmann M, Yalniz FF, Sanna A, Sallman D, Coombs CC, Renneville A, et al. Prognostic Role of Gene Mutations in Chronic Myelomonocytic Leukemia Patients Treated With Hypomethylating Agents. *EBioMedicine* (2018) 31:174–81. doi: 10.1016/j.ebiom.2018.04.018
119. Pleyer L, Leisch M, Kourakli A, Padron E, Maciejewski JP, Xicoy Cirici B, et al. Outcomes of Patients With Chronic Myelomonocytic Leukaemia Treated With Non-Curative Therapies: A Retrospective Cohort Study. *Lancet Haematol* (2021) 8(2):e135–48. doi: 10.1016/S2352-3026(20) 30374-4
120. Itzykson R, Santini V, Chaffaut C, Lionel A, Thepot S, Giagounidis A, et al. Decitabine Versus Hydroxyurea for Advanced Proliferative CMML: Results of the Emsco Randomized Phase 3 Dacota Trial. *Blood* (2020) 136(Suppl 1):53–4. doi: 10.1182/blood-2020-138680
121. Kaelin WG, McKnight SL. Influence of Metabolism on Epigenetics and Disease. *Cell* (2013) 153(1):56–69. doi: 10.1016/j.cell.2013.03.004
122. Blaschke K, Ebata KT, Karimi MM, Zepeda-Martínez JA, Goyal P, Mahapatra S, et al. Vitamin C Induces Tet-Dependent DNA Demethylation and a Blastocyst-Like State in ES Cells. *Nature* (2013) 500 (7461):222–6. doi: 10.1038/nature12362
123. DiNardo CD, Stein EM, de Botton S, Roboz GJ, Altman JK, Mims AS, et al. Durable Remissions With Ivosidenib in *IDH1* -Mutated Relapsed or Refractory AML. *N Engl J Med* (2018) 378(25):2386–98. doi: 10.1056/ NEJMoa1716984
124. Stein EM, DiNardo CD, Pollyea DA, Fathi AT, Roboz GJ, Altman JK, et al. Enasidenib in Mutant IDH2 Relapsed or Refractory Acute Myeloid Leukemia. *Blood* (2017) 130(6):722–31. doi: 10.1182/blood-2017-04-779405
125. Agathocleous M, Meacham CE, Burgess RJ, Piskounova E, Zhao Z, Crane GM, et al. Ascorbate Regulates Haematopoietic Stem Cell Function and Leukaemogenesis. *Nature* (2017) 549(7673):476–81. doi: 10.1038/nature23876
126. Huijskens MJAJ, Wodzig WKWH, Walczak M, Germeraad WTV, Bos GMJ. Ascorbic Acid Serum Levels are Reduced in Patients With Hematological Malignancies. *Results Immunol* (2016) 6:8–10. doi: 10.1016/j.rinim.2016.01.001
127. Niyongere S, Lucas N, Zhou J-M, Sansil S, Pomicter AD, Balasis ME, et al. Heterogeneous Expression of Cytokines Accounts for Clinical Diversity and Refines Prognostication in CMML. *Leukemia* (2019) 33(1):205–16. doi: 10.1038/s41375-018-0203-0
128. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield C, et al. Midostaurin Plus Chemotherapy for Acute Myeloid Leukemia With a *FLT3* Mutation. *N Engl J Med* (2017) 377(5):454–64. doi: 10.1056/NEJMoa1614359
129. Perl AE, Martinelli G, Cortes JE, Neubauer A, Berman E, Paolini S, et al. Gilteritinib or Chemotherapy for Relapsed or Refractory FLT3 -Mutated AML. *N Engl J Med* (2019) 381(18):1728–40. doi: 10.1056/NEJMoa1902688
130. Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM. K-Ras(G12C) Inhibitors Allosterically Control GTP Affinity and Effector Interactions. *Nature* (2013) 503(7477):548–51. doi: 10.1038/nature12796
131. Stieglitz E, Ward AF, Gerbing RB, Alonzo TA, Arceci RJ, Liu YL, et al. Phase II/III Trial of a Pre-Transplant Farnesyl Transferase Inhibitor in Juvenile Myelomonocytic Leukemia: A Report From the Children's Oncology Group: Pre-HSCT Farnesyl Transferase Inhibition in JMML. *Pediatr Blood Cancer* (2015) 62(4):629–36. doi: 10.1002/pbc.25342
132. Patnaik MM, Sallman DA, Sekeres MA, Luger S, Bejar R, Hobbs GS, et al. Preliminary Results From an Open-Label, Phase 2 Study of Tipifarnib in Chronic Myelomonocytic Leukemia (CMML). *Blood* (2017) 130:2963.
133. Gross AM, Wolters PL, Dombi E, Baldwin A, Whitcomb P, Fisher MJ, et al. Selumetinib in Children With Inoperable Plexiform Neurofibromas. *N Engl J Med* (2020) 382(15):1430–42. doi: 10.1056/NEJMoa1912735
134. Turner NC, Ro J, André F, Loi S, Verma S, Iwata H, et al. Palbociclib in Hormone-Receptor–Positive Advanced Breast Cancer. *N Engl J Med* (2015) 373(3):209–19. doi: 10.1056/NEJMoa1505270
135. 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(11):997–1007. doi: 10.1056/NEJMoa1315226
136. Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in Health and Disease. *Nature* (2012) 481(7381):278–86. doi: 10.1038/nature10759

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

frontiers in Oncology

CASE REPORT
published: 11 October 2021
doi: 10.3389/fonc.2021.753842

Diagnosis and Treatment of Myeloproliferative Neoplasms With PCM1-JAK2 Rearrangement: Case Report and Literature Review

Yingxin Sun [1,2†], Yifeng Cai [3†], Jia Chen [1,2], Jiannong Cen [1,2], Mingqing Zhu [1,2], Jinlan Pan [1,2], Depei Wu [1,2], Aining Sun [1,2] and Suning Chen [1,2]*

[1] Department of Hematology, First Affiliated Hospital of Soochow University, National Clinical Research Center for Hematologic Diseases, Jiangsu Institute of Hematology, Soochow University, Suzhou, China, [2] Department of Thrombosis and Hemostasis, Key Laboratory of Thrombosis and Hemostasis of Ministry of Health, Suzhou, China, [3] Department of Hematology, The Affiliated Hospital of Nantong University, Nantong University, Nantong, China

OPEN ACCESS

Edited by:
Argiris Symeonidis, University of Patras, Greece

Reviewed by:
Angelo Michele Carella, Casa Sollievo della Sofferenza (IRCCS), Italy
Yi Miao, Nanjing Medical University, China

****Correspondence:***
Suning Chen
chensuning@suda.edu.cn

[†]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: *05 August 2021*
Accepted: *22 September 2021*
Published: *11 October 2021*

Citation:
Sun Y, Cai Y, Chen J, Cen J, Zhu M, Pan J, Wu D, Sun A and Chen S (2021) Diagnosis and Treatment of Myeloproliferative Neoplasms With PCM1-JAK2 Rearrangement: Case Report and Literature Review. Front. Oncol. 11:753842. doi: 10.3389/fonc.2021.753842

Myeloproliferative neoplasm (MPN) with PCM1-JAK2 rearrangement is a rare disease with poor prognosis and lacks uniform treatment guidelines. Several studies confirmed the efficacy of ruxolitinib in hematological malignancies with PCM1-JAK2 fusion, but the efficacy is variable. Here, we report two patients diagnosed with MPN with PCM1-JAK2 fusion who were treated with ruxolitinib-based regimen, including the first case of ruxolitinib combined with pegylated interferon (Peg-IFN), and we conduct a literature review. We found that ruxolitinib combined with Peg-IFN is an effective treatment option in the case of poor efficacy of ruxolitinib monotherapy.

Keywords: myeloproliferative neoplasms, PCM1-JAK2, ruxolitinib, pegylated interferon, case report

INTRODUCTION

JAK2 components play an important role in hematopoiesis, cell proliferation, and differentiation. Abnormal activation of JAK pathway by gene mutations or rearrangements is common in Philadelphia-negative myeloproliferative neoplasm (MPN). About 75% of typical Philadelphia-negative MPN, including essential thrombocytosis (ET), polycythemia vera (PV), and primary myelofibrosis (PMF), carry a specific V617F somatic mutation in JAK2 gene (1, 2). In contrast, chromosomal translocations involving JAK2 gene are rare and have been reported in various hematological malignancies. Among them, the PCM1-JAK2 fusion gene derived from t(8;9)(p22; p24) is the most frequent (3–7). MPN with PCM1-JAK2 rearrangement is accompanied by varying degrees of eosinophilia, lymphadenopathy/hepatosplenomegaly, and myelofibrosis (8). Based on share characteristics, myeloid/lymphoid neoplasms (MLNs) with PCM1-JAK2 rearrangement has been added as a provisional entity in the 2016 World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia (9).

MLN with PCM1-JAK2 rearrangement is a rare disease with poor prognosis and lacks unified treatment guidelines. Up to now, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only way that can cure the disease. Ruxolitinib, a JAK2 inhibitor, has been approved by the United States Food and Drug Administration for the treatment of intermediate- and high-risk PMF

according to the findings of two randomized controlled trials (10, 11). However, ruxolitinib is not yet approved for MPN with PCM1-JAK2 rearrangement, although it has been shown to be effective. Here, we report two patients with MPN with PCM1-JAK2 fusion who received ruxolitinib-based regimen, one of which is the first report of ruxolitinib combined with pegylated interferon (Peg-IFN) at home and abroad.

CASE PRESENTATION

Case 1

In October 2020, a 42-year-old man was referred to our department because of leukocytosis (leukocyte 48.4 × 10^9/L) with eosinophilia (eosinophils 3.97 × 10^9/L), mononucleosis (monocyte 1.9 × 10^9/L), and anemia (hemoglobin 106 g/L). The platelet count is normal (159 × 10^9/L). The morphological test of peripheral blood showed that eosinophils accounted for 9%, monocytes accounted for 9%, and blasts accounted for 1%. Ultrasound showed that the spleen is slightly larger (length 13.2 cm) without lymphadenopathy and hepatomegaly. The bone marrow analyses showed hypercellular morphology (myeloblast 2%, monoblast 2%, promonocyte 9%, and eosinophilia) with dyshematopoiesis in granule and erythroid linages, but no evidence of myelofibrosis. Bone marrow immunohistochemistry: myeloperoxidase (3+), glycophorin A (2+), CD3 (−), CD20 (−), CD38 (−), and CD34 (−). Chromosomal analysis showed a karyotype of 46,XY,t(8;9). Targeted next-generation sequencing (NGS) was negative. RNA sequencing revealed that exon 36 of PCM1 was fused to exon 8 of JAK2. In conclusion, the patient was diagnosed with MLN with eosinophilia (MLN-Eo) and PCM1-JAK2 rearrangement according to the 2016 WHO criteria (12).

Induction treatment for the patient was hydroxyurea (HU; 500 mg qd) combined with ruxolitinib (with initiating dose of 5 mg qd and then escalated to 15 mg bid). The patient achieved complete hematologic remission (CHR) in 1 month and then accepted the maintenance therapy with ruxolitinib alone. Subsequently, his leukocytes and eosinophils progressively increased, accompanied by a reduction of platelet counts. Two and a half months after stopping HU, the patient complained of abdominal distension, and abdominal Doppler ultrasound indicated that the spleen was 3.5 cm below the ribs. PCM1-JAK2 quantitative PCR test indicated 109.17% (no data at diagnosis). In addition to ruxolitinib, Peg-IFN (90 μg s.c. qw) and HU (500 mg bid) were administered. The patient tolerated the combined treatment well. Assessment conducted 2 months later showed that leukocytes, eosinophils, and platelets were significantly improved, the spleen size returned to normal, and PCM1-JAK2 fusion transcript decreased to 37.03%. These results suggested that the combination of ruxolitinib and Peg-IFN was safe and effective. Currently, the patient is still receiving combined therapy, and he is planning to undergo haploidentical HSCT (**Figure 1A**).

Case 2

In December 2020, a 47-year-old man was referred to the hematological department complaining of fatigue, tinnitus, and dizziness for 2 months. Count of blood cells test showed leukocytes of 13.47 × 10^9/L, eosinophils of 2.52 × 10^9/L, severe anemia (hemoglobin 66 g/L), and normal platelet counts (117 × 10^9/L). Peripheral blood smear revealed the presence of immature granulocytes and erythrocytes, with teardrop-like erythrocytes and 14% eosinophils. Physical examination suggested hepatosplenomegaly. The bone marrow morphology was hypercellular and showed granulocytic proliferation with eosinophilic proliferation, reduced erythropoiesis, hyperplasia of megakaryocytes, and grade 0-1 fibrosis according to European Myelofibrosis Network criteria (13). Cytogenetic analysis demonstrated a normal male karyotype. NGS was negative. Fluorescence *in situ* hybridization study demonstrated that BCR-ABL1, BCR-JAK2, ETV6-JAK2, ETV-FLT3, ETV-ABL1, PDGFRA, PDGFRB, and FGFR1 fusion and rearrangement were all negative. The PCM1-JAK2 fusion transcript was identified by reverse transcription polymerase chain reaction (RT-PCR). The break site was located in exon 36 of PCM1 and exon 9 of JAK2. The diagnosis of MLN-Eo with PCM1-JAK2 fusion was established. The patient returned to the local hospital for treatment and was treated with HU (500 mg tid), ruxolitinib (15 mg bid), and red blood cell transfusion after patient informed consent. According to the white blood cell counts, HU was discontinued after 0.5 months. One month later, the patient's white blood cells (1.66 × 10^9/L) and eosinophils (0.30 × 10^9/L) decreased significantly, the hepatosplenomegaly was improved, and the platelet (48 × 10^9/L) decreased, so the dose of ruxolitinib was reduced. However, both white blood cells and eosinophils were progressively increased since the dose of ruxolitinib was reduced. The final dose of ruxolitinib was maintained at 10 and 15 mg alternately every day. During the treatment of ruxolitinib, there was Grade 3 leukopenia and Grade 4 thrombocytopenia. Later, the patient developed a huge spleen, which was considered to be related to the insufficient dose of ruxolitinib. In short, the patient cannot tolerate the therapeutic dose of ruxolitinib and is preparing for allo-HSCT (**Figure 1B**).

DISCUSSION

So far, 68 cases of PCM1-JAK2 fusion have been reported in hematological malignancies, most commonly in myelodysplastic syndrome (MDS)/MPN and MPN. The median age is 50 years (12–86 years), the male to female ratio is 3.17:1, 83.61% of patients have varying degrees of eosinophilia, and 50% of patients have myelofibrosis (**Table 1** and **Figure 2**). Thirteen patients were treated with ruxolitinib ± HSCT, of which 11 were myeloid neoplasms and two were lymphatic neoplasms. Twelve were responsive to ruxolitinib, and one was of uncertain efficacy; however, the duration of response varied widely (**Table 2**). The 2021 National Comprehensive Cancer Network (NCCN) guidelines for MLN-Eo with PCM1-JAK2 fusion recommend that clinical trial is the preferred treatment option for patients with chronic phase disease, and patients with chronic phase disease can be treated with tyrosine kinase inhibitor (TKI) monotherapy in the absence of a clinical trial. However, early

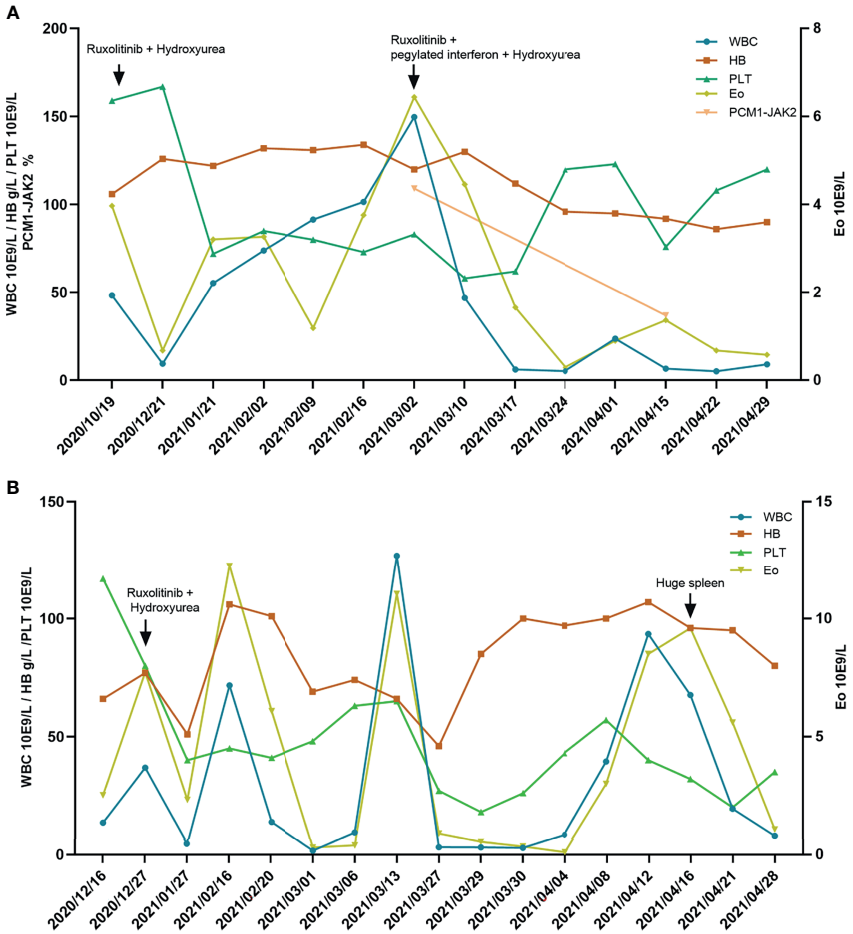


FIGURE 1 | **(A)** The blood cell and PCM1-JAK2 fusion transcript changes in patient 1 over the course of the disease. **(B)** The blood cell changes in patient 2 over the course of the disease. WBC, white blood cells; HB, hemoglobin; PLT, platelet; Eo, eosinophils.

referral to allo-HSCT should be considered for eligible patients, since TKI therapy alone does not result in durable remissions. We report two male cases diagnosed as MLN-Eo with PCM1-JAK2 fusion. Patient 1 was initially treated with ruxolitinib and HU to obtain instant CHR but later experienced progressive increase of white blood cells and eosinophils, which indicated treatment failure. The effect was regained after the addition of Peg-IFN, and PCM1-JAK2 fusion transcript decreased significantly, suggesting that molecular response was obtained. As far as we know, this is the first report of MLN-Eo and PCM1-JAK2 fusion receiving ruxolitinib combined with Peg-IFN at home and abroad. Unfortunately, limited by short follow-up time, we have not observed patients obtained complete molecular response (CMR). Patient 2 initially responded to treatment with ruxolitinib and HU, but it was discontinued due to intolerant hematological toxicity. Both cases indicated the effectiveness of ruxolitinib in MLN-Eo with PCM1-JAK2 fusion, despite different durations of response and toxicity, and both patients will undergo HSCT.

PCM1-JAK2 rearrangement has been reported in a variety of hematological neoplasms (3–7), which indicates that the PCM1-JAK2 rearrangement lacks lineage specificity. Because of the abnormality of the JAK2 signaling pathway in these diseases, treatment with ruxolitinib may be effective. Previous documents confirmed that ruxolitinib can inhibit the growth of PCM1-JAK2 transformed Ba/F3 mouse cells *in vitro* and the phosphorylation of JAK-STAT5 pathway (18). Lierman (18) and colleagues reported the first case of ruxolitinib in myeloproliferative disease with PCM1-JAK2 rearrangement. A 72-year-old male diagnosed with chronic eosinophilic leukemia (CEL) of PCM1-JAK2 rearrangement received ruxolitinib alone 10–20 mg twice daily, achieved complete cytogenetic response (CCyR) after 15 months, and was recurrence-free after 36 months (31). Subsequently, Rumi et al. (21) reported a second similar case. A 31-year-old woman was diagnosed with CEL with PCM1-JAK2 rearrangement. She was treated with ruxolitinib 15 mg twice, acquired complete clinical remission 1 year later, and attained CCyR 46 months later, with a significant decrease in the fusion transcript (31). These cases indicated that ruxolitinib is valuable for MPN with PCM1-JAK2 rearrangement and could induce long-term remission. Schwaab et al. (23) identically

TABLE 1 | Hematological neoplasms with PCM1-JAK2 fusion from literature.

Authors	Time	Journals	Number of cases
Reiter et al. (5)	2005	*Cancer Research*	7
Bousquet et al. (4)	2005	*Oncogene*	2
Murati et al. (3)	2005	*Leukemia*	4
Heiss et al. (14)	2005	*Human Pathology*	1
Adélaïde et al. (6)	2006	*Leukemia*	1
Huang et al. (15)	2008	*International Journal of Hematology*	1
Dargent et al. (16)	2011	*European Journal of Haematology*	1
Prochorec-Sobieszek et al. (17)	2012	*Leukemia & Lymphoma*	1
Lierman et al. (18)	2012	*Blood*	1
Masselli et al. (19)	2013	*British Journal of Haematology*	1
Patterer et al. (20)	2013	*Annals of Hematology*	6
Rumi et al. (21)	2013	*Journal of Clinical Oncology*	1
Saba et al. (22)	2013	*Blood*	1
Schwaab et al. (23)	2015	*Annals of Hematology*	1
Song et al. (24)	2016	*Annals of Laboratory Medicine*	1
Baer et al. (25)	2018	*Haematologica*	7
Lee et al. (26)	2018	*Annals of Laboratory Medicine*	1
Riedlinger et al. (7)	2019	*JCO Precision Oncology*	1
Salehi et al. (27)	2019	*Leukemia & Lymphoma*	1
Tang et al. (8)	2019	*Modern Pathology*	10
Schwaab et al. (28)	2020	*American Journal of Hematology*	8
Wouters et al. (29)	2021	*British Journal of Haematology*	1
Pozdnyakova et al. (30)	2021	*American Journal of Clinical Pathology*	9

confirmed that ruxolitinib was valuable in myeloid neoplasms with PCM1-JAK2 fusion, but the patient relapsed after 24 months on ruxolitinib. Recently, Schwaab et al. (28) reported a series of nine myeloid malignancy cases treated with ruxolitinib alone as first-line treatment, including eight cases of PCM1-JCK2 rearrangement and one case of BCR-JAK2 rearrangement. With a median time of 4 months (range 2–18 months), five patients achieved CHR. CCyR or CMR was observed in one patient. Their data showed that all the patients did not have long-term beneficial effects with ruxolitinib. In brief, the efficacy of ruxolitinib in MPN with PCM1-JAK2 fusion is inconsistent, with some patients surviving for a long time after ruxolitinib treatment and others relapsing early. There are also differences in the efficacy of ruxolitinib in lymphoid neoplasms with abnormal JAK2 pathway. Recent data (29) reported that an elderly woman diagnosed with B-cell acute lymphoblastic leukemia (B-ALL) with PCM1-JAK2 rearrangement failed to obtain complete cytogenetic and molecular biological response after receiving traditional chemotherapy and immunotherapy. Within 1 year after ruxolitinib 10 mg bid treatment, the PCM1-JAK2 fusion

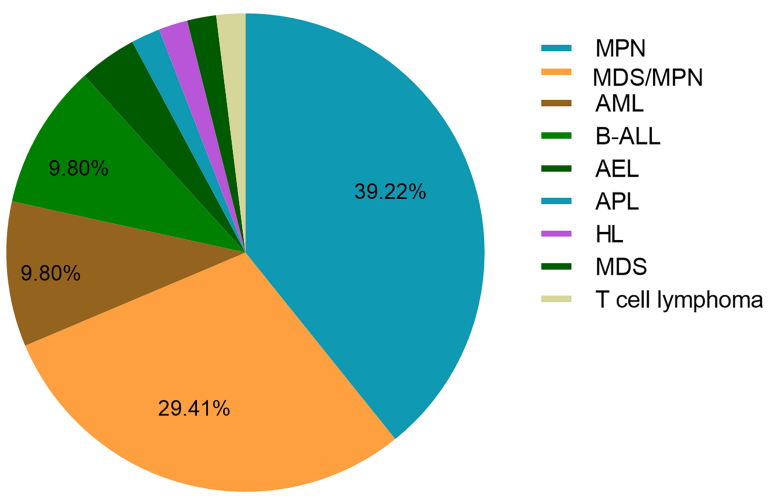


FIGURE 2 | Distribution of PCM1-JAK2 fusion in hematological neoplasms in the literature. MPNs, myeloproliferative neoplasms; MDS/MPN, myelodysplastic syndrome/myeloproliferative neoplasms; AML, acute myeloid leukemia; B-ALL, B-cell acute lymphoblastic leukemia; AEL, acute erythroid leukemia; APL, acute promyelocytic leukemia; HL, Hodgkin lymphoma.

TABLE 2 | Clinical characteristics of hematological neoplasms patients with PCM1-JAK2 rearrangement treated with ruxolitinib in the literature.

Case no.	Age	Gender	Diagnosis	WBC ($\times10^9$/L)	Eo (%)	Splenomegaly	Bone marrow	Karyotype	Treatment	Clinical course
A1	72	M	CEL, NOS	49	46.9	NA	Granulocytosis and eosinophilia	t(8;9)(p22;p24)	HU and ruxolitinib	CCyR was obtained after 15 months on ruxolitinib.
B2	50	M	MPN	9.9	6	Yes	Granulopoiesis, left-shifted and eosinophilia	t(8;9)(p22;p24)	Ruxolitinib	Alive 16 months after diagnosis.
C3	31	F	CEL, NOS	21.6	16.2	Yes	Granulopoiesis, eosinophilia, immature erythroid cells, and MF 1	t(8;9)(p22;p24)	Imatinib, HU, and ruxolitinib	No response to imatinib; then complete clinical remission was achieved after ruxolitinib.
D4	51	M	MPN	12	NA	Yes	Granulopoiesis, eosinophilia, dysplastic erythropoiesis, and MF 2	t(8;9)(p22;p24)	Ruxolitinib	CCyR was obtained after 12 months on ruxolitinib, but cytogenetic relapse occurred after 24 months.
E5	40	M	MPN	5.4	16	Yes	Eosinophils, immature erythroid precursors and MF 1–2	t(8;9)(p22;p24)	Ruxolitinib and HSCT	No disease progression during the follow-up period.
F6	76	M	CML-like MPN	28.7	5	No	Eosinophilia, left-shifted, dysplastic, and MF 2	t(8;9)(p22;p24)	Ruxolitinib	Disease progression after 1 month on ruxolitinib.
F7	70	M	aCML	29.8	1	Yes	Eosinophilia, left-shifted, dysplastic and MF 1	t(8;9)(p22;p24)	Ruxolitinib	CHR was obtained after 2 months on ruxolitinib.
F8	49	M	MDS/MPN	25.6	NA	Yes	Eosinophilia, left-shifted, dysplastic and MF 2	t(8;9;9)(p22;p24;p13)	Ruxolitinib and HSCT	CHR was obtained after 2 months on ruxolitinib, and the disease progressed 36 months later, followed by HSCT.
F9	29	M	CML-like MPN	21.7	11	Yes	NA	t(8;9)(p22;p24)	Ruxolitinib and HSCT	CHR was obtained after 2 months on ruxolitinib, followed by HSCT.
F10	50	M	MDS/MPN	12.7	13	Yes	Eosinophilia, left-shifted, dysplastic, and MF 2	t(8;9)(p22;p24)	Ruxolitinib and HSCT	CHR was obtained after 18 months on ruxolitinib, and the disease progressed 8 months later, followed by HSCT.
F11	69	F	AML-M4	10.5	1	No	Blasts 20% and MF 3	t(8;9)(p22;p24),+6,+8,+22	Ruxolitinib and azacitidine	The disease progressed after 2 months on ruxolitinib, CHR was obtained after 3 months on azacitidine, and the disease progressed again after 9 months.
F12	63	M	Pre-B-ALL	55.2	NO	No	Sheets of blasts	t(8;9)(p22;p24)	Ruxolitinib and HSCT	HSCT followed by ruxolitinib; then disease progressed.
G13	77	F	B-ALL	32.6	NO	No	87% blasts	t(8;9)(p22;p24)[1]/46,sl,der(8;9)(q10;q10),inc [5]/46,X,t(X;4)(p1?1;q13)[4]/46,XX[10]	Chemotherapy, blinatumomab, and ruxolitinib	PCM1-JAK2 fusion transcript was 23.28% after chemotherapy combined with blinatumomab, which decreased to 3.22% after the addition of ruxolitinib.

M, man; F, female; WBC, white blood cells; Eo, eosinophils; CEL, NOS, Chronic eosinophilic leukemia, not otherwise specified; CML, chronic myeloid leukemia; aCML, atypical chronic myeloid leukemia; CCyR, complete cytogenetic remission; NA, not available. Case A: (Lierman et al.) (18). Case B: (Patterer et al.) (20). Case C: (Rumi et al.) (21). Case D: (Schwaab et al.) (23). Case E: (Tang et al.) (8). Case F: (Schwaab et al.) (28). Case G: (Wouters et al.) (29).

transcript and abnormal metaphase were significantly reduced, but none of them disappeared completely. Another young female patient with relapsed and refractory B-ALL with RNPC3-JAK2 fusion did not respond to chemotherapy combined with immunotherapy and ruxolitinib (32). Mayfield et al. (33) documented that a 17-year-old B-ALL patient with JAK2 F694L mutation persisted with minimal residual disease (MRD) after standard chemotherapy, while the MRD turned out to be negative after the integration of ruxolitinib 20 mg bid for 2 weeks. The research of Ding (34) and his coworkers found that high-dose ruxolitinib combined with multidrug chemotherapy was safe and effective for children with BCR-ABL1-like ALL, and whether the combination therapy is suitable for MPN with PCM1-JAK2 fusion is worth exploring.

In summary, there have been considerable evidences that ruxolitinib is effective in hematological malignancies with abnormal JAK2 signaling pathways, although the efficacy is highly heterogeneous. The heterogeneity may be partially attributed to different somatic mutations or blastic crisis of MPN (8).

No somatic mutations were detected in both of our patients. Due to the rarity of hematological neoplasms with PCM1-JAK2 rearrangement, it is difficult to conduct large-scale clinical studies. Thus, there are no large cohort data on the molecular characterization of PCM1-JAK2-rearranged hematologic neoplasms. Baer et al. (25) found that mutation rates were 14% (1/7) for hematologic neoplasms with PCM1-JAK2 rearrangement, and the patient had TET2 somatic mutations. We speculate that somatic mutations of epigenetic regulators may be present in hematologic neoplasms with PCM1-JAK2 fusion, so hypomethylating-agent-based programs may be effective. Dargent et al. (16) described the diagnosis and treatment of a patient with MDS/MPN with PCM1-JAK2 fusion. The initial analysis of bone marrow karyotype was normal, but t(8;9) was detected by fluorescence *in situ* hybridization analysis in peripheral blood. The author suggested that t(8;9)(p22;p24) was not easy to detect in the study of G-banding and was easy to be ignored, especially in poor specimens. Tang (8) and his colleagues also noted that because t(9p24.1;V) only involved small segments of the 9p chromosome, such rearrangements were cryptic and therefore missed by routine chromosome analysis. These explain the reason for the normal karyotype of patient 2.

CONCLUSION

MLN-Eo with PCM1-JAK2 rearrangement is rare and has a poor prognosis. Existing data have shown that ruxolitinib is effective for the disease, but allo-HSCT is still the only way to cure the disease. Ruxolitinib can be used as a bridging treatment before allo-HSCT. Here, we reported the efficacy and safety of ruxolitinib in MPN with PCM1-JAK2 rearrangement. For patients with low efficacy of ruxolitinib monotherapy or rapid disease progression, the treatment options to obtain cytogenetics or molecular response prior to HSCT warrant further study. Ruxolitinib combined with Peg-IFN could be one of the candidates.

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

The studies involving human participants were reviewed and approved by the Research Ethics Committee of the First Affiliated Hospital of Soochow University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YS wrote the manuscript. SC guided the treatment of cases. YC, JC, JNC, MZ, and JP performed the research and analyzed the data. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by grants from the National Key R&D Program of China (2019YFA0111000), the National Natural Science Foundation of China (81700140, 81873449, 81900130, 81970136, 81970142, 81970142, 82000132, and 82000158), the Natural Science Foundation of the Jiangsu Higher Education Institution of China (18KJA320005), the Natural Science Foundation of Jiangsu Province (BK20190180), priority academic program development of Jiangsu Higher Education Institution, the Innovation Capability Development Project of Jiangsu Province (BM215004), the Translational Research Grant of NCRCH (2020WSB03, 2020WSB11, and 2020WSB13), the Open Project of Jiangsu Biobank of Clinical Resources (SBK202003001 and SBK202003003), Jiangsu Provincial Key Medical Center (YXZXA2016002), and National Science and Technology Major Project (2017ZX09304021).

REFERENCES

1. James C, Ugo V, Le Couédic J-P, Staerk J, Delhommeau F, Lacout C, et al. A Unique Clonal JAK2 Mutation Leading to Constitutive Signalling Causes Polycythaemia Vera. *Nature* (2005) 434:1144–8. doi: 10.1038/nature03546
2. Kralovics R, Passamonti F, Buser AS, Teo S, Tiedt R, Passweg JR, et al. A Gain-of-Function Mutation of JAK2 in Myeloproliferative Disorders. *New Engl J Med* (2005) 352:1779–90. doi: 10.1056/NEJMoa051113
3. Murati A, Gelsi-Boyer V, Adélaïde J, Perot C, Talmant P, Giraudier S, et al. PCM1-JAK2 Fusion in Myeloproliferative Disorders and Acute Erythroid Leukemia With T(8;9) Translocation. *Leukemia* (2005) 19:1692–6. doi: 10.1038/sj.leu.2403879
4. Bousquet M, Quelen C, De Mas V, Duchayne E, Roquefeuil B, Delsol G, et al. The T(8;9)(P22;P24) Translocation in Atypical Chronic Myeloid Leukaemia Yields a New PCM1-JAK2 Fusion Gene. *Oncogene* (2005) 24:7248–52. doi: 10.1038/sj.onc.1208850
5. Reiter A, Walz C, Watmore A, Schoch C, Blau I, Schlegelberger B, et al. The T (8;9)(P22;P24) Is a Recurrent Abnormality in Chronic and Acute Leukemia That Fuses PCM1 to JAK2. *Cancer Res* (2005) 65:2662–7. doi: 10.1158/0008-5472.CAN-04-4263
6. Adélaïde J, Pérot C, Gelsi-Boyer V, Pautas C, Murati A, Copie-Bergman C, et al. A T(8;9) Translocation With PCM1-JAK2 Fusion in a Patient With T-Cell Lymphoma. *Leukemia* (2006) 20:536–7. doi: 10.1038/sj.leu.2404104
7. Riedlinger GM, Chojecki A, Aviv H, Weissmann D, Joshi S, Murphy SM, et al. Hodgkin Lymphoma and Cutaneous T-Cell Lymphoma Sharing the PCM1-JAK2 Fusion and a Common T-Cell Clone. *JCO Precis Oncol* (2019) 1–8. doi: 10.1200/PO.19.00082
8. Tang G, Sydney Sir Philip JK, Weinberg O, Tam W, Sadigh S, Lake JI, et al. Hematopoietic Neoplasms With 9p24/JAK2 Rearrangement: A Multicenter Study. *Modern Pathol* (2019) 32:490–8. doi: 10.1038/s41379-018-0165-9
9. Gerds AT, Gotlib J, Bose P, Deininger MW, Dunbar A, Elshoury A, et al. Myeloid/Lymphoid Neoplasms With Eosinophilia and TK Fusion Genes,

Version 3.2021, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Cancer Network* (2020) 18:1248–69. doi: 10.6004/jnccn.2020.0042
10. Harrison C, Kiladjian J-J, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V, et al. JAK Inhibition With Ruxolitinib *Versus* Best Available Therapy for Myelofibrosis. *N Engl J Med* (2012) 366:787–98. doi: 10.1056/NEJMoa1110556
11. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, et al. A Double-Blind, Placebo-Controlled Trial of Ruxolitinib for Myelofibrosis. *N Engl J Med* (2012) 366:799–807. doi: 10.1056/NEJMoa1110557
12. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. *Blood* (2016) 127:2391–405. doi: 10.1182/blood-2016-03-643544
13. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European Consensus on Grading Bone Marrow Fibrosis and Assessment of Cellularity. *Haematologica* (2005) 90:1128–32.
14. Heiss S, Erdel M, Gunsilius E, Nachbaur D, Tzankov A. Myelodysplastic/Myeloproliferative Disease With Erythropoietic Hyperplasia (Erythroid Preleukemia) and the Unique Translocation (8;9)(P23;P24): First Description of a Case. *Hum Pathol* (2005) 36:1148–51. doi: 10.1016/j.humpath.2005.07.020
15. Huang K-P, Chase AJ, Cross NCP, Reiter A, Li T-Y, Wang T-F, et al. Evolutional Change of Karyotype With T(8;9)(P22;P24) and HLA-DR Immunophenotype in Relapsed Acute Myeloid Leukemia. *Int J Hematol* (2008) 88:197–201. doi: 10.1007/s12185-008-0113-4
16. Dargent J-L, Mathieux V, Vidrequin S, Deghorain X, Vannuffel P, Rack K. Pathology of the Bone Marrow and Spleen in a Case of Myelodysplastic/Myeloproliferative Neoplasm Associated With T(8;9)(P22;P24) Involving PCM1 and JAK2 Genes. *Eur J Haematol* (2011) 86:87–90. doi: 10.1111/j.1600-0609.2010.01525.x
17. Prochorec-Sobieszek M, Nasiłowska-Adamska B, Borg K, Kopeć I, Kos-Zakrzewska K, Juszczyński P, et al. Chronic Eosinophilic Leukemia With Erythroblastic Proliferation and the Rare Translocation T(8;9)(P22;P24) With PCM1–JAK2 Fusion Gene: A Distinct Clinical, Pathological and Genetic Entity With Potential Treatment Target? *Leukemia Lymphoma* (2012) 53:1824–7. doi: 10.3109/10428194.2012.661856
18. Lierman E, Selleslag D, Smits S, Billiet J, Vandenberghe P. Ruxolitinib Inhibits Transforming JAK2 Fusion Proteins *In Vitro* and Induces Complete Cytogenetic Remission in T(8;9)(P22;P24)/PCM1-JAK2–Positive Chronic Eosinophilic Leukemia. *Blood* (2012) 120:1529–31. doi: 10.1182/blood-2012-06-433821
19. Masselli E, Mecucci C, Gobbi G, Carubbi C, Pierini V, Sammarelli G, et al. Implication of MAPK1/MAPK3 Signalling Pathway in T(8;9)(P22;24)/PCM1-JAK2 Myelodysplastic/Myeloproliferative Neoplasms. *Br J Haematol* (2013) 162:563–6. doi: 10.1111/bjh.12392
20. Patterer V, Schnittger S, Kern W, Haferlach T, Haferlach C. Hematologic Malignancies With PCM1-JAK2 Gene Fusion Share Characteristics With Myeloid and Lymphoid Neoplasms With Eosinophilia and Abnormalities of PDGFRA, PDGFRB, and FGFR1. *Ann Hematol* (2013) 92:759–69. doi: 10.1007/s00277-013-1695-3
21. Rumi E, Milosevic JD, Casetti I, Dambruoso I, Pietra D, Boveri E, et al. Efficacy of Ruxolitinib in Chronic Eosinophilic Leukemia Associated With a PCM1-JAK2 Fusion Gene. *J Clin Oncol* (2013) 31:e269–71. doi: 10.1200/JCO.2012.46.4370
22. Saba N, Safah H. A Myeloproliferative Neoplasm With Translocation T(8;9)(P22;P24) Involving JAK2 Gene. *Blood* (2013) 122:861. doi: 10.1182/blood-2013-03-487348
23. Schwaab J, Knut M, Haferlach C, Metzgeroth G, Horny H-P, Chase A, et al. Limited Duration of Complete Remission on Ruxolitinib in Myeloid Neoplasms With PCM1-JAK2 and BCR-JAK2 Fusion Genes. *Ann Hematol* (2015) 94:233–8. doi: 10.1007/s00277-014-2221-y
24. Song I, Lee D, Lee J-H, Jang S, Huh J-R, Seo E-J. A T(8;9)(P22;P24)/PCM1-JAK2 Translocation in a Patient With Myeloproliferative Neoplasm and Myeloid Sarcoma: First Report in Korea. *Ann Lab Med* (2016) 36:79–81. doi: 10.3343/alm.2016.36.1.79
25. Baer C, Muehlbacher V, Kern W, Haferlach C, Haferlach T. Molecular Genetic Characterization of Myeloid/Lymphoid Neoplasms Associated With Eosinophilia and Rearrangement of PDGFRA, PDGFRB, FGFR1 or PCM1-JAK2. *Haematologica* (2018) 103:e348–50. doi: 10.3324/haematol.2017.187302
26. Lee J-M, Lee J, Han E, Kim M, Kim Y, Han K, et al. PCM1 - JAK2 Fusion in a Patient With Acute Myeloid Leukemia. *Ann Lab Med* (2018) 38:492–4. doi: 10.3343/alm.2018.38.5.492
27. Salehi S, Astle JM, Sadigh S, Lake J, Aikawa V, Tang G, et al. Myeloid Neoplasm With Eosinophilia and PCM1-JAK2 Associated With Acute Promyelocytic Leukemia With PML-RARA. *Leukemia Lymphoma* (2019) 60:2299–303. doi: 10.1080/10428194.2019.1581927
28. Schwaab J, Naumann N, Luebke J, Jawhar M, Somervaille TCP, Williams MS, et al. Response to Tyrosine Kinase Inhibitors in Myeloid Neoplasms Associated With *PCM1 - JAK2*, *Bcr-Jak2* And *ETV6-ABL1* Fusion Genes. *Am J Hematol* (2020) 95:824–33. doi: 10.1002/ajh.25825
29. Wouters Y, Nevejan L, Louwagie A, Devos H, Dewaele B, Selleslag D, et al. Efficacy of Ruxolitinib in B-Lymphoblastic Leukaemia With the PCM1 – JAK2 Fusion Gene. *Br J Haematol* (2021) 192:1–4. doi: 10.1111/bjh.17340
30. Pozdnyakova O, Orazi A, Kelemen K, King R, Reichard KK, Craig FE, et al. Myeloid/Lymphoid Neoplasms Associated With Eosinophilia and Rearrangements of PDGFRA, PDGFRB, or FGFR1 or With PCM1-JAK2. *Am J Clin Pathol* (2021) 155:160–78. doi: 10.1093/ajcp/aqaa208
31. Rumi E, Milosevic JD, Selleslag D, Casetti I, Lierman E, Pietra D, et al. Efficacy of Ruxolitinib in Myeloid Neoplasms With PCM1-JAK2 Fusion Gene. *Ann Hematol* (2015) 94:1927–8. doi: 10.1007/s00277-015-2451-7
32. Chen X, Wang F, Zhang Y, Ma X, Liu M, Cao P, et al. Identification of RNPC3 as a Novel JAK2 Fusion Partner Gene in B-Acute Lymphoblastic Leukemia Refractory to Combination Therapy Including Ruxolitinib. *Mol Genet Genomic Med* (2020) 8:1–8. doi: 10.1002/mgg3.1110
33. Mayfield JR, Czuchlewski DR, Gale JM, Matlawska-Wasowska K, Vasef MA, Nickl C, et al. Integration of Ruxolitinib Into Dose-Intensified Therapy Targeted Against a Novel JAK2 F694L Mutation in B-Precursor Acute Lymphoblastic Leukemia. *Pediatr Blood Cancer* (2017) 64:e26328. doi: 10.1002/pbc.26328
34. Ding YY, Stern JW, Jubelirer TF, Wertheim GB, Lin F, Chang F, et al. Clinical Efficacy of Ruxolitinib and Chemotherapy in a Child With Philadelphia Chromosome-Like Acute Lymphoblastic Leukemia With GOLGA5-JAK2 Fusion and Induction Failure. *Haematologica* (2018) 103:e427–31. doi: 10.3324/haematol.2018.192088

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Oncology

REVIEW
published: 11 November 2021
doi: 10.3389/fonc.2021.756348

Molecular Pathogenesis of *BCR-ABL*-Negative Atypical Chronic Myeloid Leukemia

Diletta Fontana[1], *Carlo Gambacorti-Passerini*[1,2] *and Rocco Piazza*[1,2,3*]

[1] Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy, [2] Hematology and Clinical Research Unit, San Gerardo Hospital, Monza, Italy, [3] Bicocca Bioinformatics, Biostatistics and Bioimaging Centre (B4), University of Milano-Bicocca, Milan, Italy

Atypical chronic myeloid leukemia is a rare disease whose pathogenesis has long been debated. It currently belongs to the group of myelodysplastic/myeloproliferative disorders. In this review, an overview on the current knowledge about diagnosis, prognosis, and genetics is presented, with a major focus on the recent molecular findings. We describe here the molecular pathogenesis of the disease, focusing on the mechanisms of action of the main mutations as well as on gene expression profiling. We also present the treatment options focusing on emerging targeted therapies.

Keywords: aCML, SETBP1, ETNK1, BCR-ABL1-negative, molecular pathogenesis, MDS/MPN

OPEN ACCESS

Edited by:
Argiris Symeonidis, University of Patras, Greece

Reviewed by:
Katerina Machova Polakova, Institute of Haematology and Blood Transfusion, Czechia
Amlan Ghosh, Presidency University, India

***Correspondence:**
Rocco Piazza
rocco.piazza@unimib.it

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

Received: *10 August 2021*
Accepted: *22 October 2021*
Published: *11 November 2021*

Citation:
Fontana D, Gambacorti-Passerini C and Piazza R (2021) Molecular Pathogenesis of BCR-ABL-Negative Atypical Chronic Myeloid Leukemia. Front. Oncol. 11:756348. doi: 10.3389/fonc.2021.756348

INTRODUCTION

Atypical chronic myeloid leukemia (aCML), *BCR-ABL1*-negative, is a rare hematological malignancy belonging to the overlap category of myelodysplastic/myeloproliferative neoplasms (MDS/MPN) of the WHO classification of myeloid neoplasms (1). The MDS/MPN category was introduced in the third edition of the WHO Classification of Tumors (2) and includes myeloid neoplasms that exhibit, at presentation, laboratory, clinical, and morphologic features that overlap between myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) (3).

Initially, aCML was described as an atypical form of chronic myeloid leukemia (CML) *BCR-ABL1*-positive, since at the onset it presents with many features of classical CML (4–6). In 2008, in order to incorporate new laboratorial as well as clinical information to refine diagnostic criteria for previously described neoplasms and to introduce newly recognized disease entities, the WHO classification was updated and published as part of the fourth edition of the WHO monograph series (7, 8). In this classification, aCML has been modified to aCML *BCR-ABL1*-negative to highlight that it was not merely a variant of classical CML (9). Finally, in 2016, after 8 years since the previous revision and owing to the massive amount of information generated by the new sequencing technologies, a revision of the 2008 WHO classification of hematopoietic neoplasms (1) was deemed necessary.

CHARACTERISTICS OF ACML AT PRESENTATION, DIAGNOSIS, AND DIFFERENTIAL DIAGNOSIS

aCML is a disorder of the elderly, since it typically affects patients with an age ranging between 60 and 76 years (10). Even though the first reports about aCML suggested a female predominance (11) or no sex predominance (12), in more recent years, reports analyzing larger cohorts of patients suggest a slight male predominance (13–15), though the biological reasons underlying aCML gender preference are poorly understood. Its estimated incidence is 1 out of 100 cases of t(9;22), *BCR-ABL1*-positive CML, meaning approximately 1 case per 1,000,000 persons per year (4, 11, 16, 17).

From a diagnostic point of view, aCML is a challenging myeloid malignancy characterized by features belonging to both myeloproliferative and myelodysplastic syndromes. As its clinical features may overlap with other myeloid malignancies, its diagnosis still relies primarily on morphological criteria, e.g., the evidence of dysgranulopoiesis in bone marrow or peripheral blood. Typically, aCML patients present clinical features similar to *BCR-ABL1*-positive CML including splenomegaly and a neutrophilic leukocytosis; they also show prominent granulocytic dysplasia (e.g., hypogranular and hypolobated neutrophils, abnormal chromatin clumping, and pseudo-Pelger–Huet neutrophils) (9). The white blood cell (WBC) count is $>13 \times 10^9$/L, with ≥10% of immature granulocytes and <20% blasts in the peripheral blood (PB) and in the bone marrow (BM) (1, 9, 18). Due to the high WBC count, it is not uncommon to observe a monocyte count $>1 \times 10^9$/L, but the percentage of monocytes at onset is always lower than 10% of the total leukocytes, which is critical to discriminate between aCML and a closely related MDS/MPN disorder, known as chronic myelomonocytic leukemia (CMML). In contrast to CML, basophilia is not prominent as basophils represent <2% of all PB white cells (9, 19). A hypercellular BM with myeloid hyperplasia and prominent granulocytic dysplasia is a consistent feature; however, trilineage dysplasia may be present (3, 20, 21). The leukocyte alkaline phosphatase level may be low, normal, or increased, therefore lacking diagnostic utility (9, 19). The recent discovery of somatic mutations occurring in *SETBP1* (22) and *ETNK1* (23) in up to a third of aCML cases (22–24) led to the insertion of these mutations in the 2016 WHO revision as supporting criteria for the diagnosis of aCML (1). However, the presence of these mutated genes does not ensure a certain diagnosis (see below and **Table 1**).

The differential diagnosis of aCML includes *BCR-ABL1*-positive CML, CMML, chronic neutrophilic leukemia (CNL), and prefibrotic primary myelofibrosis (pre-PMF) (**Table 2**). Besides the lack of the Philadelphia chromosome and the *BCR-ABL1* translocation, the major criteria that distinguish aCML from *BCR-ABL1*-positive CML are the dysgranulopoiesis, which is common in aCML but only occasionally reported in classical CML, and the normal basophil counts of the former (<2% of leukocytes) (1).

The differential diagnosis between aCML and CNL was traditionally difficult since the proportion of immature myeloid cells (promyelocytes, myelocytes, and metamyelocytes) in PB (≥10% in aCML and <10% in CNL) and the presence of dysplasia were the only distinctive features (1, 17). Currently, the distinction between the two disorders is also supported by the high frequency of *CSF3R* mutations (42) in the latter and by the higher frequency of *SETBP1* and *ETNK1* mutations in the former. However, it is important to note that none of these variants are fully restricted to one of the two disorders (43), which suggests that they represent a continuum of related diseases rather than truly distinct entities.

On the other hand, CMML can be ruled out by the lack of monocytosis (1, 3), since in CMML, monocyte count must exceed 10% of the total leukocytes. Although effective, the not uncommon detection of borderline monocyte fractions at the onset occasionally renders the application of this hard threshold troublesome.

Finally, aCML diagnosis must not meet the WHO criteria for primary myelofibrosis (PMF), polycythemia vera (PV), or essential thrombocythemia (ET). In this context, the most challenging differential diagnosis is between aCML and pre-PMF, where the lack of an overt fibrosis and the common presence of myeloid leukocytosis with immature myeloid cells in PB of pre-PMF patients may render the differential diagnosis challenging. Luckily, the availability of the three myeloproliferative gene markers, *JAK2*, *CALR*, and *MPL*,

TABLE 1 | Mutational frequencies.

	aCML	*BCR-ABL1*-positive CML	AML	MDS	CMML	CNL	MDS/MPN-U
SETBP1	7.4–48 (13, 14, 22, 25)	0 (22, 26)	0 (22)	0 (22)	4–15 (13, 22, 25)	25–41 (13, 22)	10–16 (13)
ASXL1	20–81 (13, 14, 25)	9.7 (26)	6.5–20 (27–30)	15–24 (31, 32)	14–69 (13, 25, 28)	77 (13)	64 (13)
N/K-RAS	11–27 (13, 14, 25)	0 (26)	10–18.8 (29, 30)	0 - 5 (31)	3–48 (13, 25)	10 (13)	12 (13)
ETNK1	3.7–13.3 (13, 14, 23, 25)	0 (23, 26)	0 (23)	0 (23)	0–14 (13, 23, 25)	0–2.6 (13, 23)	0–4 (13, 23)
SRSF2	14–65 (13, 14, 25)	–	6.6 (30)	14 (31)	24–51 (13, 25, 31)	43.6 (13)	48 (13)
EZH2	19–30 (13, 14, 25)	0.6 (26)	2 (33)	5–8 (31)	7–10 (13, 25)	20.5 (13)	24 (13)
RUNX1	11–15 (13, 14, 25)	2.6 (26)	5–15 (29, 30, 34)	10–20 (31)	10–37 (13, 25)	2.6 (13)	4 (13)
TET2	27–33 (13, 14, 25, 35, 36)	0.9 (26)	9–23 (29, 30, 37)	20–25 (29, 31)	48–78 (13, 25, 29)	20.5 (13)	44 (13)
CBL	8–11 (13, 14, 25)	0–0.9 (26, 38)	0.9 (30)	10 (39)	5–19 (13, 25)	5 (13)	8 (13)
CSF3R	1–22 (13, 14, 25)	–	3 (40)	1.4 (40)	0–3 (13, 25)	50–80 (13)	4 (13)
JAK2	4–11 (13, 25)	1.5 (26)	0.9 (30)	16.7 (41)	2–3 (13, 25)	7.7 (13)	8 (13)

The mutational frequencies of the genes most frequently involved in the onset or clonal evolution of aCML, BCR-ABL1-positive CML, AML, MDS, CMML, CNL, and MDS/MPN-U are reported.

TABLE 2 | Diagnostic criteria for *BCR-ABL1*-positive CML, CMML, CNL, and pre-PMF as defined by the WHO in the 2016 revision are listed.

BCR-ABL1-positive CML, accelerated phase criteria
Persistent or increasing WBC (>10 × 10^9/L), unresponsive to therapy
Persistent or increasing splenomegaly, unresponsive to therapy
Persistent thrombocytosis (>1,000 × 10^9/L), unresponsive to therapy
Persistent thrombocytopenia (<100 × 10^9/L) unrelated to therapy
20% or more basophils in the PB
10%–19% blasts in the PB and/or BM
Additional clonal chromosomal abnormalities in Ph1 cells at diagnosis that include "major route" abnormalities (second Ph, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, or abnormalities of 3q26.2
Any new clonal chromosomal abnormality in Ph1 cells that occurs during therapy
CMML diagnostic criteria
Persistent PB monocytosis ≥1 × 10^9/L, with monocytes accounting for ≥10% of the WBC count
Not meeting WHO criteria for BCR-ABL1-positive CML, PMF, PV, or ET
No evidence of PDGFRA, PDGFRB, or FGFR1 rearrangement or PCM1-JAK2 (should be specifically excluded in cases with eosinophilia)
<20% blasts in the blood and BM
Dysplasia in one or more myeloid lineages. If myelodysplasia is absent or minimal, the diagnosis of CMML may still be made if the other requirements are met and an acquired clonal cytogenetic or molecular genetic abnormality is present in hematopoietic cells or the monocytosis (as previously defined) has persisted for at least 3 months and all the other causes of monocytosis have been excluded
CNL diagnostic criteria
PB WBC ≥25 × 10^9/L
Segmented neutrophils plus band forms ≥80% of WBCs
Neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes) <10% of WBC
Myeloblasts rarely observed
Monocyte count <1 × 10^9/L
No dysgranulopoiesis
Hypercellular BM
Neutrophil granulocytes increased in percentage and number
Neutrophil maturation appears normal
Myeloblasts <5% of nucleated cells
Not meeting WHO criteria for BCR-ABL1-positive CML, PV, ET, or PMF
No rearrangement of PDGFRA, PDGFRB, or FGFR1, or PCM1-JAK2
Presence of CSF3R T618I or other activating CSF3R mutation or in the absence of a CSFR3R mutation, persistent neutrophilia (at least 3 months), splenomegaly, and no identifiable cause of reactive neutrophilia including absence of a plasma cell neoplasm or, if present, demonstration of clonality of myeloid cells by cytogenetic or molecular studies
Pre-PMF criteria (diagnosis of pre-PMF requires meeting all three major criteria and at least one minor criterion)
Major criteria
- Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis
- Not meeting the WHO criteria for BCR-ABL1-positive CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker, or absence of minor reactive BM reticulin fibrosis

Minor criteria
- Presence of at least one of the following, confirmed in two consecutive determinations:
- Anemia not attributed to a comorbid condition
- Leukocytosis ≥11 × 10^9/L
- Palpable splenomegaly
- LDH increased to above upper normal limit of institutional reference range

allows to quickly discriminate between the two conditions. Conversely, the distinction between aCML and those rare myeloproliferative cases lacking all the three markers (i.e., the so-called triple-negative myeloproliferative disorders) is currently much more blurred.

PROGNOSIS

aCML is an aggressive disease generally associated with poor outcome, with a median overall survival (OS) of 11–25 months and a 5-year OS of 25% (4, 11, 18, 19, 43–47). Fifteen percent to 40% of aCML patients progress to secondary acute myeloid leukemia (sAML), with a median time to leukemic evolution of 11.2 months (11); the remaining patients usually develop complications related to BM failure. Unfavorable prognostic factors for OS are an increased WBC count (>50 × 10^9/L) at presentation, increased immature precursors in the PB, age greater than 65 years at onset, hemoglobin (Hb) <10 g/dl, leukocyte count ≥50 × 10^9/L, and immature circulating precursors (10, 11, 15, 43, 47). In addition, mutations in *ASXL1* and *SETBP1* have been associated with a more aggressive disease (10, 48), although their prognostic impact is still unclear (14). The risk of progression to sAML seems to be higher in case of palpable hepato- or splenomegaly, monocytosis, BM blastosis >5%, marked dyserythropoiesis, and transfusional requirement (11). In a recent work, stratification based on RNA-sequencing data identified two populations in terms of overall survival, and the overexpression of *DNPH1*, *GFI1B*, and *PARP1* genes has been correlated with poor prognosis (14).

GENETICS

The molecular features of aCML include an increased frequency of karyotypic abnormalities. In up to 80% of patients with aCML, additional chromosomal abnormalities (ACA) such as trisomy 8 or 9, del(20q), and −7/7q or isochromosomes 17q are the most common ones (4, 10, 11, 24, 49), but also aberrations involving chromosomes 12, 13, 14, 19, and 21 are reported (19, 44, 46, 50). Interestingly, trisomy 8, isochromosome 17q, and trisomy 19 are the most frequent anomalies observed in *BCR-ABL1*-positive CML, which are associated with blast crisis transformation (51–53).

The most frequent somatic mutations involve *SETBP1*, *ASXL1*, *NRAS*, *KRAS*, *ETNK1*, *SRSF2*, *EZH2*, *RUNX1*, and *TET2*, while mutations in *CBL*, *CSF3R*, and *JAK2* are less frequent (10, 18, 20, 22–24, 43, 54–60). Even though mutations occurring in *SETBP1* and *ETNK1* are not univocally disease-specific, they represent the alterations most closely associated with aCML (1, 22–24, 58). *SETBP1* and *ASXL1* are considered as high-risk mutations (43, 54), while *TET2* (61), *CBL* (62), and *EZH2* (63) mutations may suggest a possible overlap between aCML and CMML at the molecular level. On the other hand, the absence of rearrangements involving *PDGFRA* or *PDGFRB* (3) and *FGFR1* (1) and the negativity for *JAK2* V617F mutation (64) all support a diagnosis of aCML. The mutation frequency of the main aCML oncogenes, compared with *BCR-ABL1*-positive CML, AML, MDS, CMML, CNL, and MDS/MPN-U, is reported in **Table 1**.

MOLECULAR LANDSCAPE OF ACML: MOLECULAR ALTERATIONS AND ASSOCIATED MOLECULAR PATHWAYS

SETBP1

The molecular lesions responsible for the onset and progression of aCML were unknown until 2013, when, by applying NGS techniques, the presence of recurrent somatic mutations in *SETBP1* was described (22, 24, 59, 65–70). *SETBP1* mutations have been identified in about one-quarter of patients affected by aCML (22), but also in 10%–16% of MDS/MPN unclassifiable cases (24) and in 4%–15% of CMML patients (59). Moreover, *SETBP1* mutations have been occasionally described in juvenile myelomonocytic leukemia (JMML) and in about 1.7%–7% of sAML arising from MPN or MDS (57). Several studies had shown that *SETBP1* mutations are associated with an adverse clinical presentation, with a higher leukocyte count, a lower Hb level, and thrombocytopenia (22, 24, 71). These data suggest that this alteration is important not only for the dissection of the mechanisms of leukemogenesis, but also because it likely provides important prognostic value (72). *SETBP1* maps on chromosome 18q21.1 and encodes for SET-binding protein 1a, a protein of 1,596 amino acids with a predicted molecular weight of 170 kDa. The protein contains a SET-binding region and a SKI homology region, in which the recurrent mutations are clustered. The latter is highly conserved among vertebrates, suggesting an important but still unknown biological function. Moreover, three AT hooks can be found in SETBP1 protein and they are likely responsible for the direct interaction occurring between SETBP1 and the genomic DNA. It is known that SETBP1 is a binding partner for the SET nuclear oncoprotein (73). In turn, SET binds and negatively regulates the phosphatase 2A (PP2A) (74) oncosuppressor, a major phosphatase implicated in many cellular processes, such as cellular proliferation (75–79). In particular, PP2A loss of function has been associated with cell transformation (80, 81). Indeed, PP2A is a tumor suppressor that acts by regulating several signaling pathways critical for malignant transformation, such as AKT and ERK1/2 (82–84). By directly interacting with SET, SETBP1 protects it from proteolytic cleavage, increasing the amount of full-length SET protein and leading to the formation of a SETBP1–SET–PP2A complex resulting in PP2A inhibition, ultimately causing increased proliferation and expansion of the leukemic clone (85). We originally demonstrated that the majority of SETBP1 somatic mutations cluster in a mutational hotspot within the SKI-homologous region of the protein, conferring a proliferative advantage to the mutated cells (22). This hotspot is part of a degron motif recognized by the F-box protein β-TrCP, one of the four subunits of the ubiquitin protein ligase complex known as SCF. Under physiological conditions, this interaction stimulates SETBP1 ubiquitination and degradation through the proteasome (**Figure 1**, upper panel). SETBP1 degron mutations severely decrease the affinity of β-TrCP to SETBP1, leading to the accumulation of SETBP1 protein, promoting its overexpression, and triggering the stabilization of SET at the protein level. The consequence of these events is the inhibition of PP2A (**Figure 1**, bottom panel). Besides the interaction with the SET–PP2A axis, SETBP1 is also able to directly interact with genomic DNA through its three conserved AT hooks (86), recruiting transcriptional modulators such as HCF1, KMT2A, PHF8, and PHF6, which belong to the SET/KMT2A (MLL1) COMPASS-like complex, forming a multiprotein complex that in turn causes the activation of gene expression. Notably, SETBP1 binds to the promoter of *MECOM*, which modulates the expression of several genes involved in the proliferation of hematopoietic stem cells and in the myeloid differentiation, upregulating it (86, 87). SETBP1 overexpression also confers self-renewal capability to myeloid progenitors *in vitro* by interacting with the homeobox A9 (*HOXA9*) and homeobox A10 (*HOXA10*) promoters (88). It is also reported to interact with the *Runx1* promoter, resulting in Runx1 downregulation (89) and impairment of the Runx1-dependent program of myeloid differentiation (90, 91). Globally, these data suggest a complex role for SETBP1 as a transcriptional modulator, likely being able to activate or repress the expression of target genes depending on the coactivator/corepressor complexes co-recruited to the target locus.

ETNK1

The *ETNK1* gene (also known as *EKI1*) maps on chromosome 12p12.1. It spans 60.5 kb and consists of eight exons and seven introns (92). *ETNK1* encodes a protein of 452 residues known as ethanolamine kinase, a cytoplasmic enzyme that catalyzes the

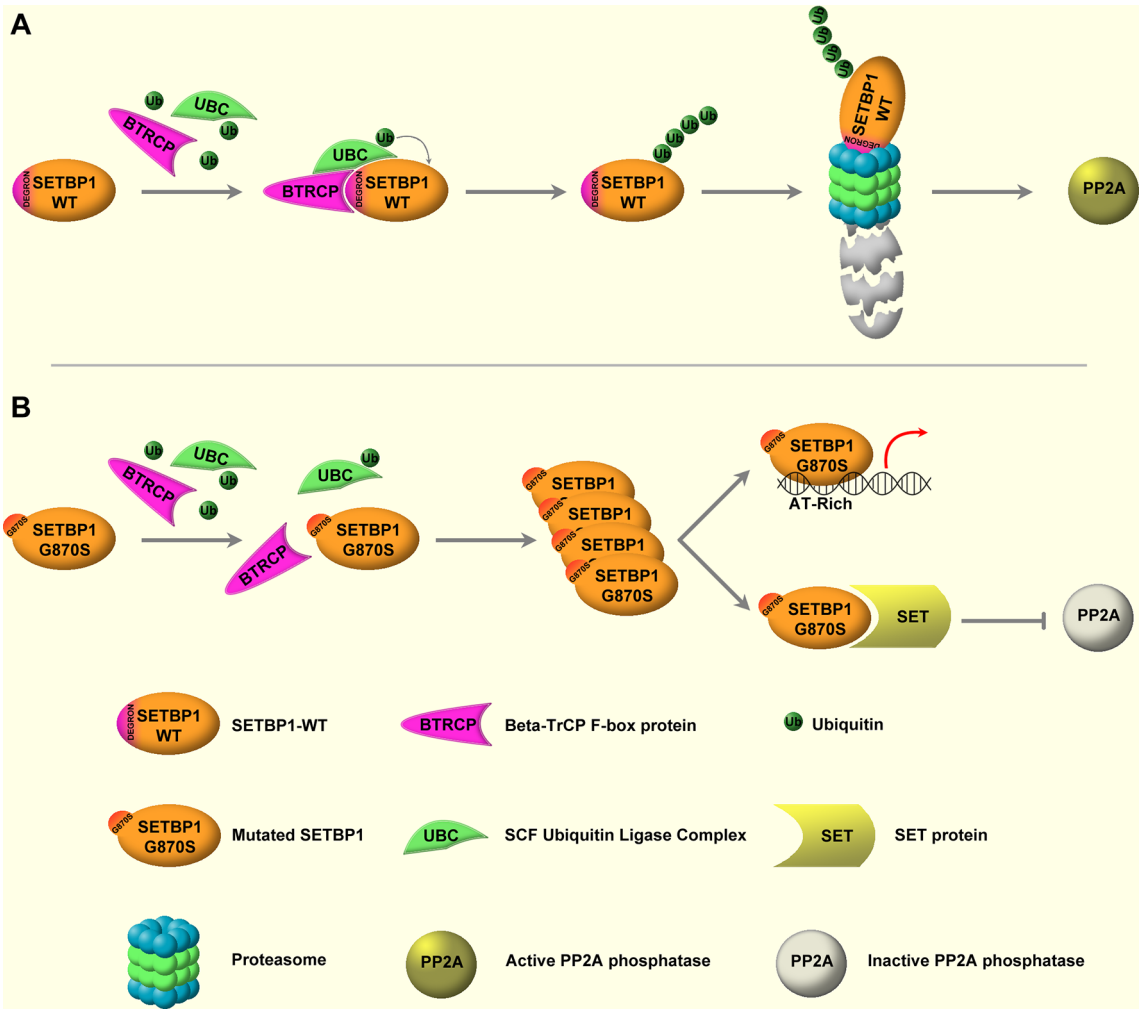


FIGURE 1 | WT and mutated SETBP1 signaling. **(A)** SETBP1–WT is under the post-translational control of the ubiquitin protein ligase complex (UBC) known as SCF, which is mediated by the β-TrCP F-box protein. β-TrCP directly interacts with the SETBP1 degron motif, mediating the recruitment of SCF and the subsequent ubiquitination of SETBP1. Following ubiquitination, SETBP1 protein is degraded through the proteasome. **(B)** Degron mutations impair the interaction between SETBP1 and β-TrCP, protecting SETBP1 from proteasomal degradation and causing accumulation of the protein. This in turn leads to overactivation of the SETBP1 downstream signaling, specifically: 1) modulation of target genes through direct binding of SETBP1 to genomic DNA, mediated by its three AT-hook domains, and 2) activation of the SETBP1–SET axis, leading to SET accumulation and inhibition of PP2A phosphatase activity.

first step of the *de novo* phosphatidylethanolamine (PE) biosynthesis through the Kennedy or cytidine diphosphate (CDP)-ethanolamine pathway (93). The Kennedy pathway is responsible for the *de novo* biosynthesis of the two major membrane phospholipids, phosphatidylcholine (PC) and PE. In particular, ETNK1 is responsible for the phosphorylation of ethanolamine to generate phosphoethanolamine (P-Et) (93). Somatic *ETNK1* mutations were originally identified by our group in 13.3% of an aCML cohort (23). ETNK1 mutations were present as a heterozygous variant in the dominant clone and affected two contiguous residues: H243Y and N244S. Mutations clustering in the same hotspot of the kinase catalytic domain (N244S, N244T, N244K, G245A, G245V) were subsequently found also in 0%–14% of CMML cases (23, 94), in 20% of patients affected by aggressive systemic mastocytosis (SM) with eosinophilia (94), and in a single case of diffuse large B-cell lymphoma (DLBCL) (95). Recently, our group demonstrated that somatic *ETNK1* mutations are responsible for a reduced activity of the enzyme, causing a decrease in P-Et synthesis (23, 96). Through the reduced competition of P-Et with succinate at mitochondrial complex II, an increased mitochondrial hyperactivation is triggered, which in turn is responsible for increased ROS production and subsequent DNA damage and accumulation of further mutations (96). Treatment with exogenous P-Et is able to restore a normal phenotype, protecting cells from ROS-mediated DNA damage (96, 97) (**Figure 2**). Notably, recent findings suggest that, whenever present, *ETNK1* mutations occur at the initial stages

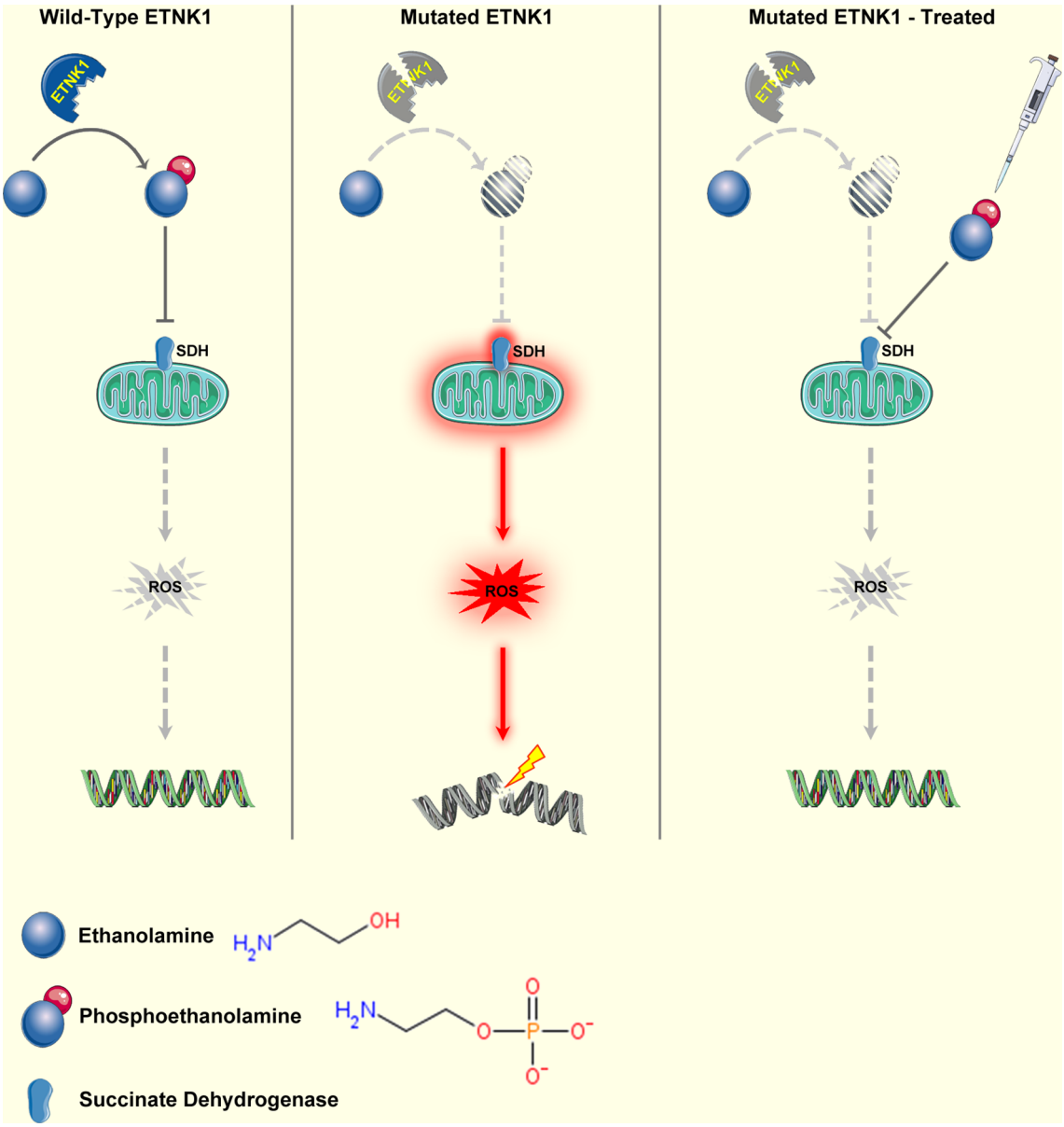


FIGURE 2 | Mechanism of ETNK1 somatic mutations. Left panel: WT ETNK1 phosphorylates ethanolamine, causing the accumulation of phosphoethanolamine. Phosphoethanolamine directly inhibits mitochondrial complex II, also known as succinate dehydrogenase, therefore downmodulating mitochondrial activity and ROS production. Middle panel: mutated ETNK1 causes a decreased production of phosphoethanolamine, which in turn leads to an increased mitochondrial activation, increased ROS production, and DNA damage. Right panel: treatment of ETNK1-mutated cells with exogenous P-Et restores the normal mitochondrial activity through direct suppression of SDH activity, normalization of ROS production, and protection of DNA from ROS-mediated damage. The image was obtained from: *Nat Commun. 2020 Nov 23;11(1):5938* under a Creative Commons Attribution 4.0 International License. Elements of the image were obtained from https://smart.servier.com/under a Creative Commons Attribution 3.0 License.

of the clonal evolution of aCML (14), preceding other driver events, such as *ASXL1* or *SETBP1*, which indirectly supports the role of *ETNK1* as an inducer of a mutator phenotype.

ASXL1

The *ASXL1* gene is located on chromosome 20q11.1, spanning 81 kb. This gene belongs to the polycomb gene family and plays a role in the recruitment of the Polycomb Repressor Complex 2 (PRC2) to its target sequences. It is also a component of the H2AK119 complex, responsible for histone H2A deubiquitination (98). *ASXL1* is mutated in more than 40% of aCML patients (14), and its mutations are associated with progression to acute phase and lower overall survival (22). It is currently known that ASXL1 contributes to the balance between the Polycomb Repressor Complex 1 (PRC1) and 2 (PRC2) in favor of the latter. Specifically, by interacting with the ubiquitin

carboxy-terminal hydrolase BAP1, ASXL1 causes H2A Lysine 119 deubiquitination, therefore directly counteracting the activity of PRC1 (99). Instead, in combination with the PRC2, it promotes H3K27 trimethylation through the recruitment of the PRC2 effectors EZH1 and EZH2 at the target site, ultimately causing gene silencing.

ASXL1 mutations are typically frameshift or nonsense mutations causing a C-terminal truncation of the ASXL1 protein. Constitutive as well as hematopoietic-lineage-restricted, homozygous ASXL1 knockout causes impairment of the bone marrow self-renewal capacity, ultimately leading to an MDS-like disease in mice (100, 101). In line with its role in promoting PRC2 activity, ASXL1 knockout confers a pan-reduction of the H3K27 trimethylation mark (27), leading to derepression of posterior *Hoxa* genes and oncogenic miR125a microRNAs. Importantly, overexpression of a truncated form of ASXL1, in combination with overexpression of BAP1, caused an important reduction in the global level of H3K27me3, together with a depletion of the H2AK119ub mark, therefore suggesting that the C-terminal truncation mutations may impair the PRC2 activity of ASXL1 while preserving its interaction with BAP1. Globally, *ASXL1* mutations act as loss-of-function events responsible for the promotion of myeloid transformation through loss of PRC2-mediated gene repression (27); however, their exact role is currently not entirely understood and likely multifaceted.

TET2

The *TET2* gene maps on chromosome 4q24, spreading over 150 kb. TET2 is responsible for the modulation of DNA hydroxymethylation by converting 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) as the first step required to promote DNA demethylation (102, 103). Mutations occurring in *TET2* are present in about 30% of aCML cases (14, 104–106); therefore, they are among the most frequent mutations occurring in this disorder, together with *SETBP1* and *ASXL1*. *TET2* mutations are invariably linked to a global decrease in the 5hmC mark, which suggests that they represent loss-of-function events. This evidence is also corroborated by the non-focal pattern of these variants, as TET2 mutations can be found throughout the entire coding region of the gene, and further supported by the frequent occurrence of nonsense and frameshift events.

TET2 plays a critical role in the bone marrow, mostly by promoting hematopoietic stem cell differentiation (107). In line with this role, it is expressed at high levels in progenitor cells and its deletion causes an increase in immature progenitor cells (35, 108), promoting myeloid as well as lymphoid malignancies in mice (109). From a prognostic point of view, the role of TET2 mutations is not univocal, but their presence probably does not negatively impact on the overall survival in most hematological malignancies (36, 110). In the context of MDS/MPN, individual reports suggest that TET2 mutations may be detrimental (111); however, its accurate prognostic role remains to be ascertained.

RAS

The *RAS* family of oncogenes comprises *HRAS*, *NRAS*, and *KRAS* genes. *HRAS* spans 3 kb and is located on chromosome 11p15.5, *NRAS* spans 7 kb and maps on chromosome 12p12.1, while *KRAS* spans more than 35 kb and is located at 1p13. These three genes share similar structures and sequences (112). The main product of the *RAS* genes is membrane-associated GTPases that control the MAP kinase cascade of serine/threonine kinases. Recurrent mutations in *RAS* genes occur in about 11%–27% of aCML patients (14, 22) and lead to a constitutively active expression of the protein. Usually aCML patients show *NRAS* or *KRAS* mutations (113, 114), and the most frequent mutations occur at codons 12, 31, and 61 (115–117).

EZH2

The *EZH2* gene is located at 7q36.1 and encodes the histone methyltransferase representing the catalytic subunit of the PRC2. In particular, EZH2 methylates histone H3 at lysine-27 (H3-K27), promoting the epigenetic silencing of genes involved in cell fate decisions (118, 119). The pattern of *EZH2* mutations is particularly complex, as EZH2 variants can be both gain (GOF) as well as loss of function (LOF), with GOF mutations, such as EZH2-Y646X, frequently found in lymphoid malignancies and, in particular, in non-Hodgkin lymphoma (120) and solid, non-hematological tumors and LOF typically found in myeloid malignancies. In the context of aCML, LOF *EZH2* mutations are seen in about 19%–30% of cases (14). The functional effect of EZH2 GOF mutations is to aberrantly increase H3K27me3, promoting transcriptional repression (121), which impairs B-cell differentiation and leads to an increased number and size of germinal centers (122). In contrast, LOF EZH2 mutations cause suppression of the H3K27me3 mark, causing overexpression of *BCAT1* and leading to enhanced branched chain amino acid metabolism and activation of mTOR signaling (123). Association of *EZH2* LOF mutations with poor prognosis was demonstrated in myelodysplastic syndromes (124), while their prognostic role in other disorders such as AML or aCML is less clear (125, 126).

RUNX1

The *RUNX1* gene, also known as *AML1*, is located at chromosome band 21q22.12 and encodes the alpha subunit of the core-binding factor (CBF) complex (127). This complex is responsible for the transcriptional modulation of critical factors involved in growth, survival, and differentiation processes; ribosome biogenesis; cell cycle regulation; and p53 and transforming growth factor β signaling pathways (128). RUNX1 contains a runt-homology domain (RHD), which is responsible for DNA binding and interaction with the heterodimeric partner CBFβ and a TAD domain characterized by the presence of motifs binding to a large number of activating and repressor proteins. RUNX1 is known to be involved in more than 50 different chromosomal translocations. The t(8;21) involving *RUNX1* and *RUNXT1*, the t(12;21) occurring in pediatric acute lymphoblastic leukemia and generating the ETV6–RUNX1 fusion, and the t(3;21) occurring in therapy-related AML and myelodysplastic syndrome and involving the *MECOM* oncogene are among those that are the most frequent. In the t(8;21), the persistence in the fusion of the RHD domain allows the binding of the protein to the normal RUNX1 gene targets. The presence of the RUNX1T1 fusion partner causes the

recruitment of corepressors carrying deacetylase activity to the target promoters, therefore impairing the normal trans-activation and changing the function of the protein into a repressor, hence causing neomorphic activity (129).

Single-nucleotide somatic mutations are also commonly found in myeloid malignancies, such as AML and MDS. They typically occur in the RHD and, with a much lower frequency, in the TAD domain and can be mono- or biallelic. *RUNX1* mutations include missense, nonsense, frameshift, deletions, and splicing mutations (130). Mechanistically, mutations occurring in the RHD domain usually inactivate the protein, while mutations occurring downstream of the RHD domain typically confer a weak dominant negative activity to the mutant (131). These mutations are functionally distinct from the chromosomal translocations and usually confer a worse prognosis. Mutations involving this gene are present in about 11%–15% of aCML cases (14), but are also found in 10%–37% of CMML patients (132–134).

SRSF2

The *SRSF2* gene is located on chromosome 17q25.1 and encodes a protein that plays a role in the splicing of primary mRNA (135, 136). This protein contains an RNA recognition motif that promotes spliceosome assembly at adjacent splice sites allowing the removal of introns from the primary transcripts (137). Moreover, it plays an active role in transcription and elongation and in coupling transcription and splicing processes (138, 139). Mutations in key factors of the spliceosome, such as *SRSF2*, *SF3B1*, *U2AF1*, and *ZRSR2*, occur in a large fraction of myelodysplastic syndromes (140).

SRSF2 contains an RNA-binding domain (RBD) responsible for the interaction with exonic splicing enhancers and an SR (serine-arginine rich) domain directly interacting with the other splicing ribonucleoproteins.

p.P95H is by far the most common mutation occurring in the *SRSF2* gene (141, 142). This mutation alters the RNA-binding affinity and specificity of the RBD domain, resulting in higher affinity for CCNG than to the standard GGNG motif, at least *in vitro* (143).

The frequency of *SRSF2* mutations in aCML is 14%–65% of cases (10, 14, 58, 144, 145). Although *SRSF2* mutations have been associated with worse survival outcomes in low-risk MDS patients (146), its prognostic role in aCML is currently unclear.

CBL

CBL is located on chromosome 11q23.3; it contains 16 exons and spans more than 110 kb (147). This gene encodes a protein that acts as an E3 ubiquitin ligase, being required for targeting substrates for degradation by the proteasome. CBL plays both positive and negative regulatory roles in tyrosine kinase signaling transduction pathways. CBL can bind to activated signaling complexes recruiting downstream signal transduction components or can target receptors that in turn trigger internalization of the receptor/ligand complex, promoting recycling or proteasomal degradation in endosomes (148–151). Besides aCML, *CBL* has also been found mutated in 5%–19% cases of CMML patients (152, 153). Moreover, mutations of *CBL* are frequently associated with uniparental disomy at 11q (14, 154).

CSF3R

The *CSF3R* gene is located at 1p34.3 and encodes the transmembrane receptor of the granulocyte colony-stimulating factor 3, which plays an essential role in the growth and differentiation of granulocytes (155, 156). Somatic *CSF3R* mutations are found in a large fraction (50%–80%) of patients affected by CNL (157, 158), and their presence is now one of the diagnostic criteria for CNL, according to the 2016 revision to the World Health Organization classification of myeloid neoplasms (1). In contrast, their association with aCML remains controversial. Although a single study reported *CSF3R* mutations to be frequent in aCML (157), several other works showed that *CSF3R* mutations are restricted to CNL and very rare in aCML (14, 22, 43, 58, 158). Currently, two types of CSF3R mutations are known: 1) extracellular domain/membrane proximal point mutations, such as the p.T618I variant, and 2) cytoplasmic truncation mutations. Mutations belonging to the first group result in a constitutive activation of the receptor, which occurs independently from the presence of the ligand. These mutations activate downstream JAK family tyrosine kinase pathways that drive the proliferation of neutrophil precursors, and are typically sensitive to JAK inhibitors. Truncation mutations instead interfere with receptor internalization and degradation, causing constitutive overexpression of CSF3R and ligand hypersensitivity, and show sensitivity to SRC kinase inhibitors (157).

GENE EXPRESSION PROFILING

To date, there are very few papers investigating the gene expression profile of aCML cases (13, 14, 25). Faisal and colleagues analyzed the mRNA expression of 26 aCML and 59 CMML cases, comparing them to a cohort of reference samples. Their analysis revealed a significant change in the expression levels of *SETBP1*, *CDKN2A*, *GATA2*, *MPL*, *TMEM14C*, *CSF3R*, and *FLT3* genes. The strongest differential expression effect was detectable in *FLT3* in CMML samples compared with aCML and references ones and in both *SETBP1* and *CSF3R* in aCML and CMML cases. These findings are in line with the mutation frequency of these genes in aCML and CMML (25). In the work of Zhang, RNA-sequencing was performed on 76 samples of aCML, CNL, CMML, and MDS/MPN unclassifiable (MDS/MPN-U). Gene expression signatures identified three main sample clusters, with different proportions of all diagnoses in each group, associated with prognostic markers (13). On the other hand, our group performed RNA-sequencing on a cohort of 43 aCML patients, and stratification based on gene expression profile identified two different populations in terms of overall survival. In this context, overexpression of three genes (*DNPH1*, *GFI1B*, and *PARP1*) was predictive of poor prognosis (14). Contrary to these disorders characterized by high heterogeneity, several profiling studies in *BCR-ABL1*-positive CML have been reported. In particular, expression profiling

analyses revealed a different signature associated with the classical t(9;22)(q34;q11) translocation or with variant t(9;22) rearrangements (159); similarly, signature analysis predicted imatinib response or resistance (160–166).

THERAPY

No drug has so far proved to be effective and no established standards of care exist for the treatment of aCML (48, 54). Moreover, no consensus recommendations such as risk-based treatment algorithms exist to help clinicians in choosing between a watch-and-wait approach and initiation of therapy. During the last years, different therapeutic approaches have been proposed, but at present, allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only potentially curative treatment option for aCML, even if only limited reports are available (167–171). However, allo-HSCT requires the presence of a suitable donor and is only available for young and middle-aged patients, since the toxicity of the transplant limits its use in the elderly (>70 years), where, especially with low-risk disease, monitoring or palliative chemotherapy may be more appropriate.

Regarding other medical therapies, different treatment strategies validated in other myeloid diseases have been evaluated in aCML. Hydroxyurea (HU) is used as a supportive care measure to control hyperleukocytosis and splenomegaly. Complete and partial hematological remissions have been reported in about 80% of patients, even if they are usually short-lived (10, 11, 19, 43, 44, 46, 47, 172–175). Moreover, complete and partial hematological remissions have also been reported after treatment with interferon alfa (IFN-alfa), even if many patients discontinued the treatment due to drug toxicity (19, 44, 172–174, 176, 177). However, both HU and IFN-alfa, despite being able to improve the WBC count, are unable to change the course of the disease and are typically used in a palliative setting where, due to the age of the patient or the presence of significant comorbidities, an allo-HSCT is not considered a valid option.

Among the other drugs that can be used in the treatment of aCML, especially in patients with high-risk disease, the hypomethylating agents (HMA), such as azacitidine or decitabine, are noteworthy (10, 47, 178–183). Indeed, based on their established activity in MDS and CMML, in which the overall response rates range from 25% to 70% and the overall survival from 12 to 37 months (184), these drugs could be used also in aCML. Nevertheless, up to now, the experience with HMA is still limited and the available data do not allow to accurately predict the efficacy of these compounds. Therefore, HMA cannot be considered as a standard of care for aCML yet and their use is off-label.

For selected patients with aggressive behavior, AML-like intensive chemotherapy is offered as a bridge to HSCT (185), even if this option has not been explored extensively.

Given the recent description of the mutational landscape of aCML, in the field of personalized therapies, different targeted drugs can be used for aCML treatment and are currently being investigated. Indeed, several actionable mutations have been identified in aCML which could be targeted, e.g., with tyrosine kinase inhibitors. Wang and colleagues administrated RAS, FLT3, MAPK, MYC, or AKT inhibitors to their patients (43). The MEK1/2 inhibitor trametinib, approved for malignant melanoma, has been used in *RAS*-mutated aCML (14, 186). Khanna and collaborators reported an aCML case with *NRAS* G12V mutation who experienced a notable response to trametinib with improvement in blood counts and 14 months of disease control (186), while another patient carrying *NRAS* G12D mutation treated with trametinib obtained a hematological response with blood cell count normalization and reduction of splenomegaly for 3 months (14).

For rare cases of aCML with JAK2 V617F mutation (62, 187, 188), ruxolitinib, a JAK2 inhibitor approved by the FDA in intermediate- to high-risk MF and PV intolerant or resistant to HU, can be used (189, 190). This drug is also effective in patients carrying *CSF3R* T618I mutation, since membrane proximal mutations result in JAK–STAT pathway activation (157), while truncation *CSF3R* mutations are reported to be sensitive to dasatinib (14, 157). An open-label, single-arm, phase II multicenter study evaluated the safety and efficacy of ruxolitinib in patients affected by CNL or aCML, regardless of their mutation status (191). Interestingly, 4/6 aCML patients carrying *CSF3R* mutations and 7/17 patients with *CSF3R* WT reached partial (PR) or complete responses (CR). PR was defined as >50% reduction of WBC, absolute neutrophil count (ANC), and granulocytic dysplasia and >25% reduction in spleen volume; CR was defined as normalization of WBC count and ANC, no evidence of granulocytic dysplasia, and normal spleen. A phase I, open-label study is currently ongoing to evaluate the safety and efficacy of TGR-1202, a PI3K-delta inhibitor, administered together with ruxolitinib in patients with MDS or MDS/MPN including aCML (ClinicalTrials.gov Identifier: NCT02493530).

Since the treatment of aCML remains a challenge, Gotlib proposed a new treatment algorithm (54), based on several decision nodes, including the potential candidacy for allogeneic hematopoietic stem cell transplantation, the results of myeloid mutation panel testing, the eligibility for enrollment in clinical trials, and the opportunity to adopt strategies used for MDS or MPN. Considering that two or more mutations in distinct genes often occur in aCML patients, combination therapies with different drugs could represent a promising approach. Since multiple actionable mutations are often present in various combinations in aCML patients, their enrollment in clinical trials should be considered whenever possible.

CONCLUSION

aCML is a *BCR-ABL1*-negative hematological disease characterized by poor survival. The challenges in the management of this leukemia comprise both the diagnosis, due to the overlap of several genetic mutations among different MDS/MPN disorders, and the treatment choices, since at present no standards of care are available, except for bone marrow transplantation that is the only curative option for younger patients. The application of NGS techniques led to the discovery of new genes involved in the onset

of the disease, which is allowing the introduction of personalized therapies for aCML patients. Further studies will be required to thoroughly assess the efficacy of these new treatments. Importantly, transcriptomic studies done at single-cell resolution may also unravel new targetable pathways that could increase the therapeutic options available for the treatment of this aggressive disorder.

AUTHOR CONTRIBUTIONS

DF: writing—original draft preparation. CG-P: funding acquisition, writing—review and editing, and supervision. RP: funding acquisition, writing—review and editing, and supervision. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. *Blood* (2016) 127(20):2391–405. doi: 10.1182/blood-2016-03-643544
2. Jaffe ES, Harris NL, Stein H, Vardiman JW. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues WHO Classification of Tumours. 3rd Edition* Vol. 3. . Lyon: IARC Press (2001).
3. Orazi A, Germing U. The Myelodysplastic/Myeloproliferative Neoplasms: Myeloproliferative Diseases With Dysplastic Features. *Leukemia* (2008) 22 (7):1308–19. doi: 10.1038/leu.2008.119
4. Vardiman JW, Bain B, Inbert M, Brunning RD, Pierre RV, Flandrin G. Atypical Chronic Myeloid Leukemia. In: E Jaffe, NL Harris, H Stein and J Vardiman, editors. *WHO Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon: IARC Press (2001). p. 53–7. Lyon: IARC Press.
5. Oscier DG. Atypical Chronic Myeloid Leukaemia, a Distinct Clinical Entity Related to the Myelodysplastic Syndrome? *Br J Haematol* (1996) 92(3):582–6. doi: 10.1046/j.1365-2141.1996.396933.x
6. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick H, et al. The Chronic Myeloid Leukaemias: Guidelines for Distinguishing Chronic Granulocytic, Atypical Chronic Myeloid, and Chronic Myelomonocytic Leukaemia. Proposals by the French-American-British Cooperative Leukaemia Group. *Br J Haematol* (1994) 87(4):746–54. doi: 10.1111/j.1365-2141.1994.tb06734.x
7. Sabattini E, Bacci F, Sagramoso C, Pileri SA. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues in 2008: An Overview. *Pathologica* (2010) 102(3):83–7.
8. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. In: *WHO Classification of Tumours, Revised*, *4th Edition*, vol. 2. . Lyon: IARC Press (2008).
9. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 Revision of the World Health Organization (WHO) Classification of Myeloid Neoplasms and Acute Leukemia: Rationale and Important Changes. *Blood* (2009) 114(5):937–51. doi: 10.1182/blood-2009-03-209262
10. Patnaik MM, Barraco D, Lasho TL, Finke CM, Reichard K, Hoversten KP, et al. Targeted Next Generation Sequencing and Identification of Risk Factors in World Health Organization Defined Atypical Chronic Myeloid Leukemia. *Am J Hematol* (2017) 92(6):542–8. doi: 10.1002/ajh.24722
11. Breccia M, Biondo F, Latagliata R, Carmosino I, Mandelli F, Alimena G. Identification of Risk Factors in Atypical Chronic Myeloid Leukemia. *Haematologica* (2006) 91(11):1566–8. doi: 10.3324/%25x
12. Hernandez J, Villaescusa T, Arefi M, López L, Garcia J. Atypical Chronic Myeloid Leukemia (Acml). *Atlas Genet Cytogenet Oncol Haematol* (2009) 13 (6):432–3. doi: 10.4267/2042/44494
13. Zhang H, Wilmot B, Bottomly D, Dao KT, Stevens E, Eide CA, et al. Genomic Landscape of Neutrophilic Leukemias of Ambiguous Diagnosis. *Blood* (2019) 134(11):867–79. doi: 10.1182/blood.2019000611
14. Fontana D, Ramazzotti D, Aroldi A, Redaelli S, Magistroni V, Pirola A, et al. Integrated Genomic, Functional, and Prognostic Characterization of Atypical Chronic Myeloid Leukemia. *Hemasphere* (2020) 4(6):e497. doi: 10.1097/HS9.0000000000000497
15. Giri S, Pathak R, Martin MG, Bhatt VR. Characteristics and Survival of BCR/ABL Negative Chronic Myeloid Leukemia: A Retrospective Analysis of the Surveillance, Epidemiology and End Results Database. *Ther Adv Hematol* (2015) 6(6):308–12. doi: 10.1177/2040620715607416
16. Oscier D. Atypical Chronic Myeloid Leukemias. *Pathol Biol (Paris)* (1997) 45 (7):587–93.
17. Cazzola M, Malcovati L, Invernizzi R. Myelodysplastic/Myeloproliferative Neoplasms. *Hematol Am Soc Hematol Educ Program* (2011) 1:264–72. doi: 10.1182/asheducation-2011.1.264
18. Gotlib J, Maxson JE, George TI, Tyner JW. The New Genetics of Chronic Neutrophilic Leukemia and Atypical CML: Implications for Diagnosis and Treatment. *Blood* (2013) 122(10):1707–11. doi: 10.1182/blood-2013-05-500959
19. Kurzrock R, Bueso-Ramos CE, Kantarjian H, Freireich E, Tucker SL, Siciliano M, et al. Bcr Rearrangement-Negative Chronic Myelogenous Leukemia Revisited. *J Clin Oncol* (2001) 19(11):2915–26. doi: 10.1200/JCO.2001.19.11.2915
20. Dao KH, Tyner JW. What's Different About Atypical CML and Chronic Neutrophilic Leukemia? *Hematol Am Soc Hematol Educ Program* (2015) 2015:264–71. doi: 10.1182/asheducation-2015.1.264
21. Thakral B, Anastasi J, Sa AW. 17 - Myeloproliferative and "Overlap" Myelodysplastic/Myeloproliferative Neoplasms. *Foundations Diagn Pathol* (2018) 488–538.e484. doi: 10.1016/B978-0-323-47913-4.00017-3
22. Piazza R, Valletta S, Winkelmann N, Redaelli S, Spinelli R, Pirola A, et al. Recurrent SETBP1 Mutations in Atypical Chronic Myeloid Leukemia. *Nat Genet* (2013) 45(1):18–24. doi: 10.1038/ng.2495
23. Gambacorti-Passerini CB, Donadoni C, Parmiani A, Pirola A, Redaelli S, Signore G, et al. Recurrent ETNK1 Mutations in Atypical Chronic Myeloid Leukemia. *Blood* (2015) 125(3):499–503. doi: 10.1182/blood-2014-06-579466
24. Meggendorfer M, Bacher U, Alpermann T, Haferlach C, Kern W, Gambacorti-Passerini C, et al. SETBP1 Mutations Occur in 9% of MDS/MPN and in 4% of MPN Cases and are Strongly Associated With Atypical CML, Monosomy 7, Isochromosome I(17)(Q10), ASXL1 and CBL Mutations. *Leukemia* (2013) 27(9):1852–60. doi: 10.1038/leu.2013.133
25. Faisal M, Stark H, Busche G, Schlue J, Teiken K, Kreipe HH, et al. Comprehensive Mutation Profiling and Mrna Expression Analysis in Atypical Chronic Myeloid Leukemia in Comparison With Chronic Myelomonocytic Leukemia. *Cancer Med* (2019) 8(2):742–50. doi: 10.1002/cam4.1946
26. Branford S, Kim DDH, Apperley JF, Eide CA, Mustjoki S, Ong ST, et al. Laying the Foundation for Genomically-Based Risk Assessment in Chronic Myeloid Leukemia. *Leukemia* (2019) 33(8):1835–50. doi: 10.1038/s41375-019-0512-y
27. Abdel-Wahab O, Adli M, LaFave LM, Gao J, Hricik T, Shih AH, et al. ASXL1 Mutations Promote Myeloid Transformation Through Loss of PRC2-Mediated Gene Repression. *Cancer Cell* (2012) 22(2):180–93. doi: 10.1016/j.ccr.2012.06.032
28. Gelsi-Boyer V, Brecqueville M, Devillier R, Murati A, Mozziconacci MJ, Birnbaum D. Mutations in ASXL1 are Associated With Poor Prognosis Across the Spectrum of Malignant Myeloid Diseases. *J Hematol Oncol* (2012) 5:12. doi: 10.1186/1756-8722-5-12
29. DiNardo CD, Cortes JE. Mutations in AML: Prognostic and Therapeutic Implications. *Hematol Am Soc Hematol Educ Program* (2016) 2016(1):348–55. doi: 10.1182/asheducation-2016.1.348
30. Hou HA, Tien HF. Genomic Landscape in Acute Myeloid Leukemia and its Implications in Risk Classification and Targeted Therapies. *J BioMed Sci* (2020) 27(1):81. doi: 10.1186/s12929-020-00674-7

31. Awada H, Thapa B, Visconte V. The Genomics of Myelodysplastic Syndromes: Origins of Disease Evolution, Biological Pathways, and Prognostic Implications. *Cells* (2020) 9(11). doi: 10.3390/cells9112512
32. Paschka P, Schlenk RF, Gaidzik VI, Herzig JK, Aulitzky T, Bullinger L, et al. ASXL1 Mutations in Younger Adult Patients With Acute Myeloid Leukemia: A Study by the German-Austrian Acute Myeloid Leukemia Study Group. *Haematologica* (2015) 100(3):324–30. doi: 10.3324/haematol.2014.114157
33. Lund K, Adams PD, Copland M. EZH2 in Normal and Malignant Hematopoiesis. *Leukemia* (2014) 28(1):44–9. doi: 10.1038/leu.2013.288
34. Marcucci G, Haferlach T, Döhner H. Molecular Genetics of Adult Acute Myeloid Leukemia: Prognostic and Therapeutic Implications. *J Clin Oncol* (2011) 29(5):475–86. doi: 10.1200/JCO.2010.30.2554
35. Figueroa ME, Lugthart S, Li Y, Erpelinck-Verschueren C, Deng X, Christos PJ, et al. DNA Methylation Signatures Identify Biologically Distinct Subtypes in Acute Myeloid Leukemia. *Cancer Cell* (2010) 17(1):13–27. doi: 10.1016/j.ccr.2009.11.020
36. Nibourel O, Kosmider O, Cheok M, Boissel N, Renneville A, Philippe N, et al. Incidence and Prognostic Value of TET2 Alterations in De Novo Acute Myeloid Leukemia Achieving Complete Remission. *Blood* (2010) 116 (7):1132–5. doi: 10.1182/blood-2009-07-234484
37. Metzeler KH, Becker H, Maharry K, Radmacher MD, Kohlschmidt J, Mrózek K, et al. ASXL1 Mutations Identify a High-Risk Subgroup of Older Patients With Primary Cytogenetically Normal AML Within the ELN Favorable Genetic Category. *Blood* (2011) 118(26):6920–9. doi: 10.1182/blood-2011-08-368225
38. Makishima H, Jankowska AM, McDevitt MA, O'Keefe C, Dujardin S, Cazzolli H, et al. CBL, CBLB, TET2, ASXL1, and IDH1/2 Mutations and Additional Chromosomal Aberrations Constitute Molecular Events in Chronic Myelogenous Leukemia. *Blood* (2011) 117(21):e198–206. doi: 10.1182/blood-2010-06-292433
39. Cuéllar C, Cánaves YP, de la Fuente EO, Sánchez JM, Rapado I, López JM, et al. Detection of CALR and CBL Mutations by Next Generation Sequencing (NGS) in Acute Myeloid Leukemia (AML) and Myelodisplasic Syndrome (MDS). *EHA Library: Abstract: PB1662* (2018).
40. Liu H, Zhang Y, Wang F, Chen X, Teng W, Wang M, et al. CSF3R Mutation in Acute Leukemia and MDS Patients. *Blood* (2017) 130:2669. doi: 10.1182/blood.V130.Suppl_1.2669.2669
41. Jekarl DW, Han SB, Kim M, Lim J, Oh EJ, Kim Y, et al. JAK2 V617F Mutation in Myelodysplastic Syndrome, Myelodysplastic Syndrome/Myeloproliferative Neoplasm, Unclassifiable, Refractory Anemia With Ring Sideroblasts With Thrombocytosis, and Acute Myeloid Leukemia. *Korean J Hematol* (2010) 45(1):46–50. doi: 10.5045/kjh.2010.45.1.46
42. Maxson JE, Tyner JW. Genomics of Chronic Neutrophilic Leukemia. *Blood* (2017) 129(6):715–22. doi: 10.1182/blood-2016-10-695981
43. Wang SA, Hasserjian RP, Fox PS, Rogers HJ, Geyer JT, Chabot-Richards D, et al. Atypical Chronic Myeloid Leukemia is Clinically Distinct From Unclassifiable Myelodysplastic/Myeloproliferative Neoplasms. *Blood* (2014) 123(17):2645–51. doi: 10.1182/blood-2014-02-553800
44. Martiat P, Michaux JL, Rodhain J. Philadelphia-Negative (Ph-) Chronic Myeloid Leukemia (CML): Comparison With Ph+ CML and Chronic Myelomonocytic Leukemia. The Groupe Francais De Cytogenetique Hematologique. *Blood* (1991) 78(1):205–11. doi: 10.1182/blood.V78.1.205.205
45. Drozd-Sokołowska J, Mądry K, Waszczuk-Gajda A, Biecek P, Szwedyk P, Budziszewska K, et al. Atypical Chronic Myeloid Leukaemia: A Case of an Orphan Disease-a Multicenter Report by the Polish Adult Leukemia Group. *Hematol Oncol* (2018) 36(3):570–5. doi: 10.1002/hon.2501
46. Hernandez JM, del Canizo MC, Cuneo A, Garcia JL, Gutierrez NC, Gonzalez M, et al. Clinical, Hematological and Cytogenetic Characteristics of Atypical Chronic Myeloid Leukemia. *Ann Oncol* (2000) 11(4):441–4. doi: 10.1023/A:1008393002748
47. Onida F, Ball G, Kantarjian HM, Smith TL, Glassman A, Albitar M, et al. Characteristics and Outcome of Patients With Philadelphia Chromosome Negative, Bcr/Abl Negative Chronic Myelogenous Leukemia. *Cancer* (2002) 95(8):1673–84. doi: 10.1002/cncr.10832
48. Dhakal P, Gundabolu K, Amador C, Rayamajhi S, Bhatt VR. Atypical Chronic Myeloid Leukemia: A Rare Entity With Management Challenges. *Future Oncol* (2018) 14(2):177–85. doi: 10.2217/fon-2017-0334
49. Orazi A, O'Malley DP, Arber DA. Illustrated Pathology of the Bone Marrow. *Cambridge Univ Press: Cambridge UK* (2006) 2006:88–92. doi: 10.1017/CBO9780511543531
50. Tefferi A, Gilliland G. Classification of Chronic Myeloid Disorders: From Dameshek Towards a Semi-Molecular System. *Best Pract Res Clin Haematol* (2006) 19(3):365–85. doi: 10.1016/j.beha.2005.07.001
51. Krishna Chandran R, Geetha N, Sakthivel KM, Suresh Kumar R, Jagathnath Krishna KMN, Sreedharan H. Impact of Additional Chromosomal Aberrations on the Disease Progression of Chronic Myelogenous Leukemia. *Front Oncol* (2019) 9:88. doi: 10.3389/fonc.2019.00088
52. Clark RE, Apperley JF, Copland M, Cicconi S. Additional Chromosomal Abnormalities at Chronic Myeloid Leukemia Diagnosis Predict an Increased Risk of Progression. *Blood Adv* (2021) 5(4):1102–9. doi: 10.1182/bloodadvances.2020003570
53. Ochi Y, Yoshida K, Huang YJ, Kuo MC, Nannya Y, Sasaki K, et al. Clonal Evolution and Clinical Implications of Genetic Abnormalities in Blastic Transformation of Chronic Myeloid Leukaemia. *Nat Commun* (2021) 12 (1):2833. doi: 10.1038/s41467-021-23097-w
54. Gotlib J. How I Treat Atypical Chronic Myeloid Leukemia. *Blood* (2017) 129 (7):838–45. doi: 10.1182/blood-2016-08-693630
55. Mughal TI, Cross NC, Padron E, Tiu RV, Savona M, Malcovati L, et al. An International MDS/MPN Working Group's Perspective and Recommendations on Molecular Pathogenesis, Diagnosis and Clinical Characterization of Myelodysplastic/Myeloproliferative Neoplasms. *Haematologica* (2015) 100(9):1117–30. doi: 10.3324/haematol.2014.114660
56. Tiu RV, Sekeres MA. Making Sense of the Myelodysplastic/Myeloproliferative Neoplasms Overlap Syndromes. *Curr Opin Hematol* (2014) 21(2):131–40. doi: 10.1097/MOH.0000000000000021
57. Zoi K, Cross NC. Molecular Pathogenesis of Atypical CML, CMML and MDS/MPN-Unclassifiable. *Int J Hematol* (2015) 101(3):229–42. doi: 10.1007/s12185-014-1670-3
58. Meggendorfer M, Haferlach T, Alpermann T, Jeromin S, Haferlach C, Kern W, et al. Specific Molecular Mutation Patterns Delineate Chronic Neutrophilic Leukemia, Atypical Chronic Myeloid Leukemia, and Chronic Myelomonocytic Leukemia. *Haematologica* (2014) 99(12):e244–6. doi: 10.3324/haematol.2014.113159
59. Makishima H, Yoshida K, Nguyen N, Przychodzen B, Sanada M, Okuno Y, et al. Somatic SETBP1 Mutations in Myeloid Malignancies. *Nat Genet* (2013) 45(8):942–6. doi: 10.1038/ng.2696
60. 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(1):144–7. doi: 10.1182/blood-2009-03-210039
61. Tefferi A, Elliot MA, Pardanani A. Atypical Myeloproliferative Disorders: Diagnosis and Management. *Mayo Clinic Proc* (2006) 81(4):553–63. doi: 10.4065/81.4.553
62. Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang LY, et al. Widespread Occurrence of the JAK2 V617F Mutation in Chronic Myeloproliferative Disorders. *Blood* (2005) 106(6):2162–8. doi: 10.1182/blood-2005-03-1320
63. Carulli G, Fabiani O, Azzara A. The Syndrome of Abnormal Chromatin Clumping in Leukocytes. *Haematologica* (1997) 82(5):635–6. doi: 10.3324/%25x
64. Fend F, Horn T, Koch I, Vela T, Orazi A. Atypical Chronic Myeloid Leukemia as Defined in the WHO Classification is a JAK2 V617F Negative Neoplasm. *Leukemia Res* (2008) 32(12):1931–5. doi: 10.1016/j.leukres.2008.04.024
65. Sakaguchi H, Okuno Y, Muramatsu H, Yoshida K, Shiraishi Y, Takahashi M, et al. Exome Sequencing Identifies Secondary Mutations of SETBP1 and JAK3 in Juvenile Myelomonocytic Leukemia. *Nat Genet* (2013) 45(8):937–41. doi: 10.1038/ng.2698
66. Inoue D, Kitaura J, Matsui H, Hou HA, Chou WC, Nagamachi A, et al. SETBP1 Mutations Drive Leukemic Transformation in ASXL1-Mutated MDS. *Leukemia* (2015) 29(4):847–57. doi: 10.1038/leu.2014.301
67. Fabiani E, Falconi G, Fianchi L, Criscuolo M, Leone G, Voso MT. SETBP1 Mutations in 106 Patients With Therapy-Related Myeloid Neoplasms. *Haematologica* (2014) 99(9):e152–3. doi: 10.3324/haematol.2014.108159
68. Patnaik MM, Itzykson R, Lasho T, Kosmider O, Finke C, Hanson C, et al. ASXL1 and SETBP1 Mutations and Their Prognostic Contribution in

Chronic Myelomonocytic Leukemia: A Two-Center Study of 466 Patients. *Leukemia* (2014) 28(11):2206–12. doi: 10.1038/leu.2014.125
69. Thol F, Suchanek KJ, Koenecke C, Stadler M, Platzbecker U, Thiede C, et al. SETBP1 Mutation Analysis in 944 Patients With MDS and AML. *Leukemia* (2013) 27(10):2072–5. doi: 10.1038/leu.2013.145
70. Wang XA, Muramatsu H, Okuno Y, Sakaguchi H, Yoshida K, Kawashima N, et al. GATA2 and Secondary Mutations in Familial Myelodysplastic Syndromes and Pediatric Myeloid Malignancies. *Haematologica* (2015) 100(10):E398–401. doi: 10.3324/haematol.2015.127092
71. Linder K, Iragavarapu C, Liu D. Mutations as a Biomarker for Myelodysplasia /Myeloproliferative Neoplasm Overlap Syndrome. *biomark Res* (2017) 5:33. doi: 10.1186/s40364-017-0113-8
72. Lasho T. Atypical CML- the Role of Morphology and Precision Genomics. *Best Pract Res Clin Haematol* (2020) 33(2):101133. doi: 10.1016/j.beha.2019.101133
73. Chen M, Yao H, Chen S, Wang Q, Wen L, Xie J, et al. Rare Occurrence of SET Binding Protein 1 Mutation in Patients With Acute Lymphoblastic Leukemia, Mixed Phenotype Acute Leukemia and Chronic Myeloid Leukemia in Blast Crisis. *Leuk Lymphoma* (2014) 55(9):2209–10. doi: 10.3109/10428194.2013.873536
74. Minakuchi M, Kakazu N, Gorrin-Rivas MJ, Abe T, Copeland TD, Ueda K, et al. Identification and Characterization of SEB, a Novel Protein That Binds to the Acute Undifferentiated Leukemia-Associated Protein SET. *Eur J Biochem* (2001) 268(5):1340–51. doi: 10.1046/j.1432-1327.2001.02000.x
75. Janssens V, Goris J. Protein Phosphatase 2A: A Highly Regulated Family of Serine/Threonine Phosphatases Implicated in Cell Growth and Signalling. *Biochem J* (2001) 353:417–39. doi: 10.1042/bj3530417
76. Wang GL, Iakova P, Wilde M, Awad S, Timchenko NA. Liver Tumors Escape Negative Control of Proliferation *via* PI3K/Akt-Mediated Block of C/EBP Alpha Growth Inhibitory Activity. *Genes Dev* (2004) 18(8):912–25. doi: 10.1101/gad.1183304
77. Kawabe T, Muslin AJ, Korsmeyer SJ. HOX11 Interacts With Protein Phosphatases PP2A and PP1 and Disrupts a G2/M Cell-Cycle Checkpoint. *Nature* (1997) 385(6615):454–8. doi: 10.1038/385454a0
78. Mumby M. PP2A: Unveiling a Reluctant Tumor Suppressor. *Cell* (2007) 130(1):21–4. doi: 10.1016/j.cell.2007.06.034
79. Westermarck J, Hahn WC. Multiple Pathways Regulated by the Tumor Suppressor PP2A in Transformation. *Trends Mol Med* (2008) 14(4):152–60. doi: 10.1016/j.molmed.2008.02.001
80. Janssens V, Goris J, Van Hoof C. Pp2a: The Expected Tumor Suppressor. *Curr Opin Genet Dev* (2005) 15(1):34–41. doi: 10.1016/j.gde.2004.12.004
81. Schönthal AH. Role of Serine/Threonine Protein Phosphatase 2A in Cancer. *Cancer Lett* (2001) 170(1):1–13. doi: 10.1016/S0304-3835(01)00561-4
82. Cristobal I, Garcia-Orti L, Cirauqui C, Alonso MM, Calasanz MJ, Odero MD. PP2A Impaired Activity is a Common Event in Acute Myeloid Leukemia and its Activation by Forskolin has a Potent Anti-Leukemic Effect. *Leukemia* (2011) 25(4):606–14. doi: 10.1038/leu.2010.294
83. Arnold HK, Sears RC. A Tumor Suppressor Role for PP2A-B56 Alpha Through Negative Regulation of C-Myc and Other Key Oncoproteins. *Cancer Metastasis Rev* (2008) 27(2):147–58. doi: 10.1007/s10555-008-9128-9
84. Sablina AA, Hahn WC. The Role of PP2A a Subunits in Tumor Suppression. *Cell Adhesion Migration* (2007) 1(3):140–1. doi: 10.4161/cam.1.3.4986
85. Cristobal I, Blanco FJ, Garcia-Orti L, Marcotegui N, Vicente C, Rifon J, et al. SETBP1 Overexpression is a Novel Leukemogenic Mechanism That Predicts Adverse Outcome in Elderly Patients With Acute Myeloid Leukemia. *Blood* (2010) 115(3):615–25. doi: 10.1182/blood-2009-06-227363
86. Piazza R, Magistroni V, Redaelli S, Mauri M, Massimino L, Sessa A, et al. SETBP1 Induces Transcription of a Network of Development Genes by Acting as an Epigenetic Hub. *Nat Commun* (2018) 9(1):2192. doi: 10.1038/s41467-018-04462-8
87. Kataoka K, Kurokawa M. Ecotropic Viral Integration Site 1, Stem Cell Self-Renewal and Leukemogenesis. *Cancer Sci* (2012) 103(8):1371–7. doi: 10.1111/j.1349-7006.2012.02303.x
88. Oakley K, Han YF, Vishwakarma BA, Chu S, Bhatia R, Gudmundsson KO, et al. Setbp1 Promotes the Self-Renewal of Murine Myeloid Progenitors via Activation of Hoxa9 and Hoxa10. *Blood* (2012) 119(25):6099–108. doi: 10.1182/blood-2011-10-388710
89. Vishwakarma BA, Nguyen N, Makishima H, Hosono N, Gudmundsson KO, Negi V, et al. Runx1 Repression by Histone Deacetylation is Critical for Setbp1-Induced Mouse Myeloid Leukemia Development. *Leukemia* (2016) 30(1):200–8. doi: 10.1038/leu.2015.200
90. Ichikawa M, Asai T, Saito T, Seo S, Yamazaki I, Yamagata T, et al. AML-1 is Required for Megakaryocytic Maturation and Lymphocytic Differentiation, But Not for Maintenance of Hematopoietic Stem Cells in Adult Hematopoiesis. *Nat Med* (2004) 10(3):299–304. doi: 10.1038/nm997
91. Growney JD, Shigematsu H, Li Z, Lee BH, Adeisperger J, Rowan R, et al. Loss of Runx1 Perturbs Adult Hematopoiesis and is Associated With a Myeloproliferative Phenotype. *Blood* (2005) 106(2):494–504. doi: 10.1182/blood-2004-08-3280
92. Lykidis A, Wang J, Karim MA, Jackowski S. Overexpression of a Mammalian Ethanolamine-Specific Kinase Accelerates the CDP-Ethanolamine Pathway. *J Biol Chem* (2001) 276(3):2174–9. doi: 10.1074/jbc.M008794200
93. Kennedy EP, Weiss SB. The Function of Cytidine Coenzymes in the Biosynthesis of Phospholipides. *J Biol Chem* (1956) 222(1):193–214. doi: 10.1016/S0021-9258(19)50785-2
94. Lasho TL, Finke CM, Zblewski D, Patnaik M, Ketterling RP, Chen D, et al. Novel Recurrent Mutations in Ethanolamine Kinase 1 (ETNK1) Gene in Systemic Mastocytosis With Eosinophilia and Chronic Myelomonocytic Leukemia. *Blood Cancer J* (2015) 5:e275. doi: 10.1038/bcj.2014.94
95. Zhou XA, Louissaint A, Wenzel A, Yang J, Martinez-Escala ME, Moy AP, et al. Genomic Analyses Identify Recurrent Alterations in Immune Evasion Genes in Diffuse Large B-Cell Lymphoma, Leg Type. *J Invest Dermatol* (2018) 138(11):2365–76. doi: 10.1016/j.jid.2018.04.038
96. Fontana D, Mauri M, Renso R, Docci M, Crespiatico I, Røst LM, et al. ETNK1 Mutations Induce a Mutator Phenotype That can be Reverted With Phosphoethanolamine. *Nat Commun* (2020) 11(1):5938. doi: 10.1038/s41467-020-19721-w
97. Fontana D, Gambacorti-Passerini C, Piazza R. Impact of ETNK1 Somatic Mutations on Phosphoethanolamine Synthesis, ROS Production and DNA Damage. *Mol Cell Oncol* (2021) 8(2):1877598. doi: 10.1080/23723556.2021.1877598
98. Carbuccia N, Murati A, Trouplin V, Brecqueville M, Adélaïde J, Rey J, et al. Mutations of ASXL1 Gene in Myeloproliferative Neoplasms. *Leukemia* (2009) 23(11):2183–6. doi: 10.1038/leu.2009.141
99. Scheuermann JC, de Ayala Alonso AG, Oktaba K, Ly-Hartig N, McGinty RK, Fraterman S, et al. Histone H2A Deubiquitinase Activity of the Polycomb Repressive Complex PR-DUB. *Nature* (2010) 465(7295):243–7. doi: 10.1038/nature08966
100. Abdel-Wahab O, Gao J, Adli M, Dey A, Trimarchi T, Chung YR, et al. Deletion of Asxl1 Results in Myelodysplasia and Severe Developmental Defects *In Vivo*. *J Exp Med* (2013) 210(12):2641–59. doi: 10.1084/jem.20131141
101. Wang J, Li Z, He Y, Pan F, Chen S, Rhodes S, et al. Loss of Asxl1 Leads to Myelodysplastic Syndrome-Like Disease in Mice. *Blood* (2014) 123(4):541–53. doi: 10.1182/blood-2013-05-500272
102. Guallar D, Bi X, Pardavila JA, Huang X, Saenz C, Shi X, et al. RNA-Dependent Chromatin Targeting of TET2 for Endogenous Retrovirus Control in Pluripotent Stem Cells. *Nat Genet* (2018) 50(3):443–51. doi: 10.1038/s41588-018-0060-9
103. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, et al. Leukemic IDH1 and IDH2 Mutations Result in a Hypermethylation Phenotype, Disrupt TET2 Function, and Impair Hematopoietic Differentiation. *Cancer Cell* (2010) 18(6):553–67. doi: 10.1016/j.ccr.2010.11.015
104. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of Genetic Lesions in 944 Patients With Myelodysplastic Syndromes. *Leukemia* (2014) 28(2):241–7. doi: 10.1038/leu.2013.336
105. Jankowska AM, Szpurka H, Tiu RV, Makishima H, Afable M, Huh J, et al. Loss of Heterozygosity 4q24 and TET2 Mutations Associated With Myelodysplastic/Myeloproliferative Neoplasms. *Blood* (2009) 113(25):6403–10. doi: 10.1182/blood-2009-02-205690
106. Reiter A, Invernizzi R, Cross NCP, Cazzola M. Molecular Basis of Myelodysplastic/Myeloproliferative Neoplasms. *Haematologica-the Hematol J* (2009) 94(12):1634–8. doi: 10.3324/haematol.2009.014001
107. Solary E, Bernard OA, Tefferi A, Fuks F, Vainchenker W. The Ten-Eleven Translocation-2 (TET2) Gene in Hematopoiesis and Hematopoietic Diseases. *Leukemia* (2014) 28(3):485–96. doi: 10.1038/leu.2013.337

108. Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Lobry D, Lobry C, et al. Tet2 Loss Leads to Increased Hematopoietic Stem Cell Self-Renewal and Myeloid Transformation. *Cancer Cell* (2011) 20(1):11–24. doi: 10.1016/j.ccr.2011.06.001
109. Li Z, Cai X, Cai CL, Wang J, Zhang W, Petersen BE, et al. Deletion of Tet2 in Mice Leads to Dysregulated Hematopoietic Stem Cells and Subsequent Development of Myeloid Malignancies. *Blood* (2011) 118(17):4509–18. doi: 10.1182/blood-2010-12-325241
110. Tefferi A, Pardanani A, Lim KH, Abdel-Wahab O, Lasho TL, Patel J, et al. TET2 Mutations and Their Clinical Correlates in Polycythemia Vera, Essential Thrombocythemia and Myelofibrosis. *Leukemia* (2009) 23(5):905–11. doi: 10.1038/leu.2009.47
111. Kosmider O, Gelsi-Boyer V, Ciudad M, Racoeur C, Jooste V, Vey N, et al. TET2 Gene Mutation is a Frequent and Adverse Event in Chronic Myelomonocytic Leukemia. *Haematologica* (2009) 94(12):1676–81. doi: 10.3324/haematol.2009.011205
112. Lowy DR, Willumsen BM. Function and Regulation of Ras. *Annu Rev Biochem* (1993) 62:851–91. doi: 10.1146/annurev.bi.62.070193.004223
113. Hirai H, Tanaka S, Azuma M, Anraku Y, Kobayashi Y, Fujisawa M, et al. Transforming Genes in Human Leukemia Cells. *Blood* (1985) 66(6):1371–8. doi: 10.1182/blood.V66.6.1371.1371
114. Hirsch-Ginsberg C, LeMaistre AC, Kantarjian H, Talpaz M, Cork A, Freireich EJ, et al. RAS Mutations are Rare Events in Philadelphia Chromosome-Negative/Bcr Gene Rearrangement-Negative Chronic Myelogenous Leukemia, But are Prevalent in Chronic Myelomonocytic Leukemia. *Blood* (1990) 76(6):1214–9. doi: 10.1182/blood.V76.6.1214.1214
115. Braun BS, Tuveson DA, Kong N, Le DT, Kogan SC, Rozmus J, et al. Somatic Activation of Oncogenic Kras in Hematopoietic Cells Initiates a Rapidly Fatal Myeloproliferative Disorder. *Proc Natl Acad Sci USA* (2004) 101(2):597–602. doi: 10.1073/pnas.0307203101
116. Chan IT, Kutok JL, Williams IR, Cohen S, Kelly L, Shigematsu H, et al. Conditional Expression of Oncogenic K-Ras From its Endogenous Promoter Induces a Myeloproliferative Disease. *J Clin Invest* (2004) 113(4):528–38. doi: 10.1172/JCI20476
117. Van Meter ME, Díaz-Flores E, Archard JA, Passegué E, Irish JM, Kotecha N, et al. K-Rasg12d Expression Induces Hyperproliferation and Aberrant Signaling in Primary Hematopoietic Stem/Progenitor Cells. *Blood* (2007) 109(9):3945–52. doi: 10.1182/blood-2006-09-047530
118. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, et al. Role of Histone H3 Lysine 27 Methylation in Polycomb-Group Silencing. *Science* (2002) 298(5595):1039–43. doi: 10.1126/science.1076997
119. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, et al. Inactivating Mutations of the Histone Methyltransferase Gene EZH2 in Myeloid Disorders. *Nat Genet* (2010) 42(8):722–6. doi: 10.1038/ng.621
120. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, et al. Somatic Mutations Altering EZH2 (Tyr641) in Follicular and Diffuse Large B-Cell Lymphomas of Germinal-Center Origin. *Nat Genet* (2010) 42(2):181–5. doi: 10.1038/ng.518
121. Yap DB, Chu J, Berg T, Schapira M, Cheng SW, Moradian A, et al. Somatic Mutations at EZH2 Y641 Act Dominantly Through a Mechanism of Selectively Altered PRC2 Catalytic Activity, to Increase H3K27 Trimethylation. *Blood* (2011) 117(8):2451–9. doi: 10.1182/blood-2010-11-321208
122. Béguelin W, Popovic R, Teater M, Jiang Y, Bunting KL, Rosen M, et al. EZH2 is Required for Germinal Center Formation and Somatic EZH2 Mutations Promote Lymphoid Transformation. *Cancer Cell* (2013) 23(5):677–92. doi: 10.1016/j.ccr.2013.04.011
123. Gu Z, Liu Y, Cai F, Patrick M, Zmajkovic J, Cao H, et al. Loss of EZH2 Reprograms BCAA Metabolism to Drive Leukemic Transformation. *Cancer Discovery* (2019) 9(9):1228–47. doi: 10.1158/2159-8290.CD-19-0152
124. Nakamura S, Takayama N, Hirata S, Seo H, Endo H, Ochi K, et al. Expandable Megakaryocyte Cell Lines Enable Clinically Applicable Generation of Platelets From Human Induced Pluripotent Stem Cells. *Cell Stem Cell* (2014) 14(4):535–48. doi: 10.1016/j.stem.2014.01.011
125. Mechaal A, Menif S, Abbes S, Safra I. EZH2, New Diagnosis and Prognosis Marker in Acute Myeloid Leukemia Patients. *Adv Med Sci* (2019) 64(2):395–401. doi: 10.1016/j.advms.2019.07.002
126. Stasik S, Middeke JM, Kramer M, Röllig C, Krämer A, Scholl S, et al. EZH2 Mutations and Impact on Clinical Outcome: An Analysis in 1,604 Patients With Newly Diagnosed Acute Myeloid Leukemia. *Haematologica* (2020) 105(5):e228–31. doi: 10.3324/haematol.2019.222323
127. Blyth K, Cameron ER, Neil JC. The RUNX Genes: Gain or Loss of Function in Cancer. *Nat Rev Cancer* (2005) 5(5):376–87. doi: 10.1038/nrc1607
128. Chuang LS, Ito K, Ito Y. RUNX Family: Regulation and Diversification of Roles Through Interacting Proteins. *Int J Cancer* (2013) 132(6):1260–71. doi: 10.1002/ijc.27964
129. Schindler JW, Van Buren D, Foudi A, Krejci O, Qin J, Orkin SH, et al. TEL-AML1 Corrupts Hematopoietic Stem Cells to Persist in the Bone Marrow and Initiate Leukemia. *Cell Stem Cell* (2009) 5(1):43–53. doi: 10.1016/j.stem.2009.04.019
130. Gaidzik VI, Teleanu V, Papaemmanuil E, Weber D, Paschka P, Hahn J, et al. RUNX1 Mutations in Acute Myeloid Leukemia are Associated With Distinct Clinico-Pathologic and Genetic Features. *Leukemia* (2016) 30(11):2282. doi: 10.1038/leu.2016.126
131. Matheny CJ, Speck ME, Cushing PR, Zhou Y, Corpora T, Regan M, et al. Disease Mutations in RUNX1 and RUNX2 Create Nonfunctional, Dominant-Negative, or Hypomorphic Alleles. *EMBO J* (2007) 26(4):1163–75. doi: 10.1038/sj.emboj.7601568
132. Kuo MC, Liang DC, Huang CF, Shih YS, Wu JH, Lin TL, et al. RUNX1 Mutations are Frequent in Chronic Myelomonocytic Leukemia and Mutations at the C-Terminal Region Might Predict Acute Myeloid Leukemia Transformation. *Leukemia* (2009) 23(8):1426–31. doi: 10.1038/leu.2009.48
133. Gelsi-Boyer V, Trouplin V, Adélaïde J, Aceto N, Remy V, Pinson S, et al. Genome Profiling of Chronic Myelomonocytic Leukemia: Frequent Alterations of RAS and RUNX1 Genes. *BMC Cancer* (2008) 8:299. doi: 10.1186/1471-2407-8-299
134. Ernst T, Chase A, Zoi K, Waghorn K, Hidalgo-Curtis C, Score J, et al. Transcription Factor Mutations in Myelodysplastic/Myeloproliferative Neoplasms. *Haematologica* (2010) 95(9):1473–80. doi: 10.3324/haematol.2010.021808
135. Fu XD, Maniatis T. The 35-Kda Mammalian Splicing Factor SC35 Mediates Specific Interactions Between U1 and U2 Small Nuclear Ribonucleoprotein Particles at the 3' Splice Site. *Proc Natl Acad Sci USA* (1992) 89(5):1725–9. doi: 10.1073/pnas.89.5.1725
136. Wahl MC, Will CL, Lührmann R. The Spliceosome: Design Principles of a Dynamic RNP Machine. *Cell* (2009) 136(4):701–18. doi: 10.1016/j.cell.2009.02.009
137. Liu HX, Chew SL, Cartegni L, Zhang MQ, Krainer AR. Exonic Splicing Enhancer Motif Recognized by Human SC35 Under Splicing Conditions. *Mol Cell Biol* (2000) 20(3):1063–71. doi: 10.1128/MCB.20.3.1063-1071.2000
138. Lin S, Coutinho-Mansfield G, Wang D, Pandit S, Fu XD. The Splicing Factor SC35 has an Active Role in Transcriptional Elongation. *Nat Struct Mol Biol* (2008) 15(8):819–26. doi: 10.1038/nsmb.1461
139. Das R, Yu J, Zhang Z, Gygi MP, Krainer AR, Gygi SP, et al. SR Proteins Function in Coupling RNAP II Transcription to Pre-Mrna Splicing. *Mol Cell* (2007) 26(6):867–81. doi: 10.1016/j.molcel.2007.05.036
140. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, et al. Frequent Pathway Mutations of Splicing Machinery in Myelodysplasia. *Nature* (2011) 478(7367):64–9. doi: 10.1038/nature10496
141. Patnaik MM, Lasho TL, Finke CM, Hanson CA, Hodnefield JM, Knudson RA, et al. Spliceosome Mutations Involving SRSF2, SF3B1, and U2AF35 in Chronic Myelomonocytic Leukemia: Prevalence, Clinical Correlates, and Prognostic Relevance. *Am J Hematol* (2013) 88(3):201–6. doi: 10.1002/ajh.23373
142. Meggendorfer M, Roller A, Haferlach T, Eder C, Dicker F, Grossmann V, et al. SRSF2 Mutations in 275 Cases With Chronic Myelomonocytic Leukemia (CMML). *Blood* (2012) 120(15):3080–8. doi: 10.1182/blood-2012-01-404863
143. Kim E, Ilagan JO, Liang Y, Daubner GM, Lee SC, Ramakrishnan A, et al. SRSF2 Mutations Contribute to Myelodysplasia by Mutant-Specific Effects on Exon Recognition. *Cancer Cell* (2015) 27(5):617–30. doi: 10.1016/j.ccell.2015.04.006
144. Meggendorfer M, Jeromin S, Haferlach C, Kern W, Haferlach T. The Mutational Landscape of 18 Investigated Genes Clearly Separates Four

Subtypes of Myelodysplastic/Myeloproliferative Neoplasms. *Haematologica* (2018) 103(5):e192–5. doi: 10.3324/haematol.2017.183160
145. Senín A, Arenillas L, Martínez-Avilés L, Fernández-Rodríguez C, Bellosillo B, Florensa L, et al. [Molecular Characterization of Atypical Chronic Myeloid Leukemia and Chronic Neutrophilic Leukemia]. *Med Clin (Barc)* (2015) 144 (11):487–90. doi: 10.1016/j.medcle.2014.03.005
146. Makishima H, Visconte V, Sakaguchi H, Jankowska AM, Abu Kar S, Jerez A, et al. Mutations in the Spliceosome Machinery, a Novel and Ubiquitous Pathway in Leukemogenesis. *Blood* (2012) 119(14):3203–10. doi: 10.1182/blood-2011-12-399774
147. Nau MM, Lipkowitz S. Comparative Genomic Organization of the Cbl Genes. *Gene* (2003) 308:103–13. doi: 10.1016/S0378-1119(03)00471-2
148. Thien CB, Walker F, Langdon WY. RING Finger Mutations That Abolish C-Cbl-Directed Polyubiquitination and Downregulation of the EGF Receptor are Insufficient for Cell Transformation. *Mol Cell* (2001) 7(2):355–65. doi: 10.1016/S1097-2765(01)00183-6
149. Fu JF, Hsu JJ, Tang TC, Shih LY. Identification of CBL, a Proto-Oncogene at 11q23.3, as a Novel MLL Fusion Partner in a Patient With De Novo Acute Myeloid Leukemia. *Genes Chromosomes Cancer* (2003) 37(2):214–9. doi: 10.1002/gcc.10204
150. Sanada M, Suzuki T, Shih LY, Otsu M, Kato M, Yamazaki S, et al. Gain-of-Function of Mutated C-CBL Tumour Suppressor in Myeloid Neoplasms. *Nature* (2009) 460(7257):904–8. doi: 10.1038/nature08240
151. Makishima H, Cazzolli H, Szpurka H, Dunbar A, Tiu R, Huh J, et al. Mutations of E3 Ubiquitin Ligase Cbl Family Members Constitute a Novel Common Pathogenic Lesion in Myeloid Malignancies. *J Clin Oncol* (2009) 27 (36):6109–16. doi: 10.1200/JCO.2009.23.7503
152. Jankowska AM, Makishima H, Tiu RV, Szpurka H, Huang Y, Traina F, et al. Mutational Spectrum Analysis of Chronic Myelomonocytic Leukemia Includes Genes Associated With Epigenetic Regulation: UTX, EZH2, and DNMT3A. *Blood* (2011) 118(14):3932–41. doi: 10.1182/blood-2010-10-311019
153. Kohlmann A, Grossmann V, Klein HU, Schindela S, Weiss T, Kazak B, et al. Next-Generation Sequencing Technology Reveals a Characteristic Pattern of Molecular Mutations in 72.8% of Chronic Myelomonocytic Leukemia by Detecting Frequent Alterations in TET2, CBL, RAS, and RUNX1. *J Clin Oncol* (2010) 28(24):3858–65. doi: 10.1200/JCO.2009.27.1361
154. Grand FH, Hidalgo-Curtis CE, Ernst T, Zoi K, Zoi C, McGuire C, et al. Frequent CBL Mutations Associated With 11q Acquired Uniparental Disomy in Myeloproliferative Neoplasms. *Blood* (2009) 113(24):6182–92. doi: 10.1182/blood-2008-12-194548
155. Beekman R, Touw IP. G-CSF and its Receptor in Myeloid Malignancy. *Blood* (2010) 115(25):5131–6. doi: 10.1182/blood-2010-01-234120
156. Liu F, Wu HY, Wesselschmidt R, Kornaga T, Link DC. Impaired Production and Increased Apoptosis of Neutrophils in Granulocyte Colony-Stimulating Factor Receptor-Deficient Mice. *Immunity* (1996) 5(5):491–501. doi: 10.1016/S1074-7613(00)80504-X
157. Maxson JE, Gotlib J, Pollyea DA, Fleischman AG, Agarwal A, Eide CA, et al. Oncogenic CSF3R Mutations in Chronic Neutrophilic Leukemia and Atypical CML. *N Engl J Med* (2013) 368(19):1781–90. doi: 10.1056/NEJMoa1214514
158. Pardanani A, Lasho TL, Laborde RR, Elliott M, Hanson CA, Knudson RA, et al. CSF3R T618I is a Highly Prevalent and Specific Mutation in Chronic Neutrophilic Leukemia. *Leukemia* (2013) 27(9):1870–3. doi: 10.1038/leu.2013.122
159. Albano F, Zagaria A, Anelli L, Coccaro N, Impera L, Minervini CF, et al. Gene Expression Profiling of Chronic Myeloid Leukemia With Variant T (9;22) Reveals a Different Signature From Cases With Classic Translocation. *Mol Cancer* (2013) 12:36. doi: 10.1186/1476-4598-12-36
160. Cammarata G, Augugliaro L, La Rosa M, Turri D, Rizzo V, Marfia A, et al. Gene Expression Profile of Chronic Myeloid Leukemia Innately Resistant to Imatinib. *Clin Leukemia* (2007) 1(4):234–42. doi: 10.3816/CLK.2007.n.014
161. Crossman LC, Mori M, Hsieh YC, Lange T, Paschka P, Harrington CA, et al. In Chronic Myeloid Leukemia White Cells From Cytogenetic Responders and non-Responders to Imatinib Have Very Similar Gene Expression Signatures. *Haematologica* (2005) 90(4):459–64.
162. Frank O, Brors B, Fabarius A, Li L, Haak M, Merk S, et al. Gene Expression Signature of Primary Imatinib-Resistant Chronic Myeloid Leukemia Patients. *Leukemia* (2006) 20(8):1400–7. doi: 10.1038/sj.leu.2404270
163. McLean LA, Gathmann I, Capdeville R, Polymeropoulos MH, Dressman M. Pharmacogenomic Analysis of Cytogenetic Response in Chronic Myeloid Leukemia Patients Treated With Imatinib. *Clin Cancer Res* (2004) 10(1 Pt 1):155–65. doi: 10.1158/1078-0432.CCR-0784-3
164. Ohno R, Nakamura Y. Prediction of Response to Imatinib by Cdna Microarray Analysis. *Semin Hematol* (2003) 40(2 Suppl 2):42–9. doi: 10.1053/shem.2003.50041
165. Tipping AJ, Deininger MW, Goldman JM, Melo JV. Comparative Gene Expression Profile of Chronic Myeloid Leukemia Cells Innately Resistant to Imatinib Mesylate. *Exp Hematol* (2003) 31(11):1073–80. doi: 10.1016/j.exphem.2003.08.006
166. Villuendas R, Steegmann JL, Pollán M, Tracey L, Granda A, Fernández-Ruiz E, et al. Identification of Genes Involved in Imatinib Resistance in CML: A Gene-Expression Profiling Approach. *Leukemia* (2006) 20(6):1047–54. doi: 10.1038/sj.leu.2404197
167. Mittal P, Saliba RM, Giralt SA, Shahjahan M, Cohen AI, Karandish S, et al. Allogeneic Transplantation: A Therapeutic Option for Myelofibrosis, Chronic Myelomonocytic Leukemia and Philadelphia-Negative/BCR-ABL-Negative Chronic Myelogenous Leukemia. *Bone Marrow Transplant* (2004) 33(10):1005–9. doi: 10.1038/sj.bmt.1704472
168. Koldehoff M, Beelen DW, Trenschel R, Steckel NK, Peceny R, Ditschkowski M, et al. Outcome of Hematopoietic Stem Cell Transplantation in Patients With Atypical Chronic Myeloid Leukemia. *Bone Marrow Transplant* (2004) 34(12):1047–50. doi: 10.1038/sj.bmt.1704686
169. Koldehoff M, Steckel NK, Hegerfeldt Y, Ditschkowski M, Beelen DW, Elmaagacli AH. Clinical Course and Molecular Features in 21 Patients With Atypical Chronic Myeloid Leukemia. *Int J Lab Hematol* (2012) 34 (1):e3–5. doi: 10.1111/j.1751-553X.2011.01351.x
170. Onida F, de Wreede LC, van Biezen A, Eikema DJ, Byrne JL, Iori AP, et al. Allogeneic Stem Cell Transplantation in Patients With Atypical Chronic Myeloid Leukaemia: A Retrospective Study From the Chronic Malignancies Working Party of the European Society for Blood and Marrow Transplantation. *Br J Haematol* (2017) 177(5):759–65. doi: 10.1111/bjh.14619
171. Lim SN, Lee JH, Kim DY, Kim SD, Kang YA, Lee YS, et al. Allogeneic Hematopoietic Cell Transplantation in Adult Patients With Myelodysplastic/Myeloproliferative Neoplasms. *Blood Res* (2013) 48 (3):178–84. doi: 10.5045/br.2013.48.3.178
172. Kurzrock R, Kantarjian HM, Shtalrid M, Gutterman JU, Talpaz M. Philadelphia Chromosome-Negative Chronic Myelogenous Leukemia Without Breakpoint Cluster Region Rearrangement: A Chronic Myeloid Leukemia With a Distinct Clinical Course. *Blood* (1990) 75(2):445–52. doi: 10.1182/blood.V75.2.445.445
173. Montefusco E, Alimena G, Lo Coco F, De Cuia MR, Wang YZ, Aloe Spiriti MA, et al. Ph-Negative and Bcr-Negative Atypical Chronic Myelogenous Leukemia: Biological Features and Clinical Outcome. *Ann Hematol* (1992) 65(1):17–21. doi: 10.1007/BF01715120
174. Costello R, Lafage M, Toiron Y, Brunel V, Sainty D, Arnoulet C, et al. Philadelphia Chromosome-Negative Chronic Myeloid Leukaemia: A Report of 14 New Cases. *Br J Haematol* (1995) 90(2):346–52. doi: 10.1111/j.1365-2141.1995.tb05157.x
175. Costello R, Sainty D, LafagePochitaloff M, Gabert J. Clinical and Biological Aspects of Philadelphia-Negative/BCR-Negative Chronic Myeloid Leukemia. *Leuk Lymphoma* (1997) 25(3-4):225–32. doi: 10.3109/10428199709114162
176. Jabbour E, Kantarjian H, Cortes J, Thomas D, Garcia-Manero G, Ferrajoli A, et al. PEG-IFN-Alpha-2b Therapy in BCR-ABL-Negative Myeloproliferative Disorders - Final Result of a Phase 2 Study. *Cancer* (2007) 110(9):2012–8. doi: 10.1002/cncr.23018
177. Gerds AT. I Walk the Other Line: Myelodysplastic/Myeloproliferative Neoplasm Overlap Syndromes. *Curr Hematol Malignancy Rep* (2014) 9 (4):400–6. doi: 10.1007/s11899-014-0233-2
178. Hausmann H, Bhatt VR, Yuan J, Maness LJ, Ganti AK. Activity of Single-Agent Decitabine in Atypical Chronic Myeloid Leukemia. *J Oncol Pharm Pract* (2016) 22(6):790–4. doi: 10.1177/1078155215605662

179. Ades L, Sekeres MA, Wolfromm A, Teichman ML, Tiu RV, Itzykson R, et al. Predictive Factors of Response and Survival Among Chronic Myelomonocytic Leukemia Patients Treated With Azacitidine. *Leuk Res* (2013) 37(6):609–13. doi: 10.1016/j.leukres.2013.01.004
180. Kantarjian HM, O'Brien S, Cortes J, Giles FJ, Faderl S, Issa JP, et al. Results of Decitabine (5-Aza-2 ' Deoxycytidine) Therapy in 130 Patients With Chronic Myelogenous Leukemia. *Cancer* (2003) 98(3):522–8. doi: 10.1002/cncr.11543
181. Tong XZ, Li J, Zhou ZH, Zheng D, Liu JR, Su C. Efficacy and Side-Effects of Decitabine in Treatment of Atypical Chronic Myeloid Leukemia. *Leuk Lymphoma* (2015) 56(6):1911–3. doi: 10.3109/10428194.2014.986477
182. Mao L, You L, Yang M, Li Y, Ye X, Tong H. The First Case of Decitabine Successfully in Treatment of Atypical Chronic Myeloid Leukemia With CEBPA Double Mutation. *Chemotherapy* (2013) 2:114. doi: 10.4172/2167-7700.1000114
183. Jiang HF, Wu ZL, Ren L, Tao DH, Tong HY. Decitabine for the Treatment of Atypical Chronic Myeloid Leukemia: A Report of Two Cases. *Oncol Lett* (2016) 11(1):689–92. doi: 10.3892/ol.2015.3977
184. Patnaik MM, Tefferi A. Chronic Myelomonocytic Leukemia: 2016 Update on Diagnosis, Risk Stratification, and Management. *Am J Hematol* (2016) 91 (6):632–42. doi: 10.1002/ajh.24396
185. Talati C, Padron E. An Exercise in Extrapolation: Clinical Management of Atypical CML, MDS/MPN-Unclassifiable, and MDS/MPN-RS-T. *Curr Hematol Malig Rep* (2016) 11(6):425–33. doi: 10.1007/s11899-016-0350-1
186. Khanna V, Pierce ST, Dao KH, Tognon CE, Hunt DE, Junio B, et al. Durable Disease Control With MEK Inhibition in a Patient With NRAS-Mutated Atypical Chronic Myeloid Leukemia. *Cureus* (2015) 7(12):e414. doi: 10.7759/cureus.414
187. Levine RL, Loriaux M, Huntly BJ, Loh ML, Beran M, Stoffregen E, et al. The JAK2V617F Activating Mutation Occurs in Chronic Myelomonocytic Leukemia and Acute Myeloid Leukemia, But Not in Acute Lymphoblastic Leukemia or Chronic Lymphocytic Leukemia. *Blood* (2005) 106(10):3377–9. doi: 10.1182/blood-2005-05-1898
188. Steensma DP, Dewald GW, Lasho TL, Powell HL, McClure RF, Levine RL, et al. The JAK2 V617F Activating Tyrosine Kinase Mutation is an Infrequent Event in Both "Atypical" Myeloproliferative Disorders and Myelodysplastic Syndromes. *Blood* (2005) 106(4):1207–9. doi: 10.1182/blood-2005-03-1183
189. Gao T, Yu C, Xia S, Liang T, Gu X, Liu Z. A Rare Atypical Chronic Myeloid Leukemia Bcr-Abl1 Negative With Concomitant. *Ther Adv Hematol* (2020) 11:2040620720927105. doi: 10.1177/2040620720927105
190. Pardanani A, Tefferi A. Is There a Role for JAK Inhibitors in BCR-ABL1-Negative Myeloproliferative Neoplasms Other Than Myelofibrosis? *Leuk Lymphoma* (2014) 55(12):2706–11. doi: 10.3109/10428194.2014.985159
191. Dao KT, Gotlib J, Deininger MMN, Oh ST, Cortes JE, Collins RH, et al. Efficacy of Ruxolitinib in Patients With Chronic Neutrophilic Leukemia and Atypical Chronic Myeloid Leukemia. *J Clin Oncol* (2020) 38(10):1006–18. doi: 10.1200/JCO.19.00895

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Oncology

REVIEW
published: 16 November 2021
doi: 10.3389/fonc.2021.778741

Hybrid or Mixed Myelodysplastic/ Myeloproliferative Disorders – Epidemiological Features and Overview

Andrea Kuendgen, Annika Kasprzak and Ulrich Germing*

Department of Hematology, Oncology, and Clinical Immunology, Heinrich-Heine-University Hospital Duesseldorf, Duesseldorf, Germany

The WHO-category Myelodysplastic/Myeloproliferative neoplasms (MDS/MPNs) recognizes a unique group of clonal myeloid malignancies exhibiting overlapping features of myelodysplastic as well as myeloproliferative neoplasms. The group consists of chronic myelomonocytic leukemia (CMML), atypical chronic myeloid leukemia, *BCR-ABL1*-negative (aCML), juvenile myelomonocytic leukemia (JMML), myelodysplastic/ myeloproliferative neoplasm with ringed sideroblasts and thrombocytosis (MDS/MPN-RS-T), and myelodysplastic/myeloproliferative neoplasms, unclassifiable (MDS/MPN-U). The most frequent entity in this category is CMML, while all other diseases are extremely rare. Thus, only very limited data on the epidemiology of these subgroups exists. An appropriate diagnosis and classification can be challenging since the diagnosis is still largely based on morphologic criteria and myelodysplastic as well as myeloproliferative features can be found in various occurrences. The diseases in this category share several features that are common in this specific WHO-category, but also exhibit specific traits for each disease. This review summarizes published data on epidemiological features and offers a brief overview of the main diagnostic criteria and clinical characteristics of the five MDS/MPN subgroups.

Keywords: MDS/MPN, overlap syndromes, CMML, MDS/MPN-RS-T, aCML, JMML, MDS/MPN-U, epidemiology

OPEN ACCESS

Edited by:
Cyrus Khandanpour, University Hospital Münster, Germany

Reviewed by:
Rosa Ayala, Research Institute Hospital 12 de Octubre, Spain
Roberto Latagliata, Unitelma Sapienza University, Italy

****Correspondence:***
Andrea Kuendgen
kuendgen@med.uni-duesseldorf.de

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

Received: *17 September 2021*
Accepted: *25 October 2021*
Published: *16 November 2021*

Citation:
Kuendgen A, Kasprzak A and Germing U (2021) Hybrid or Mixed Myelodysplastic/Myeloproliferative Disorders – Epidemiological Features and Overview. Front. Oncol. 11:778741. doi: 10.3389/fonc.2021.778741

INTRODUCTION

The World Health Organization (WHO) recognizes a group of rare clonal hematopoietic malignancies with mixed features of Myelodysplastic Syndrome (MDS) as well as Myeloproliferative Neoplasms (MPNs) (1). These malignancies are placed in a separate WHO-category named myelodysplastic/myeloproliferative neoplasms (MDS/MPN). The group consists of myeloid diseases including chronic myelomonocytic leukemia (CMML), atypical chronic myeloid leukemia, *BCR-ABL1*-negative (aCML), juvenile myelomonocytic leukemia (JMML), myelodysplastic/ myeloproliferative neoplasm with ringed sideroblasts and thrombocytosis (MDS/MPN-RS-T), and

myelodysplastic/myeloproliferative neoplasms, unclassifiable (MDS/MPN-U). Diagnosing these diseases can be challenging, as they can exhibit different features of MDS and MPNs. While the simultaneous existence of dysplasia and proliferation is mandatory, other features might be cytopenias, often in coexistence with "cytoses" and organomegaly. "MDS-like" symptoms as a result of ineffective hematopoiesis including fatigue, dyspnea, infections, and bleeding occur in parallel to the more "MPN-like" symptoms resulting from proliferative hematopoiesis, namely night sweats, weight loss, and increased risk of thromboembolic complications. Unfortunately, the morphological features of MDS/MPNs are not specific but can be found in other myeloid malignancies at presentation or as part of disease progression. Diagnostically, there is a considerable overlap between the different MDS/MPNs as well as the different myelodysplastic and myeloproliferative neoplasms. At present, no cytogenetic or molecular genetic abnormalities specific for any of the MDS/MPN subtypes exist. Nevertheless, genetic abnormalities play an important role in excluding a diagnosis of a particular MDS/MPN and some abnormalities might at least help ascertain the correct subtype (2–17).

The existence of disorders with overlapping myelodysplastic and myeloproliferative features has been described years ago. A true recognition and classification of this group of diseases, however, occurred much more recently. In 1976 the French-American-British (FAB) cooperative group introduced a classification and nomenclature of the acute myeloid and lymphoid leukemias (18). Two types of MDS, RAEB and CMML, were presented as an addendum. Then, in 1982, the FAB-group introduced a classification and nomenclature of the MDS (19). CMML was included as one of the 5 subtypes of MDS in this classification system. Only with the introduction of the WHO-classification (20) in 2001 the existence of overlap syndromes between MDS and MPN was formally recognized and CMML was moved into this new founded category of myeloid malignancies. In addition to CMML the new group included aCML, JMML, and MDS/MPN-U. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T) was initially proposed as a provisional entity in the WHO 2001 classification of myeloid neoplasms (20) and only 2016 recognized as a formal subgroup (MDS/MPN-RS-T) of MDS/MPN by the latest version of the WHO-classification (1). Additional entities that have been discussed and might represent separate entities of MDS/MPN in future classifications are MDS with isolated del(5q) and *JAK2-V617F* mutation and MDS/MPN with isolated isochromosome 17q (21–30).

The WHO-category MDS/MPN encompasses three relatively well-defined entities, namely CMML, JMML, and MDS/MPN-RS-T, the diagnostic criteria for which are easy to follow. In contrast, aCML and MDS/MPN-U are less well-defined and their diagnosis largely remains a matter of exclusion of other myeloid neoplasms (1). Main diagnostic criteria of the MDS/MPN subgroups according to WHO 2016 are depicted in **Table 1**.

The entities included within the WHO-category MDS/MPN share several common features, but also exhibit differences defining the individual disease (13, 15, 31–54). The hallmark of MDS/MPNs is the unique mixture of cytopenias and "cytoses". Therefore, the bone marrow is typically hypercellular due to the combination of a very effective "myeloproliferative" hematopoiesis and an ineffective, dysplastic hematopoiesis (54–57). Dysplasia is seen in at least one hematopoietic lineage. By definition, the diseases have further characteristics in common: The percentage of blasts in PB and BM must be <20%. Certain cytogenetic abnormalities must be ruled out to exclude other genetically defined myeloid malignancies sharing features of myelodysplastic and myeloproliferative diseases. These include *BCR-ABL1, PDGFRA, PDGFRB, PCM1-JAK2*, and *FGFR1* (1). Except JMML, MDS/MPN are diseases of the elderly and all MDS/MPN show a clear male preponderance, with the possible exception of MDS/MPN-RS-T where the gender distribution differs between publications and some even exhibit a slight predominance of the female gender (13, 15, 31–53). A high frequency of anemia is a further characteristic of most MDS/MPN, while other cytopenias are often less pronounced when compared to MDS, or "cytoses" occur. An increased WBC is frequent or mandatory in proliferative CMML, JMML, aCML, MDS/MPN-U and MDS/MPN-RS-T. Thrombocytosis is mandatory in MDS/MPN-RS-T and can occur in all other MDS/MPN subtypes as well (1, 50–54, 57).

The majority of patients show fatigue and most, maybe except MDS/MPN-RS-T, exhibit frequent general (MPN-like) symptoms like night sweats as well as symptoms of organomegaly and extramedullary disease. Spleno- and often an additional hepatomegaly are frequent clinical findings, especially in CMML and JMML. Again, an exception might be MDS/MPN-RS-T, but the data on these clinical features is unfortunately sparse regarding this rare entity (15, 17, 41, 43, 44, 46, 48–54, 58).

MDS/MPN also share a very low frequency of cytogenetic aberrations compared to MDS. In this regard the exception might be MDS/MPN-U. If cytogenetic abnormalities occur, +8 is by far the most frequent (13, 14, 17, 37, 39–44, 46, 48–54, 58). The frequency of molecular abnormalities on the other hand is very high. Such aberrations can be found in more than 90% of patients (2–17, 50–54, 58). Except in MDS/MPN-RS-T and partly in MDS/MPN-U, the frequency of JAK-2 mutations is very low when compared to classical MPNs (50–54).

Unfortunately, another feature, shared by this group of overlap diseases, is a poor response to treatment other than allogeneic stem cell transplantation. CMML, aCML, and MDS/MPN-U share a poor prognosis in general and afflicted patients are often too old for transplantation (16, 17, 31–36, 41, 43, 44, 46–54, 59–61). JMML might have a special role, as some children show spontaneous regression and otherwise most afflicted patients can be transplanted, but on the other hand the severity of the disease is obvious regarding the still unsatisfying long-term survival and the poor response to treatment other than transplantation can be observed for JMML as well (32, 37, 62). The only true exception seems to be MDS/MPN-RS-T which exhibits a low risk of progression and a long median survival time (13, 39–42). An overview of differences and similarities between the MDS/MPN subgroups is given in **Table 2**.

TABLE 1 | Main diagnostic criteria of the MDS/MPN subgroups according to WHO 2016 (1).

CMML	JMML	MDS/MPN-RS-T	aCML	MDS/MPN-U
Persistent peripheral monocytosis (≥1000/µl) with monocytes accounting for ≥10% of leukocytes (1)	Peripheral blood monocyte count (≥1000/µl) (required)	Anemia associated with erythroid lineage dysplasia, with or without multilineage dysplasia; ≥15% ringed sideroblasts	Peripheral blood leukocytosis ≥13000/µl, due to increased numbers of neutrophils and their precursors (i.e., promyelocytes, myelocytes, and metamyelocytes), with neutrophil precursors constituting ≥10% of the leukocytes	Myeloid neoplasm with mixed myeloproliferative and myelodysplastic features at onset, not meeting the WHO criteria for any other myelodysplastic/ myeloproliferative neoplasm
- Dysplasia involving ≥ myeloid lineage or - if myelodysplasia is absent or minimal, criteria (1-4) are met and - an acquired, clonal cytogenetic or molecular genetic abnormality is present in hematopoietic cells or - the monocytosis has persisted for ≥3 months and all other causes of monocytosis (i.e., malignance, infection, and inflammation) have been excluded	Splenomegaly (required)	Persistent thrombocytosis, with platelet count ≥450000/µl	- Dysgranulopoiesis, which may include abnormal chromatin clumping - No or minimal absolute basophilia; basophils constitute <2% of the peripheral blood leukocytes - No or minimal absolute monocytosis; monocytes constitute <10% of the peripheral blood leukocytes - Hypercellular bone marrow with granulocytic proliferation and granulocytic dysplasia, with or without dysplasia in the erythroid or megakaryocytic lineages	- Clinical and morphologic features of one of the myelodysplastic syndromes - Clinical and morphologic myeloproliferative features manifesting as platelet count of ≥450000/µl associated with bone marrow megakaryocyte proliferation and/or a white blood count of 13000/µl
- WHO-criteria for *BCR-ABL1*-positive chronic myeloid leukemia, primary myelofibrosis, polycythemia vera, and essential thrombocythemia are not met (2)	- No Philadelphia chromosome or *BCR-ABL1* fusion (required)	- No *BCR-ABL1* fusion - No history of myeloproliferative neoplasm, myelodysplastic syndrome (except myelodysplastic syndrome with ringed sideroblasts), or other myelodysplastic/myeloproliferative neoplasm	- WHO-criteria for *BCR-ABL1*-positive chronic myeloid leukemia, primary myelofibrosis, polycythemia vera, and essential thrombocythemia are not met	- WHO-criteria for *BCR-ABL1*-positive chronic myeloid leukemia, primary myelofibrosis, polycythemia vera, and essential thrombocythemia are not met - No history of recent cytotoxic or growth factor therapy that could explain the myelodysplastic/myeloproliferative features
- No rearrangement of *PDGFRA, PDGFRB, PCM1-JAK2*, and *FGFR1* (must be specifically excluded in cases of eosinophilia) (3)	- Somatic mutation in *PTPN11, KRAS*, or *NRAS* - Clinical diagnosis of neurofibromatosis type 1 or *NF1* mutation - Germline *CBL* mutation and loss of heterozygosity of *CBL* (1 genetic criterion is sufficient) Cases that do not meet any of the genetic criteria must meet the following criteria in addition to the clinical and hematological criteria: - Monosomy 7 or any other chromosomal abnormality or ≥2 of the following: - Increased hemoglobin F for age - Myeloid or erythroid	- SF3B1 mutation or, in the absence of SF3B1 mutation, no history of recent cytotoxic or growth factor therapy that could explain the myelodysplastic/myelo-proliferative features - no rearrangement of *PDGFRA, PDGFRB, PCM1-JAK2*, and *FGFR1* (must be specifically excluded in cases of eosinophilia) and - no t(3;3)(q21.3;q26.2), inv(3)(q21.3;q26.2), or del(5q)	- No rearrangement of *PDGFRA, PDGFRB, PCM1-JAK2*, and *FGFR1* (must be specifically excluded in cases of eosinophilia)	- No rearrangement of *PDGFRA, PDGFRB, PCM1-JAK2*, and *FGFR1* (must be specifically excluded in cases of eosinophilia) - No del(5q)

(Continued)

TABLE 1 | Continued

CMML	JMML	MDS/MPN-RS-T	aCML	MDS/MPN-U
	precursors on peripheral blood smear - Granulocyte-macrophage colony-stimulating factor (CSF2) hypersensitivity in colony assay - Hyperphosphorylation of STAT5			
Blasts constitute <20% of the cells in the peripheral blood and bone marrow (4)	Blasts constitute <20% of the cells in the peripheral blood and bone marrow (required)	<1% blasts in the peripheral blood and <5% blasts in the bone marrow	Blasts constitute <20% of the cells in the peripheral blood and bone marrow	Blasts constitute <20% of the cells in the peripheral blood and bone marrow

EPIDEMIOLOGY OF MDS/MPN

Epidemiological studies on MDS/MPNs are scarce and most of the existing data is limited to CMML. For other MDS/MPN subtypes only vague estimations exist.

Regarding CMML, Dinmohamed et al. (31) find an annual standardized incidence (ASR) rate of 0.3 per 100.000 for the period of 1989 to 2012 in the Netherlands. The ASR increased from 1989 to 2007 and remained then stable at 0.38/100 000 until 2012. The ASR was higher in males (0.42) when compared to females (0.18) and increased with age from 0.02 per 100.000 under the age of 50 years to 3.62 in patients ≥80 years old. Relative survival did not improve over time with the 5-year relative survival rate (RSR) being only 16, 20, and 20% in the three time periods investigated. Interestingly, the RSR was poor in all age groups, ranging from 12% in the group above 80 years to 21% in patients younger than 50 years of age.

In a study on the epidemiology of MDS and MPDs in the United States from 2001 to 2004, data from the NAACCR as well as the SEER programs was used by Rollison et al. (34). They found an age adjusted incidence of 0.3 per 100 000, about one-tenth that of MDS. Survival of patients with CMML was worse when compared to MDS and MPD, the 3-year survival being 21%. While 3-year survival decreased with increasing age, similar to the Dutch study survival was again poor in all age groups. 3-year survival was 12% in patients over the age of 80 and, in comparison, still only 33% in patients with an age between 50 and 59 years. This was clearly inferior when compared to MDS (37% and 54%) and MPD (66 and 89%), respectively.

Another US study on Seer data reported incidence and survival of patients with MDS and MPDs between 2001 and 2012 (32). An age adjusted incidence rate of 4.3 per 100 000 was reported for all MDS/MPN taken together. The largest proportion represented by far the patients with CMML (4.1 per 100 000). Patients with aCML had an age adjusted incidence rate of 0.1 same as patients with JMML. Incidence rates for MDS/MPN-U or MDS/MPN-RS-T were not reported in this study. Incidence rates were higher in males when compared to females for all 3 overlap syndromes reported (male: female ratio 2.31 for CMML, 2.05 for aCML, and 2.30 for JMML). Incidence increased exponentially with increasing age. This was especially apparent for CMML when compared to MPNs. For aCML and JMML such data was not available. The incidence rates for CMML were significantly lower in Hispanics (3.0), Blacks (3.1), or Asian/Pacific Islanders (2.7) compared to Non-Hispanic Whites (4.4). For the other subgroups numbers were too low for such calculations. Incidence rates for CMML did not increase significantly over the time period investigated. As in the other studies survival of CMML patients was generally very poor, but slightly better in women *vs* men and younger *vs*. older patients (5-year survival 16% males ≥60, 27% males <60 years old, 18% females ≥60, 32% females <60 years of age). For aCML 5-year survival was even worse with 11% for male patients compared to 16% for female patients over the age of 60. In children with JMML the 5-year survival rate was 56% for males and 66% for females, without treatment data given.

TABLE 2 | Differences and similarities between the MDS/MPNs.

	CMML	JMML	MDS/MPN-RS-T	aCML	MDS/MPN-U
Incidence	accounts for 12.5% of all MDS currently in the MDS registry (35) –0.3-0.4 (31, 33, 34, 62); 1 (36); 4.1 (32) cases per 100 000; For comparison: incidence MDS 3-5/100 000 (34, 63–65)	0.1 per 100 000 (32), about 1 per million children per year (66); <2-3% of all leukemias in children, –20-30% of all cases of MDS or MPD in children, 0.63 per million children per year (38) For comparison: Incidence of leukemia in children –6 per 100 000 (67) Incidence of childhood MDS 0.5-6/million population per year (37, 66, 68)	accounts for 1,45% of all MDS currently in the Duesseldorf MDS registry (35) <1% of MDS (49); 0.7% of all MDS cases (50, 69); For comparison: incidence MDS 3-5/100 000 (34, 63–65)	0.1 per 100 000 (32); 1-2% of bcr-abl positive CML (44, 70, 71) For comparison: incidence bcr-abl+ CML –0.7-1.5 per 100 000 (72, 73)	accounts for 0,05% of all MDS currently in the Duesseldorf MDS registry (35) <2% of MDS (49); For comparison: incidence MDS 3-5/100 000 (34, 63–65)
Median Age/ years	75-76 (31–33)	<1 -2 (32, 37, 38)	63-75 (13, 39–42)	62-73 (15, 32, 43–47)	65-71 (16, 17, 41, 43, 48, 49)
Gender	61-76% male (31– 36)	71-73% male (32, 38)	47-62% male (13, 39–42)	55-69% male (32, 43, 46, 47)	64-76% male (16, 17, 41, 43, 48)
Bone marrow findings	current data from the Duesseldorf registry: cellularity: 68% hypercellular 27% normocellular 5% hypocellular fibrosis: 16% (35) Degree of marrow fibrosis: 54% (74); ≥MF2 3% (75), ≥MF2 77% (76); possible presence of plasmacytoid dendritic cells (58, 77–79); Cellularity: hypocellular 5%, normocellular 30%, hypercellular 65% (50); differentiation between CMML 0, I, and II depending on marrow blast count	Hypercellular marrow, often reduced megakaryocytes, normal- moderately elevated blast count (51)	current data from the Duesseldorf registry: cellularity: 67% hypercellular 31% normocellular 2% hypocellular fibrosis: 8% (35) <5% marrow blasts [median 1-2% (13, 41, 42)], presence of >15% ringed sideroblasts, presence of large, atypical megakaryocytes, usually hypercellular marrow (13, 42)	Degree of marrow fibrosis: 37% (15), 71% (52), 31% (43); median blast count 1-3% (15, 43–46); hypercellular marrow, myeloid hyperplasia, granulocytic dysplasia prominent, erythroid hypoplasia (52, 57)	current data from the Duesseldorf registry: cellularity: 50% hypercellular 50% normocellular 0% hypocellular fibrosis: 42% (35) Degree of marrow fibrosis: 27% (43); Median marrow blast count 2-3% (16, 17, 41, 43, 48)
Peripheral cell counts	Absolute and relative monocytosis, pB blast count <20%, cytopenias may occur in all cell lines, differentiation between dysplastic and proliferative CMML by WBC (</>13000/µl); frequency dysplastic:proliferative 42%:58% (36), 50%:50% (50)	leukocytosis, monocytosis, immature monocytes, myelocytes, metamyelocytes, nucleated red cells, presence of peripheral blasts frequent (median <2%), often thrombocytopenia and moderate anemia (51)	WBC can be slightly decreased, normal or more often mild to moderately increased, thrombocytosis by definition, moderate to severe anemia (13, 39–42)	WBC increased, mostly mild to moderate anemia, absolute monocytosis is common, but the percentage of monocytes is low, no prominent basophilia (57)	WBC can be everything between decreased and strongly increased; platelet counts, and hemoglobin values can vary as well (16, 17, 43, 48)
Localization	High frequency of hepato- and splenomegaly, and extramedullary involvement (especially proliferative subtype) 27% splenomegaly in the Duesseldorf MDS registry (35)	Almost 100% hepato-splenomegaly, frequently lymphadenopathy, skin rashes, and other extramedullary involvement	Frequency of hepato-, splenomegaly 12% (41) 18% splenomegaly in the Duesseldorf MDS registry (35)	Frequency of splenomegaly 50-70% (15, 44, 46), frequency of 49% hepatomegaly (44)	Frequency of splenomegaly 23-36% (17, 41, 43, 48), Hepatomegaly 10-23% (41, 43) 62% splenomegaly in the Duesseldorf MDS registry (35)
Clinical features	Frequently fatigue, night sweats, symptoms from organomegaly, bone pain, weight loss, cachexia (58), about 30% can present with autoimmune diseases/systemic inflammatory syndromes (80–83); hyperleukocytosis/ leukostasis can occur	Increased synthesis of hemoglobin F; signs of autoimmunity in 25% of children, hypergammaglobulinemia in about 50% (37); often pulmonary infiltrates with dry cough, tachypnea, and interstitial infiltrates; frequent gut infiltrates with diarrhea and gastrointestinal infections, skin rashes, eventually features of syndromic disease like café-au-lait spots, facial dysmorphia, heart disease, failure to thrive, hearing loss, and others (51)	Frequently presenting with fatigue, increased risk of thromboembolic events, comparable to ET; "MPD-typical" general symptoms appear to be less frequent	Frequently fatigue, night sweats, symptoms from organomegaly, bone pain, weight loss, cachexia; hyperleukocytosis/leukostasis can occur	Constitutional symptoms 69% (48); 61% symptomatic (46% fatigue, 15% night sweats) (49)
Cytogenetic features	20-30% abnormal, most frequent +8, -7, del(7q), -Y (58)	About 35% abnormal, about 25% -7 (37)	About 10-20% abnormal, most common +8 (13, 14, 39–42)	20-44% abnormal, most frequent +8, del(20q) (43, 44, 46)	35-51% abnormal, most frequent +8, -7/del(7q) del(20q), and complex (17, 43, 48, 49)
Molecular genetic features	Frequency of molecular abnormalities >90% most frequent TET2 (–60%), SRSF2 (–50%), ASXL1 (–40%), RUNX1 (–15%), SETBP1 (–15%), CBL (–15%), NRAS (–15%), KRAS (–10%), SF3B1 (–5-10%), U2AF1 (–5-10%), IDH2 (–5-10%), ZRSR2 (–5%), PHF6 (–5%), PTPN11 (–5%), EZH2 (–5%), DNMT3A (–5%), FLT3 (–<5%), TP53 (–1%), IDH1 (–1%) (2–11, 58, 84); JAK-2 relatively uncommon	Mainly altered RAS pathway; about 90% of patients have mutations in either PTPN11, NRAS, KRAS, CBL, or NF1; secondary genetic alterations: ASXL1, EZH2, SETBP1, JAK3, spliceosomal genes (less frequent) (12)	SF3B1 (–85%), JAK2 (–50%), TET2 (–25%), ASXL1 (–20%), DNMT3A (–15%), SETBP1 (–10%), EZH2 (–7%), SRSF2 (–7%), U2AF1 (–5%), IDH2 (–4%), CBL (–4%), ZRSR2 (–3%), MPL (–3%), ETV6 (–3%), RUNX1 (–1%) (13, 14, 84)	SETBP1 (30%), ETNK1 (16%) ASXL1 (43%), TET2 (27%), NRAS/KRAS (22%), EZH2 (19%), RUNX1 (14%), SRSF2 (14%), CBL (8%), CREBBP (8%), CSF3R (<5%) (15),; JAK-2 relatively uncommon (84)	ASXL1 (29%), TET2 (27%), JAK2 (25%), SRSF2 (23%), EZH2 (17%), U2AF1 (13%), RUNX1 (13%), SF3B1 (12%), ZRSR2 (11%), SETBP1 (11%), NRAS (10%), DNMT3A (9%), TP53 (8%) CSF3R (5%), STAG2 (5%), CBL (4%), ETV6 (4%), NPM1 (4%), CEBPA (4%), CALR (3%), KRAS (3%), PTPN11 (3%), MPL (3%) (16) ASXL1 (56%), SRSF2 (37%), SETBP1 (21%), JAK2 (19%), NRAS (15%), TET2 (13%) (17, 84)
survival	5-year survival 20% (31); 3-year survival 21% (34); 5-year survival 16% males ≥60, 27% males <60years old, 18% females ≥60, 32% females <60 years of age (32); RSR at 3-years 27-37%, 5-years 19-23% in the US and RSR at 3-years 48-40%, 5-years 34-26% in Switzerland (33); Median OS: not reached to 18 months (CPSS) (59), 97-16 months (Mayo Molecular Model) (60), and 56-9.2 months (GFM-Model) (61) Current data from the Duesseldorf MDS registry (35): mOS: CMML 0: 33 months CMML I: 20 months CMML II: 14 months	5-year survival 56% males, 66% females (32); children with CBL-mutated JMML often experience spontaneous regression as well as a few patients with NRAS mutated JMML; the majority of patients requires allogeneic transplantation; allogeneic transplantation results in a disease-free survival of 52% (data from 2005) (62); survival at 10 years 0.39 for children after HSCT and 0.06 for children without HSCT (data from 1997) (37)	Median OS 76 months (39); median OS 80, 42, and 11 months in three different risk groups (13); median OS NR (41); median OS 88-101 month (42); median OS 10,7 years (40) Current data from the Duesseldorf MDS registry (35): mOS: 61 months	5-year survival 11% males, 16% females ≥60 of age (32); median OS 25 months (44), Median OS 24 months, 5-year survival 7% (46); median OS 10 months, 2-year survival 28% (47); median OS 12 months (43)	Median OS 19 months (41), median OS 12 months (from sample date) (16); median OS 12.4 months (from presentation) (48); median OS 26 months (17); median OS 21 months (49); median OS 22 months (43)
Leukemic transformation	AML transformation rate 39% (36); 4-year leukemic transformation rate 0-48% (59); AML transformation 19% after a median of 7 months (46); AML transformation 8, 23, and 23% at 5 yrs (63); AML transformation 13, 29, 60, and 73% (59), AML transformation 16% at a median follow up of 23 months (60) Current data from the Duesseldorf MDS registry (35): AML at 2 yrs: CMML O: 14% CMML I: 21% CMML II: 42% AML at 5 yrs: CMML O: 22% CMML I: 32% CMML II: 64%	No data available	1.8/100 patients/year (39, 40); 2% at a median follow-up of 27 months (13); AML transformation 7% (median time to AML or follow-up not given) (42) Current data from the Duesseldorf MDS registry (35): AML at 2 yrs.: 5% AML at 5 yrs.: 8%	AML transformation 40%, median time to AML 18 months (44); AML transformation 31%, median time to AML 11.5 months (46); AML transformation 9%, median time to AML 5 months (47); AML transformation 37%, median leukemia free survival 11 months (43)	AML transformation 16% after a median of 11 months (16); AML transformation 16% after a median follow up of 61 months, median LFS 24 months (17); AML transformation 23%, median LFS 19 months (43); AML transformation 54% (70% of non-RARS-T MDS/MPN-U) after a median follow-up of 20 months (49)
Subtypes and closely related diseases	CMML associated with mastocytosis, CMML and blastic plasmacytoid dendritic cell neoplasm, CMML associated with eosinophilia (77–79), t-CMML (85), pre CMML syndromes (oligo-monocytic CMML) (86, 87), CMML with JAK-2 mutation, CMML with rearranged PDGFRA, PDGFRB, FGFR1, PCM1-JAK2, other MPN (MF, PV.) with monocytosis ("MPN with CMML-like phenotype") (88)	Noonan Syndrome (51)		aCML with abnormal chromatin clumping (89–92)	MDS with del(5q) and *JAK2 V617F* mutation, MDS with isolated isochromosome (17q) (21–30)

In an investigation on the incidence of MDS in Western Greece during a 20-year period (1990-2009) Avgerinou et al. (36) found an incidence of MDS of 6 per 100.000 inhabitants. From the data given, a crude incidence of 1 per 100 000 can be calculated for CMML while the incidence is only 0.1 per 100 000 for all other MDS/MPD together. The incidence of CMML remained stable over the time period investigated. Within the period under investigation 39% of CMML patients progressed to AML.

In a comparative study between Switzerland and the US (SEER-data) Benzarti et al. described epidemiological trends regarding CMML between 1999 and 2014 (33). The age standardized incidence was similar and remained relatively stable in both countries, being 0.32 (1999-2006) and 0.38 (2007-2014) in Switzerland and 0.37 and 0.35 in the US. In both countries and time periods it was much higher in patients above the age of 75 (3.01-4.83 ≥75 *vs*. 0.17-0.25 <75 years of age) and higher in males when compared to females (0.51-0.57 *vs*. 0.17-0.25). There were an increasing proportion of older patients ≥75 years of age observed in the Swiss Cancer Registry compared to a decreasing in the US SEER database. Relative survival improved significantly in the US database (3-years 27-37%, 5-years 19-23%) and remained stable in Switzerland (3-years 48-40%, 5-years 34-26%).

In our MDS registry CMML accounts for about 12.5% of all MDS during a period from 1982 to 2020, leading to a rough incidence of 0.4 per 100.000 that remained relatively stable over the investigated time period (35).

CMML is by far the most frequent of MDS/MPNs. Published incidence rates range from 0.3-4.1 per 100 000 inhabitants with a median age above 70 years and a male predominance (31–36, 63). CMML might be described as even more heterogeneous when compared to MDS, with hematological characteristics ranging from solely dysplastic forms, presenting often cytopenic and resembling MDS with peripheral monocytosis to very proliferative forms, characterized by high white blood cell counts, but also by splenomegaly, extramedullary involvement, and strong general symptoms. Therefore, the initial distinction, as proposed by the FAB-classification (19), between dysplastic and proliferative CMML remains useful from a clinical point of view. Diagnosis is based on the presence of sustained (>3 months) peripheral blood monocytosis, along with bone marrow dysplasia. In the current WHO-classification CMML is subdivided into 3 different groups (CMML 0-II) according to blast count (1, 93). In 386 patients from our Duesseldorf registry Schuler et al. found a distribution of 26% CMML-0, 53% CMML-I, and 21% CMML-II (94).

Chromosomal abnormalities are less frequent in CMML when compared to MDS and have been described in about 10-40% of cases. On the other hand, more than 90% of CMML patients exhibit molecular mutations. These are relatively homogenous compared to other myeloid malignancies and mostly belong to a subset of 20 frequently mutated genes (58, 61, 66, 95). The clinical course of CMML patients is extremely variable, with wide differences in survival and leukemic transformation risk. Generally, survival is low around 20-35% at 5 years (35, 36, 58, 60, 93), even in lower age groups, but varies between the different prognostic risk groups. In several studies on CMML prognosis (CPSS, Mayo- Molecular Model, GFM-Model) (59–61) median survival ranged from 56 months to not reached in the best and 9-18 months in the worst prognostic group. The risk of leukemic transformation is around 15% over 3-5 years (59–61), but again varies considerably between subgroups (4-year leukemic transformation rate 0-48% [CPSS-paper) (59)].

JMML is a clonal hematopoietic stem cell disorder of childhood. It is extremely rare with an incidence rate of about 1 per 1 000 000 children under the age of 14 years (12, 37, 38, 96). Like CMML the disease is characterized by proliferation of the monocytic lineage. The age at diagnosis can vary between 1 month and early adolescence, but at least 50% of children are below 2 years old and only 5% are 5 years or older (37). Splenomegaly occurs in almost all cases, and hepatomegaly, lymphadenopathy as well as extramedullary involvement including skin, lung, and gastro-intestinal tract are common. While JMML shares a number of features with CMML, its pathobiology is unique. About a third of patients have cytogenetic abnormalities, about a quarter show monosomy 7. Molecular abnormalities occur in at least 90% of patients and usually involve the RAS pathway. About 90% of cases belong to one of 5 groups with mutations in either *PTPN11, NRAS, KRAS, CBL*, or *NF1*. The first three subtypes (*PTPN11, NRAS, KRAS*) are characterized by heterozygous somatic gain-of-function mutations in non-syndromic children, while JMML in neurofibromatosis type 1 and JMML in children with CBL-syndrome are characterized by germ line RAS disease and acquired biallelic inactivation of the *NF1* or *CBL* gene in hematopoietic cells (12, 37). Clinical presentation as well as outcome differs between these 5 JMML subtypes. Secondary genetic alterations like *ASXL1, EZH2, SETBP1, JAK3*, and mutations in spliceosomal genes often result in disease progression. Generally, a wide variation exists regarding the clinical course of the disease. In about 15% of children, most frequently in CBL mutated disease, spontaneous regression occurs. The majority of children affected by JMML, however, require allogeneic transplantation to cure the disease. An allogeneic stem cell transplantation from a histo-compatible sibling or HLA-matched unrelated donor results in a disease-free survival of 52% in a study from 2005 by Locatelli et al. (62). In an earlier study (1997) the probability of survival at 10 years was 0.39 for children having received allogeneic stem cell transplantation and 0.06 for children that did not receive HSCT (37). Variables like age, level of HbF, platelet count, or, more recently described, genome-wide DNA methylation profiles may be helpful to predict the clinical course.

aCML is a rare, *BCR-ABL1*-negative, MDS/MPN overlap syndrome characterized by leukocytosis, granulocytic dysplasia, and a dismal prognosis. It was first described as a variant CML lacking the Philadelphia chromosome, but diagnostic criteria have evolved since. However, it can still be challenging to distinguish aCML from other MPNs like chronic neutrophilic leukemia or from other MDS/MPN like MDS/MPN-U, as the

diagnosis largely relies on morphologic criteria. Its frequency is not well known, but it is estimated to account for 1-2% of *BCR-ABL1*-positive CML (~0.5-2/100000) (44, 70–73). The disorder affects elderly patients with a median age of 62-73 years and a male predominance (15, 32, 43–47, 97, 98). The clinical picture is comparable to Philadelphia positive CML including elevated WBC with co-occurrence of mature and immature cells of the granulocytic lineage, splenomegaly, and mild to moderate anemia. Typical for aCML, however, are severe dysplastic features predominantly in the granulocytic lineage. Also, in contrast to classical CML, the genetic basis of the disease is heterogeneous, with *SETBP1* and *ETNK1* mutations being recurrent, but several other mutations, typical for MDS and/or MPNs can be found as well (15). Cytogenetic abnormalities are less frequent and occur in 20-40% of patients only (43, 44, 46). Median survival varies between 10 and 25 months (43, 44, 46, 47). AML evolution occurs in about 31-40% of patients with a median time to AML of 11-18 months (43, 44, 46).

Of the MDS/MPN overlap syndromes, MDS/MPN-U is the least well defined. It encompasses such patients, that show features of myelodysplastic as well as myeloproliferative disease, who do not fit into one of the other 4 subgroups. The diagnosis is extremely rare, accounting for less than 2% of MDS (49). In the Duesseldorf MDS registry it currently accounts for only 0.05% of all MDS (35). Patients with MDS/MPN-U are relatively old with a median age of 70 years and show the male predominance that is seen in other MDS/MPN subgroups. Regarding cytogenetic features, the percentage of abnormal, including complex karyotypes is higher when compared to other MDS/MPN. Of the molecular abnormalities found in patients with MDS/MPN-U the *JAK2-V617F* mutation is relatively frequent in contrast to the other overlap syndromes except MDS-RS-T. Clinical characteristics are not well established and often seem to show similarities with one of the other MDS/MPN-subgroups. Thus, MDS/MPN-U is rather a mixture of patients not fulfilling all criteria for the diagnosis of one of the other MDS/MPN subtypes (i.e. not enough peripheral monocytes to fulfill the diagnosis CMML or slightly less than 450.000 thrombocytes not fulfilling the criteria for MDS-RS-T, WBC too low for aCML,…). This is, of course, due to the fact, that all thresholds are more or less arbitrary. This fact might be unsatisfactory, but as thresholds are necessary, a solution might be to form subgroups of "CMML-like MDS/MPN-U" and "MDS-RS-T like MDS/MPN-U" and so on or to allow the diagnosis of pre-CMML syndromes like oligo-monocytic CMML (86, 87). Patients should be checked at regular intervals whether they still fit into the MDS/MPN-U category or might be transferred into a better defined MDS/MPN subtype. With a median survival of 1-2 years, survival of patients with MDS/MPN-U is generally poor. However, some subgroups like the "MDS-RS-T like MDS/MPN-U" might do better than others.

Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (MDS-RS-T) is the latest "member" in the group of MDS/MPN. It was proposed as a provisional entity in the WHO 2001 classification of myeloid neoplasms. The latest WHO-classification has now recognized MDS/MPN-RS-T as a formal subgroup of the MDS/MPN overlap syndromes. MDS/MPN-RS-T shares clinical features with MDS-RS-SLD and essential thrombocythemia. It is characterized by the co-occurrence of ringed sideroblasts in the bone marrow (≥15%), together with an increased platelet count (≥450 000/μl) and large, atypical megakaryocytes. As for most of the other MDS/MPNs epidemiological data is scarce, due to the rarity of the disease. Its frequency can be estimated to be below or about 1% of all MDS (49, 50, 69). In our current MDS registry it accounts for 1,45% of all MDS (35). The median age at presentation ranges from 71-75 years (13, 39–42). In contrast to other MDS/MPN the male predominance seems less pronounced but varies between studies (13, 39–42). Hepato-splenomegaly and extramedullary involvement appear to be less frequent compared to other MDS/MPD, same as the "MPD-typical" constitutional symptoms, although data on these clinical features is still very limited. In addition, the prognosis is generally better than that of other MDS/MPD as it resembles the two relatively "benign" diseases MDS-RS-SLD on the one hand and ET on the other hand, leading to a relatively low risk of leukemic transformation, but also to an increased risk of thromboembolic events and an often symptomatic anemia as the typical presentation of this unique MDS/MPD subgroup. Regarding cytogenetics, about 80% of patients exhibit a normal karyotype. Gene mutations, conversely, are frequent and observed in >90% of patients (13, 14). The most frequent are *SF3B1* as well as *JAK-2* mutations. Patients with RARS-T have a shorter overall (76 *vs*. 117 months) and leukemia-free survival than patients with essential thrombocythemia along with a comparable risk of thromboembolic complications (3.6 *vs*. 3.9/100 patient years). On the other hand, they exhibit a longer survival (76 *vs*. 63 months), but a higher risk of thrombosis when compared to patients with MDS-RS (3.6 *vs*. 0.9/100 patient years) (39).

Two groups that are not recognized as (separate) entities within the MDS/MPN but show unique features and an overlap of both MDS and MPN are patients with del(5q) and *JAK2 V617F* mutation and patients with isolated isochromosome (17q). These groups are small, but usually show the typical overlapping symptoms of both myelodysplastic and myeloproliferative disease. MDS with del(5q) and *JAK2* are currently subsumed under MDS with isolated del(5q). This makes sense on the one hand since patients appear to have a comparable prognosis when compared to patients with isolated del(5q) without the *JAK2* mutation and treatment with lenalidomide appears to be active in both subgroups. However, lenalidomide shows activity in MPNs like myelofibrosis as well. On the other hand, the most recent and most extensive publication on this small subgroup of MDS patients by Sangiorgio et al. (21) shows that median cell counts regarding platelets, but also WBC, and even red blood cell counts, are higher when compared to MDS with del(5q) and *JAK2*-wildtype. 3 patients did not even meet the criteria for MDS and del(5q) because they lacked sufficient cytopenias. In addition, all 3 patients with data available showed splenomegaly, 4 of 5 patients with available bone marrow histology were hypercellular and all these patients had grade 1 or 2 fibrosis.

Dysplasia in the erythroid or granulocytic lineage was lacking. Still, megakaryocytes were not typical for MPN, but clearly exhibited dysplastic, del(5q) like features, with hypo- and monolobulated nuclei, while large hypernucleated forms existed as well. Thus, one could argue that such patients, according to their clinical presentation, might better be recognized as MDS/MPN overlap syndromes than MDS. In this study 12.7% of all MDS with isolated del(5q) were found to have a *JAK2 V617F* mutation. Others found a slightly lower frequency (22). The *JAK2 V617F* mutation identifies a subgroup of MDS patients with isolated deletion 5q and a proliferative bone marrow.

Isolated isochromosome (17q) can be a finding within complex karyotypes occurring in different myeloid malignancies, but also, rarely, exists as sole chromosomal abnormality. In this case, it often presents as MDS/MPN overlap syndrome. The median age is around 60 years, with the typical male predominance (23–30). Patients often present with anemia, leukocytosis, and splenomegaly. The bone marrow is hypercellular, often exhibiting some grade of fibrosis, and dysgranulopoiesis, including hypo- and non-segmented forms, ring nuclei, hypogranularity, and chromatin clumping is typically prominent. The blast count is usually low. Monocytosis occurs frequently, thus many cases are currently subsumed under CMML. A few might present like aCML, but most other cases can only be placed in MDS/MPN-U. As patients with this unique cytogenetic feature share many clinical features and seem to have a relatively uniform poor prognosis and high risk of leukemic evolution it is a matter of discussion, whether it might make sense to form a new, cytogenetically well-defined subgroup of MDS/MPN. However, in the Duesseldorf MDS registry currently only one patient with isolated isochromosome (17q) can be detected. This patient was diagnosed as CMML.

CONCLUSION

The WHO-category MDS/MPN encompasses a unique group of clonal myeloid neoplasms exhibiting hybrid features of myelodysplastic as well as myeloproliferative malignancies. As most entities are quite rare, epidemiological data is sparse. In adults the most frequent MDS/MPN by far is CMML, followed by MDS/MPN-RS-T. aCML and MDS/MPN-U are extremely rare diseases and not very well defined. An appropriate diagnosis and classification are difficult, but essential for further prognostication and treatment decisions. Although diagnosis of most subtypes is still largely based on morphologic criteria, diagnosing MDS/MPD properly should require a comprehensive clinical and laboratory assessment with thorough integration of morphological, immunophenotypic, genetic, as well as clinical examination. While single gene mutations might occur in different MDS/MPN or other myeloid diseases certain gene combinations may be more specific for certain subtypes and might aid in determining the correct diagnosis (69). Despite an enormous gain of knowledge regarding molecular genetics and in some subgroups pathophysiology as well we are still far from satisfactory treatment options in this rare and heterogeneous group of myeloid overlap syndromes.

AUTHOR CONTRIBUTIONS

AKu, UG, and AKa contributed to conception and design of the paper. AKu wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

REFERENCES

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. *Blood* (2016) 127:2391–405. doi: 10.1182/blood-2016-03-643544
2. Elena C, Gallì A, Such E, Meggendorfer M, Germing U, Rizzo E, et al. Integrating Clinical Features and Genetic Lesions in the Risk Assessment of Patients With Chronic Myelomonocytic Leukemia. *Blood* (2016) 128:1408–17. doi: 10.1182/blood-2016-05-714030
3. Itzykson R, Fenaux P, Bowen D, Cross NCP, Cortes J, De Witte T, et al. Diagnosis and Treatment of Chronic Myelomonocytic Leukemias in Adults: Recommendations From the European Hematology Association and the European Leukemianet. *Hemasphere* (2018) 2:e150. doi: 10.1097/HS9.0000000000000015
4. Kohlmann A, Grossmann V, Klein HU, Schindela S, Weiss T, Kazak B, et al. Next-Generation Sequencing Technology Reveals a Characteristic Pattern of Molecular Mutations in 72.8% of Chronic Myelomonocytic Leukemia by Detecting Frequent Alterations in TET2, CBL, RAS, and RUNX1. *J Clin Oncol* (2010) 28:3858–65. doi: 10.1200/JCO.2009.27.1361
5. Kohlmann A, Grossmann V, Haferlach T. Integration of Next-Generation Sequencing Into Clinical Practice: Are We There Yet? *Semin Oncol* (2012) 39:26–36. doi: 10.1053/j.seminoncol.2011.11.008
6. Patnaik MM, Lasho TL. Genomics of Myelodysplastic Syndrome/Myeloproliferative Neoplasm Overlap Syndromes. *Hematol Am Soc Hematol Educ Program* (2020) 2020:450–9. doi: 10.1182/hematology.2020000130
7. Jankowska AM, Makishima H, Tiu RV, Szpurka H, Huang Y, Traina F, et al. Mutational Spectrum Analysis of Chronic Myelomonocytic Leukemia Includes Genes Associated With Epigenetic Regulation: UTX, EZH2, and DNMT3A. *Blood* (2011) 118:3932–41. doi: 10.1182/blood-2010-10-311019
8. Cervera N, Itzykson R, Coppin E, Prebet T, Murati A, Legall S, et al. Gene Mutations Differently Impact the Prognosis of the Myelodysplastic and Myeloproliferative Classes of Chronic Myelomonocytic Leukemia. *Am J Hematol* (2014) 89:604–9. doi: 10.1002/ajh.23702
9. Smith AE, Mohamedali AM, Kulasekararaj A, Lim Z, Gäken J, Lea NC, et al. Next-Generation Sequencing of the TET2 Gene in 355 MDS and CMML Patients Reveals Low-Abundance Mutant Clones With Early Origins, But Indicates No Definite Prognostic Value. *Blood* (2010) 116:3923–32. doi: 10.1182/blood-2010-03-274704
10. Federmann B, Abele M, Rosero Cuesta DS, Vogel W, Boiocchi L, Kanz L, et al. The Detection of SRSF2 Mutations in Routinely Processed Bone Marrow Biopsies is Useful in the Diagnosis of Chronic Myelomonocytic Leukemia. *Hum Pathol* (2014) 45:2471–9. doi: 10.1016/j.humpath.2014.08.014
11. Malcovati L, Papaemmanuil E, Ambaglio I, Elena C, Galli A, Della Porta MG, et al. Driver Somatic Mutations Identify Distinct Disease Entities Within Myeloid Neoplasms With Myelodysplasia. *Blood* (2014) 124:1513–21. doi: 10.1182/blood-2014-03-560227
12. Niemeyer CM. JMML Genomics and Decisions. *Hematol Am Soc Hematol Educ Program* (2018) 2018:307–12. doi: 10.1182/asheducation-2018.1.307

13. Patnaik MM, Lasho TL, Finke CM, Hanson CA, King RL, Ketterling RP, et al. Predictors of Survival in Refractory Anemia With Ring Sideroblasts and Thrombocytosis (RARS-T) and the Role of Next-Generation Sequencing. *Am J Hematol* (2016) 91:492–8. doi: 10.1002/ajh.24332
14. Jeromin S, Haferlach T, Weissmann S, Meggendorfer M, Eder C, Nadarajah N, et al. Refractory Anemia With Ring Sideroblasts and Marked Thrombocytosis Cases Harbor Mutations in SF3B1 or Other Spliceosome Genes Accompanied by JAK2V617F and ASXL1 Mutations. *Haematologica* (2015) 100:e125–7. doi: 10.3324/haematol.2014.119032
15. Fontana D, Ramazzotti D, Aroldi A, Redaelli S, Magistroni V, Pirola A, et al. Integrated Genomic, Functional, and Prognostic Characterization of Atypical Chronic Myeloid Leukemia. *Hemasphere* (2020) 4:e497. doi: 10.1097/HS9.0000000000000497
16. Bose P, Nazha A, Komrokji RS, Patel KP, Pierce SA, Al-Ali N, et al. Mutational Landscape of Myelodysplastic/Myeloproliferative Neoplasm-Unclassifiable. *Blood* (2018) 132:2100–3. doi: 10.1182/blood-2018-05-848473
17. Mangaonkar AA, Swoboda DM, Coltro G, Lasho TL, Novotny PJ, Pophali P, et al. Clinicopathologic Characteristics, Prognostication and Treatment Outcomes for Myelodysplastic/Myeloproliferative Neoplasm, Unclassifiable (MDS/MPN-U): Mayo Clinic-Moffitt Cancer Center Study of 135 Consecutive Patients. *Leukemia* (2020) 34:656–61. doi: 10.1038/s41375-019-0574-x
18. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the Classification of the Acute Leukaemias. French-American-British (FAB) Co-Operative Group. *Br J Haematol* (1976) 33:451–8. doi: 10.1111/j.1365-2141.1976.tb03563.x
19. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the Classification of the Myelodysplastic Syndromes. *Br J Haematol* (1982) 51:189–99. doi: 10.1111/j.1365-2141.1982.tb08475.x
20. ES Jaffe, NL Harris, H Stein and JW Vardiman eds. World Health Organization Classification of Tumours. In: *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press.
21. Sangiorgio VFI, Geyer JT, Margolskee E, Al-Kawaaz M, Mathew S, Tam W, et al. Myeloid Neoplasms With Isolated Del(5q) and JAK2 V617F Mutation: A "Grey Zone" Combination of Myelodysplastic and Myeloproliferative Features? *Haematologica* (2020) 105(6):e276–9. doi: 10.3324/haematol.2019.227686
22. Ingram W, Lea NC, Cervera J, Germing U, Fenaux P, Cassinat B, et al. The JAK2 V617F Mutation Identifies a Subgroup of MDS Patients With Isolated Deletion 5q and a Proliferative Bone Marrow. *Leukemia* (2006) 20:1319–21. doi: 10.1038/sj.leu.2404215
23. McClure RF, Dewald GW, Hoyer JD, Hanson HD. Isolated Isochromosome 17q: A Distinct Type of Mixed Myeloproliferative Disorder/Myelodysplastic Syndrome With an Aggressive Clinical Course. *Br J Haematol* (1999) 106:445–54. doi: 10.1046/j.1365-2141.1999.01537.x
24. Kanagal-Shamanna R, Luthra R, Yin CC, Patel KP, Takahashi K, Lu X, et al. Myeloid Neoplasms With Isolated Isochromosome 17q Demonstrate a High Frequency of Mutations in SETBP1, SRSF2, ASXL1 and NRAS. *Oncotarget* (2016) 7:14251–8. doi: 10.18632/oncotarget.7350
25. Kanagal-Shamanna R, Bueso-Ramos CE, Barkoh B, Lu G, Wang S, Garcia-Manero G, et al. Myeloid Neoplasms With Isolated Isochromosome 17q Represent a Clinicopathologic Entity Associated With Myelodysplastic/Myeloproliferative Features, a High Risk of Leukemic Transformation, and Wild-Type TP53. *Cancer* (2012) 118(11):2879–88. doi: 10.1002/cncr.26537
26. Koczkodaj D, Muzyka-Kasietczuk J, Chocholska S, Podhorecka M. Prognostic Significance of Isochromosome 17q in Hematologic Malignancies. *Oncotarget* (2021) 12:708–18. doi: 10.18632/oncotarget.27914
27. Ganguly S, Uvodich M, Dias A, McGuirk JP. Clinical Characteristics and Treatment Outcome of Patients With Isochromosome 17q (I17q) Abnormality and Myeloid Neoplasms: A Single Center Experience. *Leuk Res Rep* (2018) 10:55–6. doi: 10.1016/j.lrr.2018.06.002
28. Fioretos T, Strömbeck B, Sandberg T, Johansson B, Billström R, Borg A, et al. Isochromosome 17q in Blast Crisis of Chronic Myeloid Leukemia and in Other Hematologic Malignancies is the Result of Clustered Breakpoints in 17p11 and is Not Associated With Coding TP53 Mutations. *Blood* (1999) 94:225–32. doi: 10.1182/blood.V94.1.225.413k24_225_232
29. Weh HJ, Kuse R, Hossfeld DK. Acute Nonlymphocytic Leukemia (ANLL) With Isochromosome I(17q) as the Sole Chromosomal Anomaly: A Distinct Entity? *Eur J Haematol* (1990) 44:312–4. doi: 10.1111/j.1600-0609.1990.tb00401.x
30. Solé F, Torrabadella M, Granada I, Florensa L, Vallespi T, Ribera JM, et al. Isochromosome 17q as a Sole Anomaly: A Distinct Myelodysplastic Syndrome Entity? *Leuk Res* (1993) 17:717–20. doi: 10.1016/0145-2126(93)90080-5
31. Dinmohamed AG, Brink M, Visser O, Sonneveld P, van de Loosdrecht AA, Jongen-Lavrencic M, et al. Trends in Incidence, Primary Treatment and Survival in Chronic Myelomonocytic Leukaemia: A Population-Based Study of 1359 Patients Diagnosed in the Netherlands From 1989 to 2012. *Br J Haematol* (2015) 171:436–9. doi: 10.1111/bjh.13414
32. Srour SA, Devesa SS, Morton LM, Check DP, Curtis RE, Linet MS, et al. Incidence and Patient Survival of Myeloproliferative Neoplasms and Myelodysplastic/Myeloproliferative Neoplasms in the United States, 2001-12. *Br J Haematol* (2016) 174:382–96. doi: 10.1111/bjh.14061
33. Benzarti S, Daskalakis M, Feller A, Bacher VU, Schnegg-Kaufmann A, Rüfer A, et al. Trends of Incidence and Survival of Patients With Chronic Myelomonocytic Leukemia Between 1999 and 2014: A Comparison Between Swiss and American Population-Based Cancer Registries. *Cancer Epidemiol* (2019) 59:51–7. doi: 10.1016/j.canep.2019.01.003
34. Rollison DE, Howlader N, Smith MT, Strom SS, Merritt WD, Ries LA, et al. Epidemiology of Myelodysplastic Syndromes and Chronic Myeloproliferative Disorders in the United States, 2001-2004, Using Data From the NAACCR and SEER Programs. *Blood* (2008) 112:45–52. doi: 10.1182/blood-2008-01-134858
35. Germing U. *Data From the Duesseldorf MDS Registry as at 2021*. Duesseldorf: Duesseldorf MDS Registry (2021).
36. Avgerinou C, Alamanos Y, Zikos P, Lampropoulou P, Melachrinou M, Labropoulou V, et al. The Incidence of Myelodysplastic Syndromes in Western Greece is Increasing. *Ann Hematol* (2013) 92:877–87. doi: 10.1007/s00277-013-1712-6
37. Niemeyer CM, Arico M, Basso G, Biondi A, Cantu Rajnoldi A, Creutzig U, et al. Chronic Myelomonocytic Leukemia in Childhood: A Retrospective Analysis of 110 Cases. European Working Group on Myelodysplastic Syndromes in Childhood (EWOG-MDS). *Blood* (1997) 89:3534–43. doi: 10.1182/blood.V89.10.3534
38. Passmore SJ, Chessells JM, Kempski H, Hann IM, Brownbill PA, Stiller CA. Paediatric Myelodysplastic Syndromes and Juvenile Myelomonocytic Leukaemia in the UK: A Population-Based Study of Incidence and Survival. *Br J Haematol* (2003) 121:758–67. doi: 10.1046/j.1365-2141.2003.04361.x
39. Broseus J, Florensa L, Zipperer E, Schnittger S, Malcovati L, Richebourg S, et al. Clinical Features and Course of Refractory Anemia With Ring Sideroblasts Associated With Marked Thrombocytosis. *Haematologica* (2012) 97:1036–41. doi: 10.3324/haematol.2011.053918
40. Broséus J, Alpermann T, Wulfert M, Florensa Brichs L, Jeromin S, Lippert E, et al. Age, JAK2(V617F) and SF3B1 Mutations are the Main Predicting Factors for Survival in Refractory Anaemia With Ring Sideroblasts and Marked Thrombocytosis. *Leukemia* (2013) 27:1826–31. doi: 10.1038/leu.2013.120
41. Atallah E, Nussenzveig R, Yin CC, Bueso-Ramos C, Tam C, Manshouri T, et al. Prognostic Interaction Between Thrombocytosis and JAK2 V617F Mutation in the WHO Subcategories of Myelodysplastic/Myeloproliferative Disease-Unclassifiable and Refractory Anemia With Ringed Sideroblasts and Marked Thrombocytosis. *Leukemia* (2008) 22:1295–8. doi: 10.1038/sj.leu.2405054
42. Wang SA, Hasserjian RP, Loew JM, Sechman EV, Jones D, Hao S, et al. Refractory Anemia With Ringed Sideroblasts Associated With Marked Thrombocytosis Harbors JAK2 Mutation and Shows Overlapping Myeloproliferative and Myelodysplastic Features. *Leukemia* (2006) 20:1641–4. doi: 10.1038/sj.leu.2404316
43. Wang SA, Hasserjian RP, Fox PS, Rogers HJ, Geyer JT, Chabot-Richards D. Atypical Chronic Myeloid Leukemia is Clinically Distinct From Unclassifiable Myelodysplastic/Myeloproliferative Neoplasms. *Blood* (2014) 123:2645–51. doi: 10.1182/blood-2014-02-553800
44. Breccia M, Biondo F, Latagliata R, Carmosino I, Mandelli F, Alimena G. Identification of Risk Factors in Atypical Chronic Myeloid Leukemia. *Haematologica* (2006) 91:1566–8. doi: 10.3324/%25x
45. Hernández JM, del Cañizo MC, Cuneo A, García JL, Gutiérrez NC, González M, et al. Clinical, Hematological and Cytogenetic Characteristics of Atypical

Chronic Myeloid Leukemia. *Ann Oncol* (2000) 11:441–4. doi: 10.1023/a:1008393002748
46. Onida F, Ball G, Kantarjian HM, Smith TL, Glassman A, Albitar M, et al. Characteristics and Outcome of Patients With Philadelphia Chromosome Negative, Bcr/Abl Negative Chronic Myelogenous Leukemia. *Cancer* (2002) 95:1673–84. doi: 10.1002/cncr.10832
47. Kong JH, Nam H, Go TH, Hyun SY, Shim KY. Characteristics and Survival of Patients With Atypical Chronic Myeloid Leukemia. *Blood Res* (2019) 54:233–6. doi: 10.5045/br.2019.54.3.233
48. DiNardo CD, Daver N, Jain N, Pemmaraju N, Bueso-Ramos C, Yin CC, et al. Myelodysplastic/Myeloproliferative Neoplasms, Unclassifiable (MDS/MPN, U): Natural History and Clinical Outcome by Treatment Strategy. *Leukemia* (2014) 28:958–61. doi: 10.1038/leu.2014.8
49. Cannella L, Breccia M, Latagliata R, Frustaci A, Alimena G. Clinical and Prognostic Features of Patients With Myelodysplastic/Myeloproliferative Syndrome Categorized as Unclassified (MDS/MPD-U) by WHO Classification. *Leuk Res* (2008) 32:514–6. doi: 10.1016/j.leukres.2007.07.004
50. Orazi A, Germing U. The Myelodysplastic/Myeloproliferative Neoplasms: Myeloproliferative Diseases With Dysplastic Features. *Leukemia* (2008) 22:1308–19. doi: 10.1038/leu.2008.119
51. Clara JA, Sallman DA, Padron E. Clinical Management of Myelodysplastic Syndrome/Myeloproliferative Neoplasm Overlap Syndromes. *Cancer Biol Med* (2016) 13:360–72. doi: 10.20892/j.issn.2095-3941.2016.0043
52. Hall J, Foucar K. Diagnosing Myelodysplastic/Myeloproliferative Neoplasms: Laboratory Testing Strategies to Exclude Other Disorders. *Int J Lab Hematol* (2010) 32:559–71. doi: 10.1111/j.1751-553X.2010.01251.x
53. Cazzola M, Malcovati L, Invernizzi R. Myelodysplastic/Myeloproliferative Neoplasms. *Hematol Am Soc Hematol Educ Program* (2011) 2011:264–72. doi: 10.1182/asheducation-2011.1.264
54. Mughal TI, Cross NC, Padron E, Tiu RV, Savona M, Malcovati L, et al. An International Mds/Mpn Working Group's Perspective and Recommendations on Molecular Pathogenesis, Diagnosis and Clinical Characterization of Myelodysplastic/Myeloproliferative Neoplasms. *Haematologica* (2015) 100:1117–30. doi: 10.3324/haematol.2014.114660
55. Germing U, Strupp C, Knipp S, Kuendgen A, Giagounidis A, Hildebrandt B, et al. Chronic Myelomonocytic Leukemia in the Light of the WHO Proposals. *Haematologica* (2007) 92:974–7. doi: 10.3324/haematol.11051
56. Niemeyer CM, Flotho C. Juvenile Myelomonocytic Leukemia: Who's the Driver at the Wheel? *Blood* (2019) 133:1060–70. doi: 10.1182/blood-2018-11-844688
57. Xubo G, Xingguo L, Xianguo W, Rongzhen X, Xibin X, Lin W, et al. The Role of Peripheral Blood, Bone Marrow Aspirate and Especially Bone Marrow Trephine Biopsy in Distinguishing Atypical Chronic Myeloid Leukemia From Chronic Granulocytic Leukemia and Chronic Myelomonocytic Leukemia. *Eur J Haematol* (2009) 83:292–301. doi: 10.1111/j.1600-0609.2009.01283.x
58. Patnaik MM, Tefferi A. Chronic Myelomonocytic Leukemia: 2020 Update on Diagnosis, Risk Stratification and Management. *Am J Hematol* (2020) 95:97–115. doi: 10.1002/ajh.25684
59. Such E, Germing U, Malcovati L, Cervera J, Kuendgen A, Della Porta MG, et al. Development and Validation of a Prognostic Scoring System for Patients With Chronic Myelomonocytic Leukemia. *Blood* (2013) 121:3005–15. doi: 10.1182/blood-2012-08-452938
60. Patnaik MM, Padron E, LaBorde RR, Lasho TL, Finke CM, Hanson CA, et al. Mayo Prognostic Model for WHO-Defined Chronic Myelomonocytic Leukemia: ASXL1 and Spliceosome Component Mutations and Outcomes. *Leukemia* (2013) 27:1504–10. doi: 10.1038/leu.2013.88
61. Itzykson R, Kosmider O, Renneville A, Gelsi-Boyer V, Meggendorfer M, Morabito M, et al. Prognostic Score Including Gene Mutations in Chronic Myelomonocytic Leukemia. *J Clin Oncol* (2013) 31(19):2428–36. doi: 10.1200/JCO.2012.47.3314
62. Locatelli F, Nöllke P, Zecca M, Korthof E, Lanino E, Peters C, et al. Hematopoietic Stem Cell Transplantation (HSCT) in Children With Juvenile Myelomonocytic Leukemia (JMML): Results of the EWOG-MDS/EBMT Trial. *Blood* (2005) 105:410–9. doi: 10.1182/blood-2004-05-1944
63. Neukirchen J, Schoonen WM, Strupp C, Gattermann N, Aul C, Haas R, et al. Incidence and Prevalence of Myelodysplastic Syndromes: Data From the Dusseldorf MDS-Registry. *Leuk Res* (2011) 35:1591–6. doi: 10.1016/j.leukres.2011.06.001
64. Ma X, Does M, Raza A, Mayne ST. Myelodysplastic Syndromes: Incidence and Survival in the United States. *Cancer* (2007) 109:1536–42. doi: 10.1002/cncr.22570
65. Maynadié M, Verret C, Moskovtchenko P, Mugneret F, Petrella T, Caillot D, et al. Epidemiological Characteristics of Myelodysplastic Syndrome in a Well-Defined French Population. *Br J Cancer* (1996) 74:288–90. doi: 10.1038/bjc.1996.354
66. Merlevede J, Droin N, Qin T, Meldi K, Yoshida K, Morabito M, et al. Mutation Allele Burden Remains Unchanged in Chronic Myelomonocytic Leukaemia Responding to Hypomethylating Agents. *Nat Commun* (2016) 7:10767. doi: 10.1038/ncomms10767
67. Barrington-Trimis JL, Cockburn M, Metayer C, Gauderman WJ, Wiemels J, McKean-Cowdin R. Trends in Childhood Leukemia Incidence Over Two Decades From 1992 to 2013. *Int J Cancer* (2017) 140:1000–8. doi: 10.1002/ijc.30487
68. Xavier AC, Kutny M, Costa LJ. Incidence and Outcomes of Paediatric Myelodysplastic Syndrome in the United States. *Br J Haematol* (2018) 180:898–901. doi: 10.1111/bjh.14460
69. Gattermann N, Billiet J, Kronenwett R, Zipperer E, Germing U, Nollet F, et al. High Frequency of the JAK2 V617F Mutation in Patients With Thrombocytosis (Platelet Count>600x109/L) and Ringed Sideroblasts More Than 15% Considered as MDS/MPD, Unclassifiable. *Blood* (2007) 109:1334–5. doi: 10.1182/blood-2006-05-022491
70. Oscier D. Atypical Chronic Myeloid Leukemias. *Pathol Biol (Paris)* (1997) 45:587–93.
71. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) Classification of the Myeloid Neoplasms. *Blood* (2002) 100:2292–302. doi: 10.1182/blood-2002-04-1199
72. Hehlmann R, Hochhaus A, Baccarani M. European Leukemianet. Chronic Myeloid Leukaemia. *Lancet* (2007) 370:342–50. doi: 10.1016/S0140-6736(07)61165-9
73. Rohrbacher M, Hasford J. Epidemiology of Chronic Myeloid Leukaemia (CML). *Best Pract Res Clin Haematol* (2009) 22:295–302. doi: 10.1016/j.beha.2009.07.007
74. Petrova-Drus K, Chiu A, Margolskee E, Barouk-Fox S, Geyer J, Dogan A, et al. Bone Marrow Fibrosis in Chronic Myelomonocytic Leukemia is Associated With Increased Megakaryopoiesis, Splenomegaly and With a Shorter Median Time to Disease Progression. *Oncotarget* (2017) 8:103274–82. doi: 10.18632/oncotarget.21870
75. Gur HD, Loghavi S, Garcia-Manero G, Routbort M, Kanagal-Shamanna R, Quesada A, et al. Chronic Myelomonocytic Leukemia With Fibrosis is a Distinct Disease Subset With Myeloproliferative Features and Frequent JAK2 P.V617F Mutations. *Am J Surg Pathol* (2018) 42:799–806. doi: 10.1097/PAS.0000000000001058
76. Khan M, Muzzafar T, Kantarjian H, Badar I, Short N, Wang X, et al. Association of Bone Marrow Fibrosis With Inferior Survival Outcomes in Chronic Myelomonocytic Leukemia. *Ann Hematol* (2018) 97:1183–91. doi: 10.1007/s00277-018-3289-6
77. Vitte F, Fabiani B, Bénet C, Dalac S, Balme B, Delattre C, et al. Specific Skin Lesions in Chronic Myelomonocytic Leukemia: A Spectrum of Myelomonocytic and Dendritic Cell Proliferations: A Study of 42 Cases. *Am J Surg Pathol* (2012) 36:1302–16. doi: 10.1097/PAS.0b013e31825dd4de
78. Lucas N, Duchmann M, Rameau P, Noël F, Michea P, Saada V, et al. Biology and Prognostic Impact of Clonal Plasmacytoid Dendritic Cells in Chronic Myelomonocytic Leukemia. *Leukemia* (2019) 33:2466–80. doi: 10.1038/s41375-019-0447-3
79. Lebecque B, Vial JP, Pigneux A, Lechevalier N. Chronic Myelomonocytic Leukaemia Followed by Blastic Plasmacytoid Dendritic Cell Neoplasm. *Br J Haematol* (2019) 185:398. doi: 10.1111/bjh.15794
80. Zahid MF, Barraco D, Lasho TL, Finke C, Ketterling RP, Gangat N, et al. Spectrum of Autoimmune Diseases and Systemic Inflammatory Syndromes in Patients With Chronic Myelomonocytic Leukemia. *Leuk Lymphoma* (2017) 58:1488–93. doi: 10.1080/10428194.2016.1243681
81. Peker D, Padron E, Bennett JM, Zhang X, Horna P, Epling-Burnette PK, et al. A Close Association of Autoimmune-Mediated Processes and Autoimmune Disorders With Chronic Myelomonocytic Leukemia: Observation From a Single Institution. *L.Acta Haematol* (2015) 133:249–56. doi: 10.1159/000365877

82. Elbæk MV, Sørensen AL, Hasselbalch HC. Chronic Inflammation and Autoimmunity as Risk Factors for the Development of Chronic Myelomonocytic Leukemia? *Leuk Lymphoma* (2016) 57:1793–9. doi: 10.3109/10428194.2016.1143938
83. Elbæk MV, Sørensen AL, Hasselbalch HC. Cardiovascular Disease in Chronic Myelomonocytic Leukemia: Do Monocytosis and Chronic Inflammation Predispose to Accelerated Atherosclerosis? *Ann Hematol* (2019) 98:101–9. doi: 10.1007/s00277-018-3489-0
84. Palomo L, Meggendorfer M, Hutter S, Twardziok S, Ademà V, Fuhrmann I, et al. Molecular Landscape and Clonal Architecture of Adult Myelodysplastic/Myeloproliferative Neoplasms. *Blood* (2020) 136:1851–62. doi: 10.1182/blood.2019004229
85. Patnaik MM, Vallapureddy R, Yalniz FF, Hanson CA, Ketterling RP, Lasho TL, et al. Therapy Related-Chronic Myelomonocytic Leukemia (CMML): Molecular, Cytogenetic, and Clinical Distinctions From *De Novo* CMML. *Am J Hematol* (2018) 93:65–73. doi: 10.1002/ajh.24939
86. Schuler E, Frank F, Hildebrandt B, Betz B, Strupp C, Rudelius M, et al. Myelodysplastic Syndromes Without Peripheral Monocytosis But With Evidence of Marrow Monocytosis Share Clinical and Molecular Characteristics With CMML. *Leuk Res* (2018) 65:1–4. doi: 10.1016/j.leukres.2017.12.002
87. Valent P. Oligo-Monocytic CMML and Other Pre-CMML States: Clinical Impact, Prognostication and Management. *Best Pract Res Clin Haematol* (2020) 33:101137. doi: 10.1016/j.beha.2019.101137
88. Heibl S, Gisslinger B, Jäger E, Barna A, Gurbisz M, Stegemann M, et al. Clinical, Hematologic, Biologic and Molecular Characteristics of Patients With Myeloproliferative Neoplasms and a Chronic Myelomonocytic Leukemia-Like Phenotype. *Cancers (Basel)* (2020) 12:1891. doi: 10.3390/cancers12071891
89. Brizard A, Huret JL, Lamotte F, Guilhot F, Benz-Lemoine E, Giraud C, et al. Three Cases of Myelodysplastic-Myeloproliferative Disorder With Abnormal Chromatin Clumping in Granulocytes. *Br J Haematol* (1989) 72:294–5. doi: 10.1111/j.1365-2141.1989.tb07703.x
90. Felman P, Bryon PA, Gentilhomme O, Ffrench M, Charrin C, Espinouse D, et al. The Syndrome of Abnormal Chromatin Clumping in Leucocytes: A Myelodysplastic Disorder With Proliferative Features? *Br J Haematol* (1988) 70:49–54. doi: 10.1111/j.1365-2141.1988.tb02433.x
91. Gustke SS, Becker GA, Garancis JC, Geimer NF, Pisciotta AV. Chromatin Clumping in Mature Leukocytes: A Hitherto Unrecognized Abnormality. Abnormal Chromatin Clumping in Leucocytes: A Clue to a New Subtype of Myelodysplastic Syndrome. *Blood* (1970) 35:637–58. doi: 10.1182/blood.V35.5.637.637
92. Jaén A, Irriguible D, Milla F, Vallespi T, Torrabadella M, Abella E, et al. Abnormal Chromatin Clumping in Leucocytes: a Clue to a New Subtype of Myelodysplastic Syndrome. *Eur J Haematol* (1990) 45:209–14. doi: 10.1111/j.1600-0609.1990.tb00459.x
93. Germing U, Kündgen A, Gattermann N. Risk Assessment in Chronic Myelomonocytic Leukemia (CMML). *Leuk Lymphoma* (2004) 45(7):1311–8. doi: 10.1080/1042819042000207271
94. Schuler E, Schroeder M, Neukirchen J, Strupp C, Xicoy B, Kündgen A, et al. Refined Medullary Blast and White Blood Cell Count Based Classification of Chronic Myelomonocytic Leukemias. *Leuk Res* (2014) 38:1413–9. doi: 10.1016/j.leukres.2014.09.003
95. Meggendorfer M, Roller A, Haferlach T, Eder C, Dicker F, Grossmann V, et al. SRSF2 Mutations in 275 Cases With Chronic Myelomonocytic Leukemia (CMML). *Blood* (2012) 120:3080–8. doi: 10.1182/blood-2012-01-404863
96. Hasle H, Aricò M, Basso G, Biondi A, Cantù Rajnoldi A, Creutzig U, et al. Myelodysplastic Syndrome, Juvenile Myelomonocytic Leukemia, and Acute Myeloid Leukemia Associated With Complete or Partial Monosomy 7. European Working Group on MDS in Childhood (EWOG-MDS). *Leukemia* (1999) 13:376–85. doi: 10.1038/sj.leu.2401342
97. Piazza R, Valletta S, Winkelmann N, Redaelli S, Spinelli R, Pirola A, et al. Recurrent SETBP1 Mutations in Atypical Chronic Myeloid Leukemia. *Nat Genet* (2013) 45:18–24. doi: 10.1038/ng.2495
98. Gotlib J, Maxson JE, George TI, Tyner JW. The New Genetics of Chronic Neutrophilic Leukemia and Atypical CML: Implications for Diagnosis and Treatment. *Blood* (2013) 122:1707–11. doi: 10.1182/blood-2013-05-500959

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Oncology

REVIEW
published: 17 November 2021
doi: 10.3389/fonc.2021.722507

Atypical Chronic Myelogenous Leukemia, *BCR-ABL1* Negative: Diagnostic Criteria and Treatment Approaches

Panagiotis T. Diamantopoulos * *and Nora-Athina Viniou*

First Department of Internal Medicine, Laikon General Hospital, National and Kapodistrian University of Athens, Athens, Greece

Atypical chronic myelogenous leukemia (aCML), *BCR/ABL1* negative is a rare myelodysplastic/myeloproliferative neoplasm, usually manifested with hyperleukocytosis without monocytosis or basophilia, organomegaly, and marked dysgranulopoiesis. In this review, we will discuss the classification and diagnostic criteria of aCML, as these have been formulated during the past 30 years, with a focus on the recent advances in the molecular characterization of the disease. Although this entity does not have a definitive molecular profile, its molecular characterization has contributed to a better understanding and more accurate classification and diagnosis of aCML. At the same time, it has facilitated the identification of adverse prognostic factors and the stratification of patients according to their risk for leukemic transformation. What is more, the molecular characterization of the disease has expanded our therapeutic choices, thoroughly presented and analyzed in this review article.

Keywords: atypical chronic myelogenous leukemia, myelodysplastic syndrome/myeloproliferative neoplasm, diagnostic criteria, treatment, molecular characterization

OPEN ACCESS

Edited by:
Argiris Symeonidis, University of Patras, Greece

Reviewed by:
Deepshi Thakral, All India Institute of Medical Sciences, India
Nicholas Cross, University of Southampton, United Kingdom

***Correspondence:**
Panagiotis T. Diamantopoulos
pandiamantopoulos@gmail.com

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

Received: *08 June 2021*
Accepted: *02 November 2021*
Published: *17 November 2021*

Citation:
Diamantopoulos PT and Viniou N-A (2021) Atypical Chronic Myelogenous Leukemia, BCR-ABL1 Negative: Diagnostic Criteria and Treatment Approaches. Front. Oncol. 11:722507. doi: 10.3389/fonc.2021.722507

INTRODUCTION

Atypical chronic myelogenous leukemia (aCML), *BCR/ABL1* negative is a rare disorder classified into the category of myelodysplastic/myeloproliferative neoplasms (MDS/MPN), according to the 2016 revision of the World Health organization (WHO) classification of myeloid neoplasms and acute leukemia (1). It is, by definition, a *BCR-ABL1*-negative clonal disorder sharing myelodysplastic and myeloproliferative features. Due to its rarity, the diagnostic criteria for aCML have been changing since its first description while there are no established standards of care for patients with this condition; hence we will focus on the diagnostic criteria of the disease, new molecular characteristics that have emerged during the last few years, some of which have already been incorporated in the latest diagnostic criteria of the WHO, the proposed risk stratification systems, and the available treatment approaches for aCML.

EPIDEMIOLOGY, CLINICAL AND LABORATORY CHARACTERISTICS

The incidence of aCML is low, a fact leading to limited knowledge about this disease entity. Its true incidence is largely unknown, since it is usually estimated in comparison to that of (*BCR-ABL1*-positive) CML at about one to two cases per 100 cases of CML (2). The cases reported so far concern adults. Patients usually present in the seventh or eighth decade of life and there is an apparent male predominance (1:1 to 4:1 in several case series).

The patients usually present with organomegaly and hyperleukocytosis. Splenomegaly has been reported in 50% to 75% of the patients while in one patient series, hepatomegaly has been reported to be present in 49% of the patients. The median white blood cell (WBC) count at presentation has been reported to fluctuate between $23x10^9$/L to $97x10^9$/L in several case series. The patients are usually moderately to severely anemic [median hemoglobin (Hb) level, 8.6 g/dL to 11.7 g/dL], while about two thirds of them are transfusion dependent. The platelet count may be decreased or normal but can also be found increased in several cases (3). Monocyte and basophile counts are usually within normal range while immature myeloid precursors (promyelocytes, myelocytes, metamyelocytes) are usually present in the peripheral blood smear (10% to 20%), but peripheral blasts are generally absent or low. The neutrophils are highly dysplastic in most cases with typical pseudo-Pelger-Huet changes and hypogranularity of the cytoplasm while dyserythropoiesis and dysmegakaryopoiesis may also be present (3). The bone marrow is hypercellular, with an increased myeloid-to-erythroid ratio (usually up to 10:1) while marrow fibrosis may be present. By definition, blood or bone marrow blasts are less than 20%..

CLASSIFICATION AND DIAGNOSTIC CRITERIA

In the late 80s, the first reports of the disease came to light, with the scientific community wondering whether a Philadelphia-negative CML exists (4). This lead to the identification of "masked" or "hidden" cases of *BCR/ABL1* translocation (5–8), and to the understanding that "CML-like" changes can be found without the diagnostic hallmark of CML. At the same time, cases with features compatible with chronic neutrophilic leukemia (CNL) (9) or chronic myelomonocytic leukemia (10, 11) but atypical presentation were identified, leading to the initial descriptions of the clinical and laboratory characteristics of the disease that eventually led to its classification in the group of chronic myeloid leukemias (12). The French-American-British (FAB) Cooperative Leukaemia Group also classified the disease in 1994 as a subtype of chronic myeloid leukemia with distinct features such as the low basophil and monocyte count, granulocytic dysplasia, presence of immature granulocytes up to 10%-20% of WBC, blasts <2%, and the absence of Philadelphia (Ph) chromosome and the *BCR-ABL1* fusion gene (13). The WHO 2001 classification had minimal differences from the original FAB classification, mainly referring to the marked multilineage dysplasia and the blast percentage in the bone marrow at <20%. In 2008, the new WHO classification added the threshold of $13x10^9$/L to define leukocytosis while it also proposed the limit of 10% of total WBC for monocytes. Moreover, it limited dysplasia to dysgranulopoiesis and for the first time it required the absence of other rearrangements besides that of *BCR-ABL1* (2). Finally, the latest WHO 2016 classification (1) emphasized molecular changes in newly described genes (*ETNK1*, *SETBP1*) and the absence of rearrangement of PDGFRA, PDGFRB, FGFR1, and PCM1-JAK2. Moreover, for the first time, it was added as an exclusion criterion that WHO criteria for other myeloproliferative neoplasms should not be met. Finally, it is the first time that marrow cellularity is commented on. Nevertheless, there is no quantification of bone marrow cellularity and dysplasia in any of the available diagnostic criteria as is usually the case in other disease entities. Although a major feature of aCML, prominent dysgranulopoiesis is not further specified qualitatively and, especially, quantitatively. Granulocytic dysplasia includes pseudo-Pelger-Huët cells, other types of abnormal segmentation of the nucleus or abnormal chromatin clumping, and reduced cytoplasmic granularity. Although, dysgranulopoiesis is the main morphologic feature of the disorder, dyserythropoiesis and, especially, dysmegakaryopoiesis are not rare, although more subtle. These defining morphologic features help distinguish aCML from other entities such as CML and CNL where dysplastic features are minimal or absent (14). A comprehensive list of the diagnostic criteria for aCML as these were shaped during the last 35 years is presented in **Table 1**.

In conclusion, the defining characteristics of aCML are granulocytic proliferation with marked dysgranulopoiesis, along with minimal or absent monocytosis and absence of basophilia, without *BCR/ABL1* translocation or rearrangements of genes that define other hematologic neoplasms. Because of the rarity of aCML, it remains virtually an exclusion diagnosis that should be made when other MDS and MPN can be safely ruled out. In recent years, the molecular profile of the disease is being increasingly outlined through the accumulation of data on the detection of recurrent molecular abnormalities that may contribute to the differential diagnosis of aCML. These molecular changes may be incorporated in the diagnostic criteria for the disease in the future.

The cytogenetic abnormalities in aCML, apart from the lack of the Philadelphia chromosome, are not disease-specific and include trisomy 8, deletion Y, deletion 20q, isochromosome 17 (q), and other cytogenetic changes along with complex karyotype that are usually found in MDS. Nevertheless, about 50% to 80% of the cases have normal karyotypes (15, 16).

Although the presence of several molecular changes can help to rule out the diagnosis of aCML, there is no definitive molecular profile of the disease. During the last few years, recurrent mutations have been increasingly identified in patients with aCML, but the percentage of patients bearing those mutations vary significantly among different studies. Frequently mutated genes (i.e. >20% of

TABLE 1 | Diagnostic criteria of atypical chronic myeloid leukemia, *BCR/ABL1* negative.

Feature	FAB (1994)	WHO 2001	WHO 2008	WHO 2016
Ph chromosome	Absent	Absent	Absent	Absent
BCR/ABL1	Absent	Absent	Absent	Absent
Leukocytosis	Present	Persistent	Persistent (≥13x10^9/L)	Persistent (≥13x10^9/L)
Basophil count	<2%	<2%	<2%	<2%
Monocyte count	≥3% and <10%	<1x10^9/L	<1x10^9/L and <10% of leukocytes	<10% of leukocytes
Multilineage dysplasia	NA	Marked	NA	Dyserythropoiesis and dysmegakaryopoiesis may be present
Dysgranulopoiesis	++	NA	Marked	Present
Immature granulocytes	10-20%	>10%	NA	≥ 10% leukocytes
Blasts	>2%	<20% (bone marrow)	NA	<20% (blood and bone marrow)
Bone marrow cellularity	NA	NA	NA	Hypercellular bone marrow
Other molecular characteristics	NA	NA	No rearrangements of *PDGFRA*, *PDGFRB*, and *FGFR1*	No rearrangements of *PDGFRA*, *PDGFRB*, *FGFR1*, and *PCM1-JAK2* Emphasis on molecular changes (*ETNK1*, *SETBP1*)
Other factors	NA	NA	NA	WHO criteria for other MPNs not met

FAB, French-American-British; WHO, World Health Organization; Ph, Philadelphia; MPN, myeloproliferative neoplasm; NA, not available; MPN, myeloproliferative neoplasm.

cases) are *SETBP1*, *ASXL1*, *NRAS/KRAS*, *SRSF2*, and *TET2* while a variety of genes, including *CSF3R*, *CBL*, *EZH2*, *ETNK1*, *U2AF1* and others (14, 15, 17–22) are found mutated in a lower frequency (<10% of cases). The genes found recurrently mutated in aCML, along with their chromosomal location, normal function, affected exons, mutation types, and clinical implications are listed in **Table 2** (23). Moreover, an algorithm for the diagnosis of aCML incorporating molecular data has been proposed and can be found in **Figure 1**. Since some of the mutated genes found in patients with aCML may be targetable, below we will discuss frequently mutated genes that may affect treatment decision. A list of actionable and non-actionable mutations along with potential targeted therapies can be found in **Table 3**.

SET Binding Protein 1 (SETBP1)

The most frequently identified mutated gene in aCML, in up to one third of cases, is *SETBP1* (15, 20, 21, 30). SETBP1 binds to SET which inhibits the tumor suppressor PP2A. This binding protects SET from cleavage, thus repressing PP2A activity. Mutations of *SETBP1* lead to ubiquitination and, thus, degradation of the protein, increasing SET expression, and, consequently, cellular proliferation through inhibition of PP2A. *SETPB1* mutations have been correlated with more pronounced dysplasia, higher WBC counts, more severe anemia and thrombocytopenia, as well as a worse prognosis (20, 21). Moreover, *SETBP1* mutations have been associated with *ASXL1* and *CBL* mutations while they have been found to be mutually exclusive with *JAK2* and *TET2* mutations (21). *SETBP1* mutations have been incorporated as a supportive criterion for aCML in the WHO 2016 diagnostic criteria.

Ethanolamine Kinase 1 (ETNK1)

ETNK1 is responsible for the phosphorylation of ethanolamine to phosphoethanolamine as part of the Kennedy pathway, which is the main metabolic route for the synthesis of phosphatidylethanolamine and phosphatidylcholine (31). The presence of phosphatidylethanolamine is crucial for cytokinesis and cells lacking phosphatidylethanolamine are unable to complete the mitotic process while reduced intracellular phosphoethanolamine causes hyperactivation of the mitochondria, ROS production, and Histone H2AX phosphorylation, ultimately leading to DNA damage. Recurrent somatic mutations of *ETNK1* have been found in about 13% of patients with aCML (32). A recent study on 43 aCML samples identified *ETNK1* mutations in 16.2% of the analyzed samples while it also suggested that whereas *ETNK1* mutations are an early event in the clonal evolution history of the disease, *SETBP1* mutations are usually a late event (33). Although the presence of *ETNK1* mutations has been considered as a relatively specific finding for aCML, leading to their inclusion in the 2016 WHO classification as a support criterion for the diagnosis of the disease, they have also been found in chronic myelomonocytic leukemia (CMML) and systemic mastocytosis with eosinophilia (34) while they were recently described in diffuse large B-cell lymphoma (35). Nevertheless, the interest in *ETNK1* mutations remains high due to the fact that phosphoethanolamine, the metabolic product of ETNK1, has been shown to negatively control mitochondrial activity, thus restoring a normal phenotype (24).

Colony Stimulating Factor 3 Receptor (CSF3R)

Although, in an early study, *CSF3R* mutations had been reported in 59% of patients with aCML and CNL (25, 36), further studies did not confirm this high frequency, and it is now believed that *CSF3R*-mutated aCML is in fact rare (19, 37). Nevertheless, these mutations have been proposed to activate either the JAK-STAT pathway (*CSF3R* membrane proximal mutation), or the SRC-kinase signaling pathway (*CSF3R* truncating mutation), thus being targetable with ruxolitinib or dasatinib, respectively (38). Moreover, due to the extremely high frequency of CSF3R mutations in CNL (39), these mutations constitute a criterion for the diagnosis of CNL and, along with other morphological and clinical features, help distinguish CNL from aCML In fact,

TABLE 2 | Recurrently mutated genes in aCML.

Gene	Chromosomal location	Normal function	Mutations	Clinical implications
SETBP1	18q12.3	It encodes a protein containing a ski homology region, a SET-binding region, and 3 nuclear localization signals. The protein binds to the SET nuclear oncogene which is involved in DNA replication. The SETBP1 protein is thought to control genes involved in developmental processes (e.g. nerve cell migration in the brain during fetal development).	Exon count: 15 Nonsense substitution A>C **Missense substitution A>G** **Synonymous substitution A>T** Inframe deletion C>G Frameshift deletion G>A Inframe insertion C>A Frameshift insertion C>T Complex mutation G>C • c.2602G>A, p.Asp868Asn (sAML, MDS, CMML1/2, CML-BP) • c.2608G>A, p.Gly870Ser (sAML, MDS, CMML1/2, CML-BP) • c.2640C>A, p.Asp880Glu (sAML) • c.2638G>A, p.Asp880Asn (CMML1) • c.2612T>C, p.Ile871Thr (sAML, pAML,	Severe anemia and thrombocytopenia Poor prognosis
ETNK1	12p12.1-p11.2	It catalyzes the first step of the *de novo* phosphatidylethanolamine biosynthesis pathway, responsible for the phosphorylation of ethanolamine to phosphoethanolamine.	Exon count: 13 Nonsense substitution A>C **Missense substitution A>G** Synonymous substitution A>T Inframe deletion C>G Frameshift deletion G>A	Association with altered sensitivity to the AKT kinase inhibitor capivasertib
CSF3R	1p34.3	Essential for granulocytic maturation. Plays a crucial role in the proliferation, differentiation, and survival of cells of the neutrophilic lineage. May function in some adhesion or recognition events at the cell surface.	Exon Count: 19 Nonsense substitution A>C **Missense substitution A>G** Synonymous substitution A>T Inframe deletion C>G Frameshift deletion G>A Inframe insertion C>A Frameshift insertion C>T Complex mutation G>C	Transformation to AML Congenital neutropenia
SRSF2	17q25.1	Necessary for pre-mRNA splicing. Required for formation of the earliest ATP-dependent splicing complex and interacts with spliceosome components bound to both the 5'- and 3'-splice sites during spliceosome assembly.	Exon count: 5 Nonsense substitution A>C **Missense substitution A>G** Synonymous substitution A>T Inframe deletion C>G Frameshift deletion G>A Inframe insertion C>A Frameshift insertion C>T Complex mutation G>C	Poor prognosis in MDS No prognosis impact in CMML
NRAS	1p13.2	Oncogene encoding a membrane protein that shuttles between the Golgi apparatus and the plasma membrane. The protein has intrinsic GTPase activity; it is activated by a guanine nucleotide-exchange factor and inactivated by a GTPase activating protein	Exon count: 7 Nonsense substitution A>C **Missense substitution A>G** Synonymous substitution A>T Inframe deletion C>G Frameshift deletion G>A Inframe insertion C>A Frameshift insertion C>T Complex mutation G>C	Associated with altered sensitivity to selumetinib, cediranib, ibrutinib, and dasatinib. Mutations which change amino-acids 12, 13 or 61 enhance the potential of Ras to transform cultured cells and are implicated in a variety of human tumors.
ASXL1	20q11.21	It encodes a chromatin-binding protein, member of the Polycomb group of proteins involved in transcriptional regulation mediated by ligand-bound nuclear hormone receptors, such as retinoic acid receptors (RARs) and peroxisome proliferator-activated receptor gamma (PPARG). It is thought to disrupt chromatin in localized areas, enhancing transcription of certain genes while repressing the transcription of others.	Exon count: 18 **Nonsense substitution A>C** **Missense substitution A>G** Synonymous substitution A>T Inframe deletion C>G **Frameshift deletion G>A** Inframe insertion C>A **Frameshift insertion C>T** Complex mutation G>C	Mutations in *ASXL1* are associated with altered sensitivity to 46 drugs among them olaparib, venetoclax, and pevonedistat

(Continued)

TABLE 2 | Continued

Gene	Chromosomal location	Normal function	Mutations	Clinical implications
TET2	4q24	It plays a key role in active DNA demethylation. It is also involved in the recruitment of the O-GlcNAc OGT to CpG-rich transcription start sites of active genes, thereby promoting histone H_2B GlcNAcylation by OGT.	Exon count: 15 **Nonsense substitution A>C** **Missense substitution A>G** Synonymous substitution A>T Inframe deletion C>G **Frameshift deletion G>A** Inframe insertion C>A **Frameshift insertion C>T** Complex mutation G>C	Associated with altered sensitivity to bexarotene, Ara-G, tretinoin and VNLG/124.

SETBP1, SET Binding Protein 1; sAML, secondary acute myeloid leukemia; MDS, myelodysplastic syndrome; CMML, chronic myelomonocytic leukemia; pAML, primary acute myeloid leukemia; ETNK1, ethanolamine kinase 1; CSF3R, colony stimulating factor 3 receptor; SRSF2, serine and arginine rich splicing factor 2; NRAS, neuroblastoma RAS viral oncogene; ASXL1, Additional Sex Combs Like 1; TET2, Ten-eleven Translocation 2; OGT, O-linked N-acetylglucosamine transferase; Ara-G, 9-β-D- arabinofuranosylguanine, VNLG/124, 4-(butanoyloxymethyl)phenyl(2 E,4 E,6 E,8 E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoate
Frequently encountered mutations are reported in bold.

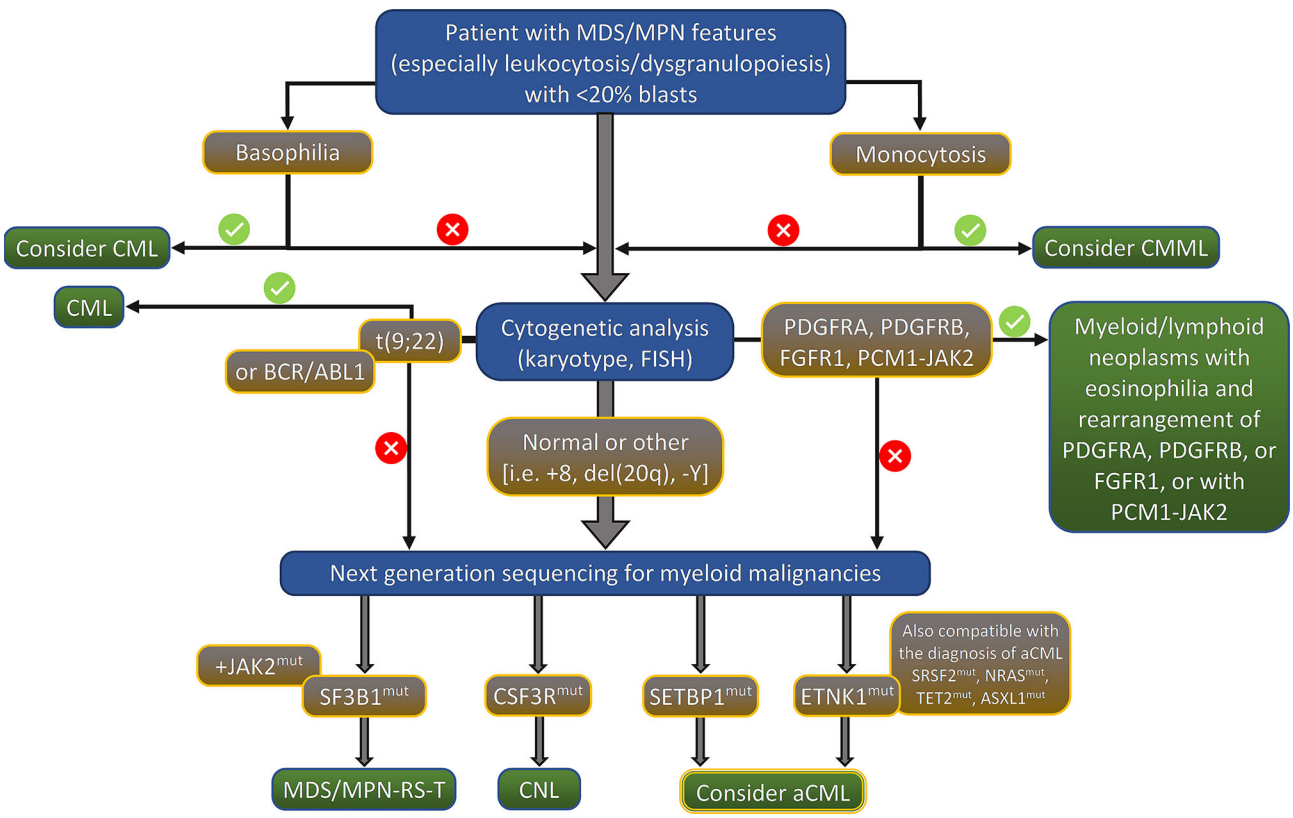


FIGURE 1 | Proposed algorithm for the diagnosis of aCML. MDS/MPN, myelodysplastic syndrome/myeloproliferative neoplasm; CML, chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; FISH, fluorescent *in situ* hybridization; RS, ring sideroblasts; T, thrombocytosis; CNL, chronic neutrophilic leukemia; aCML, atypical chronic myelogenous leukemia, BCR/ABL1 negative.

the identification of a CSF3R mutation in a patient with hepatosplenomegaly, hyperleukocytosis with prominence of neutrophils, low percentage of peripheral blood immature granulocytes and myeloblasts, along with minimal or absent dysgranulopoiesis in the bone marrow strongly favors the diagnosis of CNL over aCML.

Serine and Arginine Rich Splicing Factor 2 (SRSF2)

SRSF2 mutations have been reported in 12%, 13.5%, and 40% of aCML cases in three studies (15, 33, 37). In the study by Patnaik et al., the presence of *SRSF2* mutations in three of the examined cases did not affect OS, while one patient had a concomitant

TABLE 3 | Actionable and non-actionable mutations in aCML.

Mutated gene	Actionable	Potential targeted therapy	Comments
SETBP1	No	NA	No reports on the development of agents targeting SETBP1.
ETNK1	Possibly	P-Et	It has been shown in ETNK1 overexpression models and patient samples that P-Et is able to fully counteract the metabolic effects of ETNK1 overexpression (24).
CSF3R	Yes	Ruxoitinib for *CSF3R* membrane proximal mutation	After a few case reports on the successful use of ruxolitinib in patients with CSF3R mutations, a phase II study including 23 patients with aCML, six of whom carried an activating mutation of CSF3R reported an overall response rate of only 8.7% (18).
		Dasatinib for *CSF3R* truncating mutation	*In vitro* studies with dasatinib on cell lines with truncating mutations of *CSF3R* have shown sensitivity of the cells to the drug (25, 26), but no *in vivo* reports have supported these findings.
SRSF2	Possibly	CTX-712	CTX-712 is a novel Clk Inhibitor that has been found to exert an anti-tumor effect in an SRSF2-mutated xenograft model (26).
NRAS	Yes	Trametinib	A phase I/II nonrandomized study has shown clinical activity of trametinib in several RAS-mutated myeloid malignancies (27).
ASXL1	Possibly	BAP1 catalytic inhibitors	BAP1 catalytic inhibitors have been shown to inhibit truncated-ASXL1-driven leukemic gene expression and halt tumor progression *in vivo* (28).
TET2	Possibly	TET-selective small molecule inhibitor	Treatment with a TET-selective small molecule inhibitor has been found to suppress the clonal evolution of TET2 mutant cells in murine models and human leukemia xenografts (29).

aCML, atypical chronic myelogenous leukemia; BCR/ABL1 negative; P-Et, phosphoethanolamine SETBP1, SET Binding Protein 1; NA, not applicable; ETNK1, ethanolamine kinase 1; CSF3R, colony stimulating factor 3 receptor; SRSF2, serine and arginine rich splicing factor 2; NRAS, neuroblastoma RAS viral oncogene; ASXL1, Additional Sex Combs Like 1; BAP1, BRCA1 associated protein 1; TET2, Ten-eleven translocation.

TET2 mutation. On the other hand, in the study by Meggendorfer et al., it was shown that *SRSF2* mutations tended to coexist with *SETBP1* and *ASXL1* mutations.

RAS Mutations

NRAS mutations resulting is constitutive activation of the MAPK pathway, thus, promoting malignant cell survival and proliferation, have been detected in up to one third of patients with aCML (19, 40). Moreover, it has been shown that enhanced MAPK signaling is crucial to leukemogenesis by *CSF3R* mutants in CNL (41).

Other Gene Mutations

Mutations of *ASXL1* and *TET2* are found in over 15% of cases with aCML. In one study, *TET2* mutations were correlated with lower OS along with advanced age and low hemoglobin level. On the other hand, *ASXL1* is considered to be the most commonly mutated gene in aCML (28%), but it has also been shown not to affect overall survival (OS) despite the detrimental effect on survival in CMML, CNL, and PMF (37). Mutations of *ASXL1* have been found to accompany spliceosome gene mutations in as high as 65% of cases (21), a fact supporting the hypothesis of serial accumulation of mutations in the malignant clone, that seems to apply to all MDS/MPNs (42). It should be noted though, that the correlation of OS with the mutational status has been based in small patient series and should, therefore, be considered with caution.

TREATMENT APPROACHES FOR ACML

As already mentioned, there is no standard of care for the management of patients with aCML. The rarity of the disease has led to its misclassification, resulting in the lack of a universally accepted risk stratification system that would lead to the formulation of recommendations for risk-based treatment strategies. Treatment choices are based on the results of small trials and patient series while some classical therapeutic options have never actually been studied and their use is only based on their efficacy in other MDS/MPN. Recently, the molecular profile of the disease has led to the emergence of new treatment options based on targetable mutations, but their use is mostly based on preclinical data or case reports. Most patients require some kind of treatment since their initial presentation, due to the accelerating leukocytosis and the deteriorating anemia, splenomegaly, and constitutional symptoms, thus a wait-and-watch strategy is rarely advisable.

As already mentioned, there is a lack of risk stratification systems for aCML, mainly due to the rarity of the condition and to the absence of large studies evaluating prognosis. Nevertheless, there have been some efforts to identify adverse prognostic factors and to stratify the patients according to their risk for AML transformation and death. In one of the largest studies in aCML, the authors analyzed the prognostic characteristics of 76 treatment-naïve patients with *BCR/ABL1* negative CML. Multivariate analysis identified age >65 years, hemoglobin level <10 g/dL, and WBC>$50x10^9$/L as independent prognostic factors of poor survival (43). In the same study it was shown that treatment did not significantly affect survival. Moreover, a simple scoring system assigning one point to each one of the three above-mentioned independent prognostic factors was designed to stratify patients according to their expected survival. The system stratified the patients into two risk-groups (low risk with 0-1 point and high risk with >1 points), with a corresponding median survival of 38 months *versus* 9 months ($p<0.01$). Although this risk stratification system has not been widely used, the value of its prognostic parameters has been confirmed in more recent studies. Thus, in a study of 55 patients with aCML (44) multivariate analysis associated shorter survival with age >65 years, female sex, WBC>$50x10^9$/L, and presence of circulating immature precursors while the hemoglobin level did not retain its statistical significance. The authors also evaluated the risk of

leukemic transformation that was found to be higher in patients with palpable hepatosplenomegaly, monocytosis, bone marrow blasts >5%, marked dyserythropoiesis, and transfusion dependence. Moreover, they tried to validate the prognostic scoring system by Onida et al. and reported that, in their cohort, it could not identify poor survival but identified patients at higher risk for AML transformation. A smaller study reported longer survival rates in patients with normal platelet counts and hemoglobin level >10 g/dL (45) while in a study of 65 patients, WBC>50x10^9/L, and a high blood immature myeloid cell count and bone marrow blast count (as continuous variables) were correlated with lower OS in univariate analysis. However, no multivariate analysis was carried out (19). Finally, in the most recent study in aCML, age >67 years, hemoglobin level <10 g/dL, and *TET2* mutations were correlated with lower survival rates in multivariate analysis (37). The authors also provided a two-group prognostic model based on the above parameters, with a median OS of 18 and seven months, respectively. **Table 4** summarizes the proposed prognostic factors for aCML by the above referenced studies.

In conclusion, most of the studies reporting on prognostic factors in aCML agree that advanced age, anemia, hyperleukocytosis, and presence of immature myeloid cells in the peripheral blood adversely affect OS. It is becoming more and more obvious that the underlying molecular mechanisms may be well correlated with prognosis; thus, further analysis of the molecular footprint of the disease will allow the design of more accurate risk stratification systems that will define the treatment needs of each patient. The available treatment options for patients with aCML are presented in detail in the following paragraphs.

Allogeneic Hematopoietic Stem Cell Transplantation (Allo-HSCT)

Allo-HSCT should be considered in all eligible patients, given the unfavorable prognosis of aCML. Nevertheless, the age of the patients is usually a prohibitive factor while at the same time there is limited reliable data on the efficacy of allo-HSCT in patients with aCML. Several small studies of retrospective nature have been published. In a study of nine transplanted patients (four from HLA-identical siblings, four from HLA-compatible unrelated donors and one from a twin sibling) followed for a median period of 55 months, all nine achieved complete chimerism and remained in complete remission (CR) while, with the exception of one patient who died from cerebral toxoplasmosis, the remaining eight were alive at the time of the analysis (46). In another study of seven patients with aCML treated with allo-HSCT, after a follow-up period of 17.5 months, only two were alive; one had died due to aCML, and the remaining four due to transplantation related complications (47). In an analysis of 42 cases reported to the European Society for Blood and Marrow Transplantation (EBMT) registry, donors were HLA-identical siblings in 64% and matched unrelated in 36% of the cases. A CR was observed in 87% of patients and the 5-year relapse-free survival was 36%. Younger patients with low EBMT risk scores were found to be the best candidates for allo-HSCT (43). In a recent study of 14 patients with aCML treated with allo-HSCT, 13/14 had received first-line therapy (10/14 with hydroxyurea) with variable responses, 8/13 had received second-line therapy before transplant, and 4/8 third-line therapy while at least two had progressed to acute myelogenous leukemia (AML) before allo-HSCT. Five, seven, and two patients received an allograft from HLA-matched related, unrelated marrow, and unrelated cord blood donors, respectively. A myeloablative regimen was used in 11/14 patients. Among patients with neutrophil engraftment, 9/13 achieved a CR and the 1-year OS was 54.4%, being higher in univariate analysis in patients with related donors, <5% myeloblasts in the peripheral blood, and a Karnofsky performance status of ≤80% (48).

Questions on the donor source, the correct timing (i.e. upfront transplantation *versus* transplantation after initial treatment to reduce the disease burden), the intensity of the

TABLE 4 | Factors correlated with lower overall survival in patients with atypical chronic myelogenous leukemia, *BCR/ABL1* negative.

	Onida et al	Breccia et al	Wang et al	Hernandez et al	Patnaik et al
Number of patients, N	76	55	65	11	25
Median OS (months)	24	25	21.4	14	10.8
Age (years)	>65	>65	–	–	>67
Gender	–	F	–	–	NS
Hemoglobin level (g/dL)	<10	NS	–	<10	<10
Dyserythropoiesis	–	present*	–	–	–
Transfusion dependence	–	present*	–	–	NS
WBC (x10^9/L)	>50	>50	>50	–	NS
Monocyte count	>1.0x10^9/L	3-8%*		–	–
Platelet count (x10^9/L)	–	–	NS	<140	NS
Blood immature myeloid cells (%)	>10	present	↑	–	NS
Bone marrow blasts (%)	–	>5*	↑	–	NS
LDH level (U/mL)	>2000	–	NS	–	–
Hepatosplenomegaly	–	Present*	–	–	NS
Gene mutations	–	–	–	–	TET2

**Risk factor for acute myeloid leukemia transformation.*

OS, overall survival; F, female; NS, not significant; WBC, white blood cell; LDH, lactated dehydrogenase; ↑, high (as a continuous variable).

conditioning regimen, the impact of previously administered treatments, and the possible impact of the molecular profile of the patients still remain unanswered. Nevertheless, the advanced age of most patients with aCML probably dictates the use of reduced intensity conditioning regimens while the molecular profile of the patients may be useful for the monitoring of minimal residual disease (49) and as a guide for post-transplant maintenance in cases with targetable mutations.

Hypomethylating Agents (HMAs)

The use of HMAs in patients with aCML should be a case-by-case approach since patients with striking myeloproliferative features are less likely to respond to HMAs. Although data on the efficacy and safety of HMAs in aCML is limited, the rationale for their use is sound, based on their established activity in CMML and other MDS/MPN. Early data on the use of decitabine in seven patients with aCML and a median age of 67 years showed an OS rate of only 13 months and a 14% 2-year survival rate (50). In a study of 76 patients with aCML treated with several regimens (5 patients treated with an HMA), OS was not affected by the treatment choice, but no information is specifically provided for the prognosis of patients treated with HMAs (51). Decitabine has also been administered in four patients with aCML and after a median of 2.5 cycles of treatment and a median follow-up of 13 months, three patients were still alive (one of them eventually treated with allo-HSCT) (52). Finally, in a report of five patients with aCML treated with HMAs, the best achieved response was stable disease (37), while a few case reports give mixed results on the efficacy of HMAs. Thus, a general rule would be that the use of HMAs in patients with aCML should be restricted in elderly patients with prominent dysplastic and more subtle myeloproliferative features, or as a bridge to a transplant.

Cytoreduction and Intensive Chemotherapy

Historically, hydroxyurea has been the mainstay of treatment for patients with aCML and other MDS/MPNs. In recent reports, it remains the most widely used factor for the management of myeloproliferation in aCML and it can effectively control leukocytosis and splenomegaly, but responses are typically short-lived. In an early study on 11 patients with aCML, nine were treated with hydroxyurea achieving a partial remission (8). In a study of 55 patients with aCML, 48 (87.3%) patients had been treated with hydroxyurea, but the effect of treatment on OS was not further discussed by the authors (44). Furthermore, in a study on the characteristics and outcome of 76 patients with aCML, the authors stated that 53% of the patients had already been treated with hydroxyurea or busulfan at the time of their referral to the study center while nine of them were also treated with hydroxyurea after their referral. Although there was no specific mention of the outcome per regimen, the authors reported that there was no significant difference in OS between treated and untreated patients (43). Finally, in a more recent study of 25 patients, hydroxyurea had been administered in 15 (60.0%) patients, but treatment outcomes were not evaluated (37). Busulfan and low-dose cytarabine have also been used in the treatment of patients with aCM while intensive chemotherapy is not a standard treatment option for patients with aCML and should be reserved for patients with leukemic transformation.

Ruxolitinib

Activating mutations in *CSF3R* have been found to drive myeloproliferative disorders resembling aCML and CNL in mouse models, where these mutations are sensitive to JAK inhibition that may effectively reduce the WBC count and the spleen size (53). Preliminary data has shown that treatment of aCML/CNL cells carrying activating mutations of *CSF3R* with ruxolitinib resulted in inhibition of cell growth while a patient with CNL bearing the *CSF3R* T618I mutation was effectively treated with ruxolitinib (25). Although the experience with ruxolitinib was limited to case reports, a recent phase II study was conducted to investigate the hematologic response to ruxolitinib in patients with aCML/CNL. The study included 23 patients with aCML, six of whom carried an activating mutation of *CSF3R* (T618I, T640N, or T615A). Response (PR and CR) was reported in only two (8.7%) patients while no serious adverse events attributed to ruxolitinib were observed (54). The results of this trial reduced the initial enthusiasm on the efficacy of targeting *CSF3R* mutations with ruxolotinib in aCML, especially since these mutations proved to be rather rare in this condition. The use of ruxolitinib as a bridge to transplant, though, is still a valid choice in an effort to reduce the WBC count and splenomegaly following the paradigm of myelofibrosis although clinical trials are still lacking.

Dasatinib

CSF3R truncation mutations have been shown to activate the SRC family pathway offering an option of targeted therapy with dasatinib. This SRC family kinase inhibitor used in CML has been proposed to potentially have therapeutic value in aCML with *CSF3R* truncation mutations since *in vitro* studies of cell lines with such mutations have shown sensitivity of the cells to the drug (18, 25). However, these preclinical data have not been supported by *in vivo* reports to date.

Trametinib

The mitogen-activated protein kinase kinase (MEK) inhibitor trametinib, approved for the treatment of advanced or metastatic melanoma, has been proven active against *NRAS*-mutated AML in human cell lines and murine models (55). Moreover, it has been shown that MEK inhibition with trametinib is sufficient to suppress CNL induced by *CSF3R* mutations, highlighting a MAPK-dependent mechanism of CSF3R-induced pathogenesis that is valid at least in CNL (41). A case report of a patient with aCML responding to trametinib (56) and a phase I/II nonrandomized study showing clinical activity of trametinib in several RAS-mutated myeloid malignancies (27) have confirmed these preclinical data although the beneficial effect of the drug in reducing the blast count was not translated into a survival benefit. Further studies with trametinib as monotherapy or in combination with other targeted therapies are needed to test its efficacy in aCML.

Other Agents

Interferon has been used in aCML with mixed results. In an early study of 14 patients, interferon-α was administered to seven patients following treatment with hydroxyurea, and CR was achieved in five (57). In another study on the characteristics and outcome of patients with aCML, interferon-α and interferon-γ were used as single-agent therapy in 17 patients with aCML; nevertheless, response rates were not presented (43). Moreover, among 11 patients with aCML, six were treated with interferon-α, two of whom achieved a CR lasting for 9 and 40 months (8). In another early study, out of 14 patients treated with interferon-α, six (43%) of them responded (five with a CR) with a duration of response ranging from 1 to >100 months (58). Finally, among five patients with aCML treated with peg-interferon-α-2b in the context of a phase II study, two responded to treatment achieving a CR after three months of treatment (59). The above-referenced results show that the use of interferon-α and its pegylated formulations should be further investigated.

Supportive and Palliative Treatment

Supportive treatment should be offered to all patients in the form of erythropoiesis-stimulating agents (ESAs) and red blood cell transfusions. The use of ESAs in terms of efficacy and safety has never been specifically studied for aCML. Thus, factors predicting response to ESAs have not been established. Moreover, heavily transfused patients should probably also receive iron chelation therapy, depending on their estimated prognosis, but this strategy is not supported by any published data. Finally, the use of corticosteroids, danazol, and thalidomide or lenalidomide to treat anemia has never been studied or reported in patients of aCML. Splenectomy or splenic irradiation have been scarcely used in the past as palliative measures in patients with aCML, with no disease improvement (8, 50, 60). Thus, they should be avoided since any possible benefit is cancelled out by potentially severe complications, such as bleeding, thrombosis, infection, and potential acceleration of leukocytosis and/or hepatomegaly (22).

Conclusions on Treatment Approach

Based on the available data, the general principals of the management of patients with aCML can be summarized into the following.

a. Allo-HSCT should be offered to all eligible patients.
b. Myeloid mutation panel testing should be performed to all patients to detect potentially targetable mutations.
c. Although clinical trials focusing on patients with aCML are rare, inclusion in a clinical trial should be an early choice, especially for patients without targetable mutations.
d. In patients not eligible for allo-HSCT, the treatment should focus on addressing the patient's major clinical issues (constitutional symptoms, anemia, organomegaly) and potentially on decreasing the possibility of progression to AML although supporting data about the latter is lacking.
e. Treatment strategies applicable to patients with MDS or MPN can be selected on a case-by-case approach.
f. Given the poor prognosis of aCML, initiation of treatment is generally favored over a watch-and-wait strategy.

The prognosis of patients with aCML remains poor and cases with long survival are the exception to the rule. Further identification of the molecular footprint of the disease may allow for the emergence of new targeted treatment choices that may reduce the risk for AML transformation and prolong survival.

AUTHOR CONTRIBUTIONS

PD has drafted the manuscript and N-AV has critically revised the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We would like to thank Ms Evita Alexopoulos for copyediting the final manuscript.

REFERENCES

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. *Blood* (2016) 127(20):2391–405. doi: 10.1182/blood-2016-03-643544
2. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 Revision of the World Health Organization (WHO) Classification of Myeloid Neoplasms and Acute Leukemia: Rationale and Important Changes. *Blood* (2009) 114(5):937–51. doi: 10.1182/blood-2009-03-209262
3. Drozd-Sokołowska JE, Waszczuk-Gajda A, Mądry K, Dwilewicz-Trojaczek J. Atypical Chronic Myeloid Leukaemia - A Rare Subtype of Myelodysplastic/Myeloproliferative Neoplasm. *Contemp Oncol (Pozn)* (2018) 22(1):14–9. doi: 10.5114/wo.2018.74388
4. Iavorkovskiĭ LI, Iavorkovskiĭ LL. [Does Ph'-Negative Chronic Myeloleukemia Exist]? . *Gematol Transfuziol* (1989) 34(11):3–10.
5. van der Plas DC, Hermans AB, Soekarman D, Smit EM, de Klein A, Smadja N, et al. Cytogenetic and Molecular Analysis in Philadelphia Negative CML. *Blood* (1989) 73(4):1038–44. doi: 10.1182/blood.V73.4.1038.1038
6. Sessarego M, Mareni C, Vimercati R, Defferrari R, Origone P, Damasio E, et al. Translocation T(9;9)(P13;Q34) in Philadelphia-Negative Chronic Myeloid Leukemia With Breakpoint Cluster Region Rearrangement. *Cancer Genet Cytogenet* (1989) 43(1):51–6. doi: 10.1016/0165-4608(89)90126-X
7. Selleri L, Emilia G, Luppi M, Temperani P, Zucchini P, Tagliafico E, et al. Chronic Myelogenous Leukemia With Typical Clinical and Morphological Features can be Philadelphia Chromosome Negative and "Bcr Negative". *Hematol Pathol* (1990) 4(2):67–77.
8. Kurzrock R, Kantarjian HM, Shtalrid M, Gutterman JU, Talpaz M. Philadelphia Chromosome-Negative Chronic Myelogenous Leukemia Without Breakpoint Cluster Region Rearrangement: A Chronic Myeloid Leukemia With a Distinct Clinical Course. *Blood* (1990) 75(2):445–52. doi: 10.1182/blood.V75.2.445.445
9. Zoumbos NC, Symeonidis A, Kourakli-Symeonidis A. Chronic Neutrophilic Leukemia With Dysplastic Features. A New Variant of the Myelodysplastic Syndromes. *Acta Haematol* (1989) 82(3):156–60. doi: 10.1159/000205367
10. Kantarjian HM, Kurzrock R, Talpaz M. Philadelphia Chromosome-Negative Chronic Myelogenous Leukemia and Chronic Myelomonocytic Leukemia. *Hematol Oncol Clin North Am* (1990) 4(2):389–404. doi: 10.1016/S0889-8588(18)30494-5

11. Michaux JL, Martiat P. Chronic Myelomonocytic Leukaemia (CMML)–a Myelodysplastic or Myeloproliferative Syndrome? *Leuk Lymphoma* (1993) 9 (1-2):35–41. doi: 10.3109/10428199309148501
12. Shepherd PC, Ganesan TS, Galton DA. Haematological Classification of the Chronic Myeloid Leukaemias. *Baillieres Clin Haematol* (1987) 1(4):887–906. doi: 10.1016/S0950-3536(87)80031-8
13. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick H, et al. The Chronic Myeloid Leukaemias: Guidelines for Distinguishing Chronic Granulocytic, Atypical Chronic Myeloid, and Chronic Myelomonocytic Leukaemia. Proposals by the French-American-British Cooperative Leukaemia Group. *Br J Haematol* (1994) 87(4):746–54. doi: 10.1111/j.1365-2141.1994.tb06734.x
14. Dao KH, Tyner JW. What's Different About Atypical CML and Chronic Neutrophilic Leukemia? *Hematol Am Soc Hematol Educ Program* (2015) 2015:264–71. doi: 10.1182/asheducation-2015.1.264
15. Meggendorfer M, Haferlach T, Alpermann T, Jeromin S, Haferlach C, Kern W, et al. Specific Molecular Mutation Patterns Delineate Chronic Neutrophilic Leukemia, Atypical Chronic Myeloid Leukemia, and Chronic Myelomonocytic Leukemia. *Haematologica* (2014) 99(12):e244–6. doi: 10.3324/haematol.2014.113159
16. Sadigh S, Hasserjian RP, Hobbs G. Distinguishing Atypical Chronic Myeloid Leukemia From Other Philadelphia-Negative Chronic Myeloproliferative Neoplasms. *Curr Opin Hematol* (2020) 27(2):122–7. doi: 10.1097/MOH.0000000000000565
17. Gotlib J, Maxson JE, George TI, Tyner JW. The New Genetics of Chronic Neutrophilic Leukemia and Atypical CML: Implications for Diagnosis and Treatment. *Blood* (2013) 122(10):1707–11. doi: 10.1182/blood-2013-05-500959
18. Zoi K, Cross NC. Molecular Pathogenesis of Atypical CML, CMML and MDS/MPN-Unclassifiable. *Int J Hematol* (2015) 101(3):229–42. doi: 10.1007/s12185-014-1670-3
19. Wang SA, Hasserjian RP, Fox PS, Rogers HJ, Geyer JT, Chabot-Richards D, et al. Atypical Chronic Myeloid Leukemia Is Clinically Distinct From Unclassifiable Myelodysplastic/Myeloproliferative Neoplasms. *Blood* (2014) 123(17):2645–51. doi: 10.1182/blood-2014-02-553800
20. Piazza R, Valletta S, Winkelmann N, Redaelli S, Spinelli R, Pirola A, et al. Recurrent SETBP1 Mutations in Atypical Chronic Myeloid Leukemia. *Nat Genet* (2013) 45(1):18–24. doi: 10.1038/ng.2495
21. Meggendorfer M, Bacher U, Alpermann T, Haferlach C, Kern W, Gambacorti-Passerini C, et al. SETBP1 Mutations Occur in 9% of MDS/MPN and in 4% of MPN Cases and Are Strongly Associated With Atypical CML, Monosomy 7, Isochromosome I(17)(Q10), ASXL1 and CBL Mutations. *Leukemia* (2013) 27(9):1852–60. doi: 10.1038/leu.2013.133
22. Gotlib J. How I Treat Atypical Chronic Myeloid Leukemia. *Blood* (2017) 129 (7):838–45. doi: 10.1182/blood-2016-08-693630
23. Tate JG, Bamford S, Jubb HC, Sondka Z, Beare DM, Bindal N, et al. COSMIC: The Catalogue Of Somatic Mutations In Cancer. *Nucleic Acids Research* (2019) 47: (D1):D941–7. doi: 10.1093/nar/gky1015
24. Fontana D, Mauri M, Renso R, Docci M, Crespiatico I, Røst LM, et al. ETNK1 Mutations Induce a Mutator Phenotype That can be Reverted With Phosphoethanolamine. *Nat Commun* (2020) 11(1):5938. doi: 10.1038/s41467-020-19721-w
25. Maxson JE, Gotlib J, Pollyea DA, Fleischman AG, Agarwal A, Eide CA, et al. Oncogenic CSF3R Mutations in Chronic Neutrophilic Leukemia and Atypical CML. *N Engl J Med* (2013) 368(19):1781–90. doi: 10.1056/NEJMoa1214514
26. Yoda A, Morishita D, Mizutani A, Satoh Y, Ochi Y, Nannya Y, et al. CTX-712, a Novel Clk Inhibitor Targeting Myeloid Neoplasms With SRSF2 Mutation. *Blood* (2019) 134(Supplement_1):404.
27. Borthakur G, Popplewell L, Boyiadzis M, Foran J, Platzbecker U, Vey N, et al. Activity of the Oral Mitogen-Activated Protein Kinase Kinase Inhibitor Trametinib in RAS-Mutant Relapsed or Refractory Myeloid Malignancies. *Cancer* (2016) 122(12):1871–9. doi: 10.1002/cncr.29986
28. Wang L, Birch NW, Zhao Z, Nestler CM, Kazmer A, Shilati A, et al. Epigenetic Targeted Therapy of Stabilized BAP1 in ASXL1 Gain-of-Function Mutated Leukemia. *Nat Cancer* (2021) 2:515–26. doi: 10.1038/s43018-021-00199-4
29. Guan Y, Tiwari AD, Phillips JG, Hasipek M, Grabowski DR, Pagliura S, et al. A Therapeutic Strategy for Preferential Targeting of TET2 Mutant and TET-Dioxygenase Deficient Cells in Myeloid Neoplasms. *Blood Cancer Discovery* (2021) 2(2):146–61. doi: 10.1158/2643-3230.BCD-20-0173
30. Makishima H, Yoshida K, Nguyen N, Przychodzen B, Sanada M, Okuno Y, et al. Somatic SETBP1 Mutations in Myeloid Malignancies. *Nat Genet* (2013) 45(8):942–6. doi: 10.1038/ng.2696
31. Kennedy EP, Weiss SB. The Function of Cytidine Coenzymes in the Biosynthesis of Phospholipides. *J Biol Chem* (1956) 222(1):193–214. doi: 10.1016/S0021-9258(19)50785-2
32. Gambacorti-Passerini CB, Donadoni C, Parmiani A, Pirola A, Redaelli S, Signore G, et al. Recurrent ETNK1 Mutations in Atypical Chronic Myeloid Leukemia. *Blood* (2015) 125(3):499–503. doi: 10.1182/blood-2014-06-579466
33. Fontana D, Ramazzotti D, Aroldi A, Redaelli S, Magistroni V, Pirola A, et al. Integrated Genomic, Functional, and Prognostic Characterization of Atypical Chronic Myeloid Leukemia. *Hemasphere* (2020) 4(6):e497. doi: 10.1097/HS9.0000000000000497
34. Lasho TL, Finke CM, Zblewski D, Patnaik M, Ketterling RP, Chen D, et al. Novel Recurrent Mutations in Ethanolamine Kinase 1 (ETNK1) Gene in Systemic Mastocytosis With Eosinophilia and Chronic Myelomonocytic Leukemia. *Blood Cancer J* (2015) 5(1):e275. doi: 10.1038/bcj.2014.94
35. Zhou XA, Louissaint AJr., Wenzel A, Yang J, Martinez-Escala ME, Moy AP, et al. Genomic Analyses Identify Recurrent Alterations in Immune Evasion Genes in Diffuse Large B-Cell Lymphoma, Leg Type. *J Invest Dermatol* (2018) 138(11):2365–76. doi: 10.1016/j.jid.2018.04.038
36. Langabeer SE, McCarron SL, Haslam K, O'Donovan MT, Conneally E. The CSF3R T618I Mutation as a Disease-Specific Marker of Atypical CML Post Allo-SCT. *Bone Marrow Transplant* (2014) 49(6):843–4. doi: 10.1038/bmt.2014.35
37. Patnaik MM, Barraco D, Lasho TL, Finke CM, Reichard K, Hoversten KP, et al. Targeted Next Generation Sequencing and Identification of Risk Factors in World Health Organization Defined Atypical Chronic Myeloid Leukemia. *Am J Hematol* (2017) 92(6):542–8. doi: 10.1002/ajh.24722
38. Schwartz LC, Mascarenhas J. Current and Evolving Understanding of Atypical Chronic Myeloid Leukemia. *Blood Rev* (2019) 33:74–81. doi: 10.1016/j.blre.2018.07.004
39. Pardanani A, Lasho TL, Laborde RR, Elliott M, Hanson CA, Knudson RA, et al. CSF3R T618I Is a Highly Prevalent and Specific Mutation in Chronic Neutrophilic Leukemia. *Leukemia* (2013) 27(9):1870–3. doi: 10.1038/leu.2013.122
40. Patnaik MM, Lasho TL. Genomics of Myelodysplastic Syndrome/Myeloproliferative Neoplasm Overlap Syndromes. *Hematol Am Soc Hematol Educ Program* (2020) 2020(1):450–9. doi: 10.1182/hematology.2020000130
41. Rohrabaugh S, Kesarwani M, Kincaid Z, Huber E, Leddonne J, Siddiqui Z, et al. Enhanced MAPK Signaling Is Essential for CSF3R-Induced Leukemia. *Leukemia* (2017) 31(8):1770–8. doi: 10.1038/leu.2016.376
42. Deininger MWN, Tyner JW, Solary E. Turning the Tide in Myelodysplastic/Myeloproliferative Neoplasms. *Nat Rev Cancer* (2017) 17(7):425–40. doi: 10.1038/nrc.2017.40
43. Onida F, de Wreede LC, van Biezen A, Eikema DJ, Byrne JL, Iori AP, et al. Allogeneic Stem Cell Transplantation in Patients With Atypical Chronic Myeloid Leukaemia: A Retrospective Study From the Chronic Malignancies Working Party of the European Society for Blood and Marrow Transplantation. *Br J Haematol* (2017) 177(5):759–65. doi: 10.1111/bjh.14619
44. Breccia M, Biondo F, Latagliata R, Carmosino I, Mandelli F, Alimena G. Identification of Risk Factors in Atypical Chronic Myeloid Leukemia. *Haematologica* (2006) 91(11):1566–8.
45. Hernández JM, del Cañizo MC, Cuneo A, García JL, Gutiérrez NC, González M, et al. Clinical, Hematological and Cytogenetic Characteristics of Atypical Chronic Myeloid Leukemia. *Ann Oncol* (2000) 11(4):441–4. doi: 10.1023/A:1008393002748
46. Koldehoff M, Beelen DW, Trenschel R, Steckel NK, Peceny R, Ditschkowski M, et al. Outcome of Hematopoietic Stem Cell Transplantation in Patients With Atypical Chronic Myeloid Leukemia. *Bone Marrow Transplant* (2004) 34(12):1047–50. doi: 10.1038/sj.bmt.1704686
47. Mittal P, Saliba RM, Giralt SA, Shahjahan M, Cohen AI, Karandish S, et al. Allogeneic Transplantation: A Therapeutic Option for Myelofibrosis, Chronic Myelomonocytic Leukemia and Philadelphia-Negative/BCR-ABL-Negative

Chronic Myelogenous Leukemia. *Bone Marrow Transplant* (2004) 33 (10):1005–9. doi: 10.1038/sj.bmt.1704472
48. Itonaga H, Ota S, Ikeda T, Taji H, Amano I, Hasegawa Y, et al. Allogeneic Hematopoietic Stem Cell Transplantation for the Treatment of BCR-ABL1-Negative Atypical Chronic Myeloid Leukemia and Chronic Neutrophil Leukemia: A Retrospective Nationwide Study in Japan. *Leuk Res* (2018) 75:50–7. doi: 10.1016/j.leukres.2018.11.003
49. Langabeer SE, Comerford CM, Quinn J, Murphy PT. Molecular Profiling and Targeted Inhibitor Therapy in Atypical Chronic Myeloid Leukaemia in Blast Crisis. *J Clin Pathol* (2017) 70(12):1089. doi: 10.1136/jclinpath-2017-204621
50. Kantarjian HM, O'Brien S, Cortes J, Giles FJ, Faderl S, Issa JP, et al. Results of Decitabine (5-Aza-2'deoxycytidine) Therapy in 130 Patients With Chronic Myelogenous Leukemia. *Cancer* (2003) 98(3):522–8. doi: 10.1002/cncr.11543
51. Onida F, Ball G, Kantarjian HM, Smith TL, Glassman A, Albitar M, et al. Characteristics and Outcome of Patients With Philadelphia Chromosome Negative, Bcr/Abl Negative Chronic Myelogenous Leukemia. *Cancer* (2002) 95(8):1673–84. doi: 10.1002/cncr.10832
52. Tong X, Li J, Zhou Z, Zheng D, Liu J, Su C. Efficacy and Side-Effects of Decitabine in Treatment of Atypical Chronic Myeloid Leukemia. *Leuk Lymphoma* (2015) 56(6):1911–3. doi: 10.3109/10428194.2014.986477
53. Fleischman AG, Maxson JE, Luty SB, Agarwal A, Royer LR, Abel ML, et al. The CSF3R T618I Mutation Causes a Lethal Neutrophilic Neoplasia in Mice That Is Responsive to Therapeutic JAK Inhibition. *Blood* (2013) 122 (22):3628–31. doi: 10.1182/blood-2013-06-509976
54. Dao KT, Gotlib J, Deininger MMN, Oh ST, Cortes JE, Collins RHJr., et al. Efficacy of Ruxolitinib in Patients With Chronic Neutrophilic Leukemia and Atypical Chronic Myeloid Leukemia. *J Clin Oncol* (2020) 38(10):1006–18. doi: 10.1200/JCO.19.00895
55. Burgess MR, Hwang E, Firestone AJ, Huang T, Xu J, Zuber J, et al. Preclinical Efficacy of MEK Inhibition in Nras-Mutant AML. *Blood* (2014) 124(26):3947–55. doi: 10.1182/blood-2014-05-574582
56. Khanna V, Pierce ST, Dao KH, Tognon CE, Hunt DE, Junio B, et al. Durable Disease Control With MEK Inhibition in a Patient With NRAS-Mutated Atypical Chronic Myeloid Leukemia. *Cureus* (2015) 7(12):e414. doi: 10.7759/cureus.414
57. Costello R, Lafage M, Toiron Y, Brunel V, Sainty D, Arnoulet C, et al. Philadelphia Chromosome-Negative Chronic Myeloid Leukaemia: A Report of 14 New Cases. *Br J Haematol* (1995) 90(2):346–52. doi: 10.1111/j.1365-2141.1995.tb05157.x
58. Kurzrock R, Bueso-Ramos CE, Kantarjian H, Freireich E, Tucker SL, Siciliano M, et al. BCR Rearrangement-Negative Chronic Myelogenous Leukemia Revisited. *J Clin Oncol* (2001) 19(11):2915–26. doi: 10.1200/JCO.2001.19.11.2915
59. Jabbour E, Kantarjian H, Cortes J, Thomas D, Garcia-Manero G, Ferrajoli A, et al. PEG-IFN-Alpha-2b Therapy in BCR-ABL-Negative Myeloproliferative Disorders: Final Result of a Phase 2 Study. *Cancer* (2007) 110(9):2012–8. doi: 10.1002/cncr.23018
60. Martiat P, Michaux JL, Rodhain J. Philadelphia-Negative (Ph-) Chronic Myeloid Leukemia (CML): Comparison With Ph+ CML and Chronic Myelomonocytic Leukemia. The Groupe Français De Cytogénétique Hématologique. . *Blood* (1991) 78(1):205–11.

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Oncology

BRIEF RESEARCH REPORT
published: 25 November 2021
doi: 10.3389/fonc.2021.715217

An Abnormal Host/Microbiomes Signature of Plasma-Derived Extracellular Vesicles Is Associated to Polycythemia Vera

Monica Barone[1,2†], Martina Barone[3†], Francesca Ricci[4], Giuseppe Auteri[3], Giulia Corradi[3], Francesco Fabbri[5], Valentina Papa[6], Erika Bandini[5], Giovanna Cenacchi[6], Pier Luigi Tazzari[4], Nicola Vianelli[7], Silvia Turroni[2], Michele Cavo[3], Francesca Palandri[7], Marco Candela[2‡] and Lucia Catani[3‡]*

[1] Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy, [2] Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy, [3] Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Azienda Ospedaliero-Universitaria di Bologna, Istituto di Ematologia "Seràgnoli", Dipartimento di Medicina Specialistica, Diagnostica e Sperimentale, Università di Bologna, Bologna, Italy, [4] Servizio di Immunoematologia e Trasfusionale, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, [5] Biosciences Laboratory, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy, [6] Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy, [7] Istituto di Ematologia "Seràgnoli", Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

Polycythemia Vera (PV) is a myeloproliferative neoplasm with increased risk of thrombosis and progression to myelofibrosis. Chronic inflammation is commonly observed in myeloproliferative neoplasms including PV. The inflammatory network includes the extracellular vesicles (EVs), which play a role in cell-cell communication. Recent evidence points to circulating microbial components/microbes as potential players in hemopoiesis regulation. To address the role of EVs in PV, here we investigated phenotype and microbial DNA cargo of circulating EVs through multidimensional analysis. Peripheral blood and feces were collected from PV patients (n=38) and healthy donors (n=30). Circulating megakaryocyte (MK)- and platelet (PLT)-derived EVs were analyzed by flow cytometry. After microbial DNA extraction from feces and isolated EVs, the 16S rDNA V3-V4 region was sequenced. We found that the proportion of circulating MK-derived EVs was significantly decreased in PV patients as compared with the healthy donors. By contrast, the proportion of the PLT-derived EVs was increased. Interestingly, PV was also associated with a microbial DNA signature of the isolated EVs with higher diversity and distinct microbial composition than the healthy counterparts. Of note, increased proportion of isolated lipopolysaccharide-associated EVs has been demonstrated in PV patients. Conversely, the gut microbiome profile failed to identify a distinct layout between PV patients and healthy donors. In conclusion, PV is associated with circulating EVs harbouring abnormal phenotype and dysbiosis signature with a potential role in the (inflammatory) pathogenesis of the disease.

Keywords: polycythemia vera, cancer, extracellular vesicles, microbial DNA cargo, gut microbiota

OPEN ACCESS

Edited by:
Alessandro Isidori, AORMN Hospital, Italy

Reviewed by:
Douglas Tremblay, Icahn School of Medicine at Mount Sinai, United States
Abhik Saha, Presidency University, India

***Correspondence:**
Francesca Palandri
francesca.palandri@unibo.it

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

‡These authors have contributed equally to this work and share last authorship

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

Received: *26 May 2021*
Accepted: *08 November 2021*
Published: *25 November 2021*

Citation:
Barone M, Barone M, Ricci F, Auteri G, Corradi G, Fabbri F, Papa V, Bandini E, Cenacchi G, Tazzari PL, Vianelli N, Turroni S, Cavo M, Palandri F, Candela M and Catani L (2021) An Abnormal Host/Microbiomes Signature of Plasma-Derived Extracellular Vesicles Is Associated to Polycythemia Vera. Front. Oncol. 11:715217. doi: 10.3389/fonc.2021.715217

INTRODUCTION

Polycythemia Vera (PV) is a chronic myeloproliferative neoplasm (MPN) characterized by clonal expansion of the erythrocyte mass. There is often concurrent stimulation of myeloid and megakaryocytic lineages leading to increased white blood cell and platelet production, respectively. Molecular pathogenesis relies on constitutive activation of the JAK-STAT pathway that is responsible for abnormal myeloproliferation and increased production of circulating inflammatory cytokines (1, 2). Clinical phenotype includes increased risk of thrombosis and progression toward myelofibrosis and/or leukemia. PV management is primarily focused on minimizing the thrombotic risk, representing the main cause of morbidity and mortality (3, 4). All MPNs are associated with an important inflammatory response as demonstrated by the presence of high plasma levels of inflammatory cytokines as well as constitutional symptoms alleviated by anti-inflammatory therapies. These inflammatory cytokines are synthetized by the mutated and non-mutated hematopoietic cells as well as by non-hematopoietic cells such as mesenchymal stromal cells (5–8). Inflammation is an important thrombotic risk factor in PV (3, 4, 9).

Over the past few years, extracellular vesicles (EVs) have emerged as key modulators of immunity and inflammation (10). EVs from both immune and nonimmune cells, such as mesenchymal stem cells and endothelial cells, contribute to antigen-specific and nonspecific immune regulation. They also likely play a role in modulating inflammatory and autoimmune diseases (11). EVs are membrane-surrounded particles that are released by a broad variety of cells, either eukaryotic or prokaryotic, with effects on cell signaling. EV cargos are enriched in nucleic acids, proteins, and lipids and these bioactive molecules can be delivered to recipient cells to influence their biological properties and modify surrounding microenvironment or distant targets. Most circulating EVs are of megakaryocyte (MK-EVs) and platelet (PLT-EVs) origin. Recently, the number as well as the cargo, including proteins, microRNA and long non-coding RNA, have been reported to be upregulated in the EVs of patients with blood cancers, suggesting that circulating EVs might be a diagnostic marker for these disorders (12, 13).

Interestingly, evidence indicative of the presence of a microbial component in the blood of healthy human individuals is steadily accumulating (14, 15). Furthermore, EVs may contain a range of immunostimulatory microbe-associated molecules, including Gram-negative bacteria derived-lipopolysaccharide (LPS), whose circulating levels have been reported to be increased in patients with intestinal barrier dysfunction (16, 17). Of note, it has been hypothesized that peculiar cargo of microbiomes components in EVs may control for their overall inflammatory potential.

Based on this, here we hypothesized that the combined evaluation of circulating EVs and the carried microbiomes components could provide a signature for PV patients and possibly contribute to add further knowledge of the pathogenesis of the disease.

METHODS

The "EV-PV" study is a monocenter clinical-biological study promoted by the Institute of Hematology "L. and A. Seràgnoli", S. Orsola-Malpighi Bologna University Hospital, and performed in collaboration with the Department of Pharmacy and Biotechnology, University of Bologna. After approval by the local Ethics Committee, the EV-PV study was conducted according to the Helsinki declaration. Informed consent was obtained from all subjects.

Collection of Peripheral Blood and Platelet Poor Plasma Preparation

Briefly, EDTA-anticoagulated peripheral blood and fecal samples were collected from PV patients, regardless of the time of diagnosis, and from age- and sex-matched healthy donors (HD). PV was diagnosed according to the 2016 WHO classification (18). After discarding the first 2 ml of blood, Platelet Poor Plasma (PPP) was obtained (within 2 hours from blood collection) after two consecutive centrifugations at 2500 × g for 15 min at room temperature. PPP was then aliquoted and stored at -80°C until testing.

Identification and Characterization of Circulating EVs by Flow Cytometry

Circulating MK (CD61+/CD62P-)- and PLT (CD61+/CD62P+) -EVs were analyzed by flow cytometry (Navios, Beckman Coulter, Milan, Italy) in PPP, after thawing at 37°C. PPP (100 µl) was incubated at 4°C for 15 min with antibodies, then diluted 1:3 and acquired immediately (List of monoclonal antibodies according to EVs subtype is shown in **Table S2**). To detect EVs, the instrument was calibrated with MegaMix Beads (Stagò, Marseille, France). Fluorescence gated polystyrene beads of different sizes were used to determine the gates identifying big (500-900 nm), small (200-300 nm) and nano (100-160 nm) EVs, as previously described (19). The Violet Side Scatter laser (VSSC) was used as a trigger signal to discriminate noise. Our analysis was focused on big EVs that were identified by using the size and ability to bind specific monoclonal antibodies. Matched isotype controls were used to select the cut-off. By using the defined gate for big EVs, all events positive for marker staining were recorded and expressed as a percentage of positive EVs.

Circulating EV Isolation, Enumeration, Morphology, and Phenotype Characterization

EVs were isolated from thawed PPP (2 ml at 37°C) by ultracentrifugation at 100,000 × g for 2 hours at 4°C with Optima L-90 K ultracentrifuge (Beckman Coulter) equipped with Type 50.2 Ti rotor, as previously described (20). After centrifugation, pelleted EVs were resuspended and washed with twice filtered (filter pore size, 0.22 µm) Dulbecco's PBS (DPBS; Sigma Aldrich). Finally, EVs were resuspended in saline buffer solution with 1% DMSO and stored at − 80°C until use. EV enumeration and purity were assessed by using the NanoSight technology (NanoSight NS300-Malvern Panalytical Ltd., Royston, United Kingdom) and nanoparticle tracking analysis

software (NTA Proprietary Software-Malvern Panalytical Ltd.). Transmission electron microscopy analysis of the isolated EVs was also performed. Samples were processed for negative staining by adsorption, washing and staining steps. The suspension (10 µl) was placed on a carbon-coated grid and after 30 sec of adsorption, it was slowly and gently removed by bibulous pieces of paper without touching the grid directly. Then, a series of droplets of distilled water were used to remove interfering salts. Ten microliters of water 1% uranyl acetate solution, pH 4.4 were used for negative staining, 5-10 sec. Stain droplets were gently removed as described above. After drying, grids were observed by a Philips CM 100 (TSS microscopy, Hillsboro, OR, USA), recorded by digital camera (Olympus, Milan, Italy), and digitally measured by iTem software. Isolated EVs were characterized by cytofluorimetric analysis using monoclonal antibodies against tetraspanins (CD9, CD63, CD81), CD61, CD62P, and lipopolysaccharide (LPS) (List of monoclonal antibodies according to EVs subtype is shown in **Table S2**). Conjugated mouse isotypic IgG was used as a control. Briefly, EVs (2×108) were stained at 4°C for 15 min, then diluted 1:3 and acquired immediately, as above described. Tetraspanins analysis was focused on total isolated EVs while the other markers were analyzed on big EVs.

Microbial DNA Extraction From Feces and Isolated EVs

Microbial DNA was extracted from feces (250 mg) and isolated EVs (2ml of PPP) using the repeated bead-beating plus columns method, as previously described with a few modifications (21). In brief, all samples were suspended in 1 ml of lysis buffer (500 mM NaCl, 50 mM Tris-HCl pH 8, 50 mM EDTA, and 4% (w/v) SDS), added with four 3-mm glass beads and 0.5 g of 0.1-mm zirconia beads (BioSpec Products, Bartlesville, OK) and bead-beaten in a FastPrep instrument (MP Biomedicals, Irvine, CA) at 5.5 movements/sec for 1 min. Only one homogenization step was performed for EVs samples, while for stool samples it was repeated three times, incubating the samples on ice for 5 min between treatments. After incubation at 95°C for 15 min, all samples were centrifuged at 13,000 rpm for 5 min. Nucleic acids were precipitated by adding 260 µl of 10 M ammonium acetate and one volume of isopropanol. The pellets were then washed with 70% ethanol and suspended in 100 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). RNA was removed by treatment with 2 µl of 10 mg/ml DNase-free RNase at 37°C for 15 min. Protein removal and column-based DNA purification were performed by using the DNeasy Blood and Tissue kit (QIAGEN, Hilden, Germany) and following the manufacturer's instructions. Template-free controls (i.e. RPMI medium and extraction kit reagents) were processed as well, in the same way as the samples. DNA was quantified with the NanoDrop ND-10000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

16S rRNA Gene Amplification and Sequencing

The V3-V4 hypervariable region of the 16S rRNA gene was amplified from DNA extracted from isolated EVs, stool samples and no-template controls, by using the 341F and 785R primers with Illumina overhang adapter sequences, as previously described by Klindworth et al. (22). PCR reactions were performed by using KAPA HiFi HotStart ReadyMix (Roche, Mannheim, Germany), in a Thermal Cycler T (Biometra, Göttingen, Germany) with the following gradient: 3 min at 95°C for the initial denaturation, 25 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 30 sec, and a final elongation step at 72°C for 5 min. PCR products of around 460 bp were purified using a magnetic bead-based system (Agencourt AMPure XP, Beckman Coulter), and a limited-cycle PCR using Nextera Technology was performed to obtain the indexed library, followed by a second clean-up step as described above. Indexed libraries were pooled at equimolar concentration of 4 nM, denatured with NaOH 0.2 N, and diluted to 5 pM before loading onto the Illumina MiSeq flow cell. The 2 × 250 bp paired-end sequencing protocol was performed according to the manufacturer's instructions (Illumina, San Diego, CA). Sequencing reads were deposited in the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA) under the following project number: PNRxxx.

Bioinformatics and Biostatistics

Statistical analysis was performed at the Department of Pharmacy and Biotechnology and at the biostatistics laboratory of the MPN Unit at the Institute of Hematology "L. and A. Seràgnoli", IRCCS Azienda Ospedaliero-Universitaria di Bologna.

Raw sequences were processed using a pipeline that combines PANDAseq (23) and QIIME 2 (24). After length (min/max = 350/500 bp) and quality filtering (default parameters), cleaned reads were binned into amplicon sequence variants (ASVs) using DADA2 (25, 26). Taxonomy was assigned through the VSEARCH algorithm (27), using the Greengenes database as a reference (release May 2013). All singleton ASVs were discarded. Moreover, for EVs, ASVs were considered putative contaminants and therefore removed when their mean relative abundance did not exceed 20% of that in controls, similarly to what was previously applied by Dash and colleagues (28). Alpha diversity was evaluated using two different metrics: Shannon and inverse Simpson (1/D). The Jaccard similarity index was used to construct Principal Coordinates Analysis (PCoA) plots.

Statistical analyses were performed using R 3.6.1, using R Studio 1.2.1335 and the packages vegan (29), made4 (30) and stats (31). The significance of data separation in the PCoA was tested by means of a permutation test with pseudo-F ratio (function adonis of the vegan package). Wilcoxon rank-sum test was used to assess significant differences between groups (for intra- and inter-individual diversity as well as taxon relative abundance), while Kruskal-Wallis test was used for multiple comparisons. P values were corrected for multiple testing using the Benjamini-Hochberg method, and a false discovery rate (FDR) ≤0.05 was considered statistically significant.

As for the phenotype of EVs, statistical analysis was performed with GraphPad (GraphPad Software Inc., La Jolla, CA). The differences between the groups were analyzed with Mann-Whitney, Chi-square or Spearman's correlation tests, as

appropriate. P values were considered significant when ≤0.05 (2-tailed).

RESULTS

Study Cohort

Thirty-eight PV patients (35 JAK2V617F and 3 JAK2Exon12-mutated) were included into the study after a median time from PV diagnosis of 2.6 years (range, 0.1-13.6). At the time of enrollment, all patients had received phlebotomies and antiplatelet therapy (mainly low-dose aspirin); hydroxyurea was ongoing in 81.6% of patients. No patients had received therapy with anagrelide, busulfan, interferons or ruxolitinib. Peripheral blood/fecal samples were also collected in 30 HD. Compared to PV patients, HD presented significantly lower platelet/leukocytes/hematocrit levels and were less frequently treated with low-dose aspirin (**Table S1**).

Characterization of PLT-EVs, MK-EVs, and LPS-EVs

The profile of PLT-EVs and MK-EVs was assessed by flow cytometry in the plasma of PV patients and HD (**Figure S1A**). Plasma MK-EVs were significantly decreased in PV patients ($p<0.001$; **Figure 1A**); by contrast, PLT-EVs were significantly increased ($p<0.001$; **Figure 1B**). Both in PV and HD, the proportion of plasma MK-EVs and PLT-EVs was not influenced by sex or age >60 years. No differences were also observed between PV patients with/without previous thrombosis or HU treated/untreated.

We also analyzed the phenotype, size, and morphology of the isolated EVs. Interestingly, compared to HD, PV patients showed increased proportion of the isolated LPS-associated EVs ($p<0.05$; **Figure 1C** and **Figure S1B**). The tetraspanins (CD81, CD63 and CD9) were highly expressed by the majority of the isolated EVs from both patients and HD (**Figures S2A**–**D**). Consistent with the circulating EVs data, the proportion of isolated MK-EVs and PLT-EVs of PV patients was decreased and increased, respectively, compared to those of HD ($p<0.01$; **Figures S2E, F**). At variance with the concentration (**Figure S3A**), the isolated EVs of PV patients were significantly smaller compared to those of the HD counterpart ($p<0.001$; **Figures S3B, C**). Transmission electron microscopy analysis showed round-shaped EVs in both PV patients and HD (**Figure S3D**).

Characterization of EV-Associated Microbial DNA

Isolated EVs were subjected to 16S rRNA gene-based NGS to evaluate whether microbial DNA can be detected and whether its

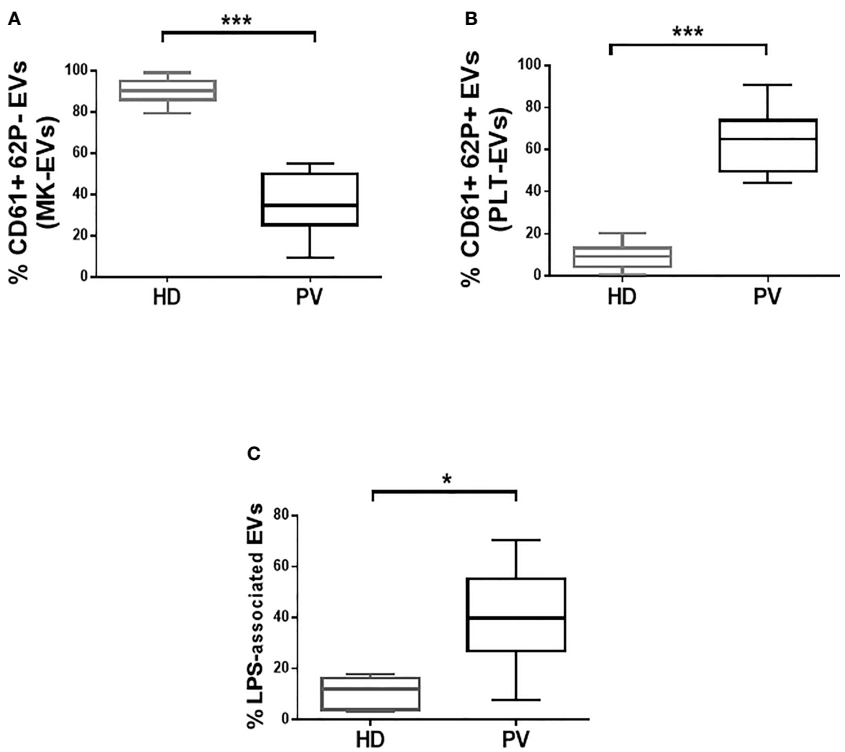


FIGURE 1 | Proportion of plasma MK- and PLT-EVs, and of isolated LPS-associated EVs in patients with PV and HD. **(A, B)** MK-EVs and PLT-EVs profile of PV patients (n = 38) and HD (n = 30). Data are expressed as percentage of MK-EVs and PLT-EVs and presented as min to max with median (Mann-Whitney test; *p < 0.05; ***p < 0.001). **(C)** Proportion of isolated LPS-associated EVs of PV patients (n = 28) and HD (n = 20). Data are expressed as percentage and presented as min to max with median (Mann-Whitney test; *p < 0.05).

pattern may represent a PV-specific signature. A total of 1,799,864 sequence reads, with an average of 22,220 (± 5,974, SD) reads per sample, were obtained and analyzed. According to the inverse Simpson and Shannon indices, the EVs-associated microbial diversity was higher in PV patients compared to HD ($p<0.001$, **Figure 2A**). Principal Coordinates Analysis (PCoA) based on Jaccard similarity between the genus-level profiles showed separation between PV patients and HD ($p=0.001$; **Figure 2B**). Interestingly, taxonomic comparisons revealed numerous differences between the EVs-associated microbial DNA of PV patients and HD. PV patients were depleted in Proteobacteria-related DNA while enriched in that of Actinobacteria and Cyanobacteria, compared to HD ($p<0.001$; $p<0.01$; **Figure 2C**). At the family level, the most discriminating taxa (with mean relative abundance ≥1% in at least one of the study groups) were *Rhodobacteraceae* ($p<0.01$), whose proportions were greater in PV patients, and *Caulobacteraceae* ($p<0.05$) and *Bradyrhizobiaceae* ($p<0.001$), which were underrepresented in PV patients (**Figure 2D**). Genus-level data showed depletion of *Bradyrhizobium* in PV patients ($p<0.001$; **Figure 2E**).

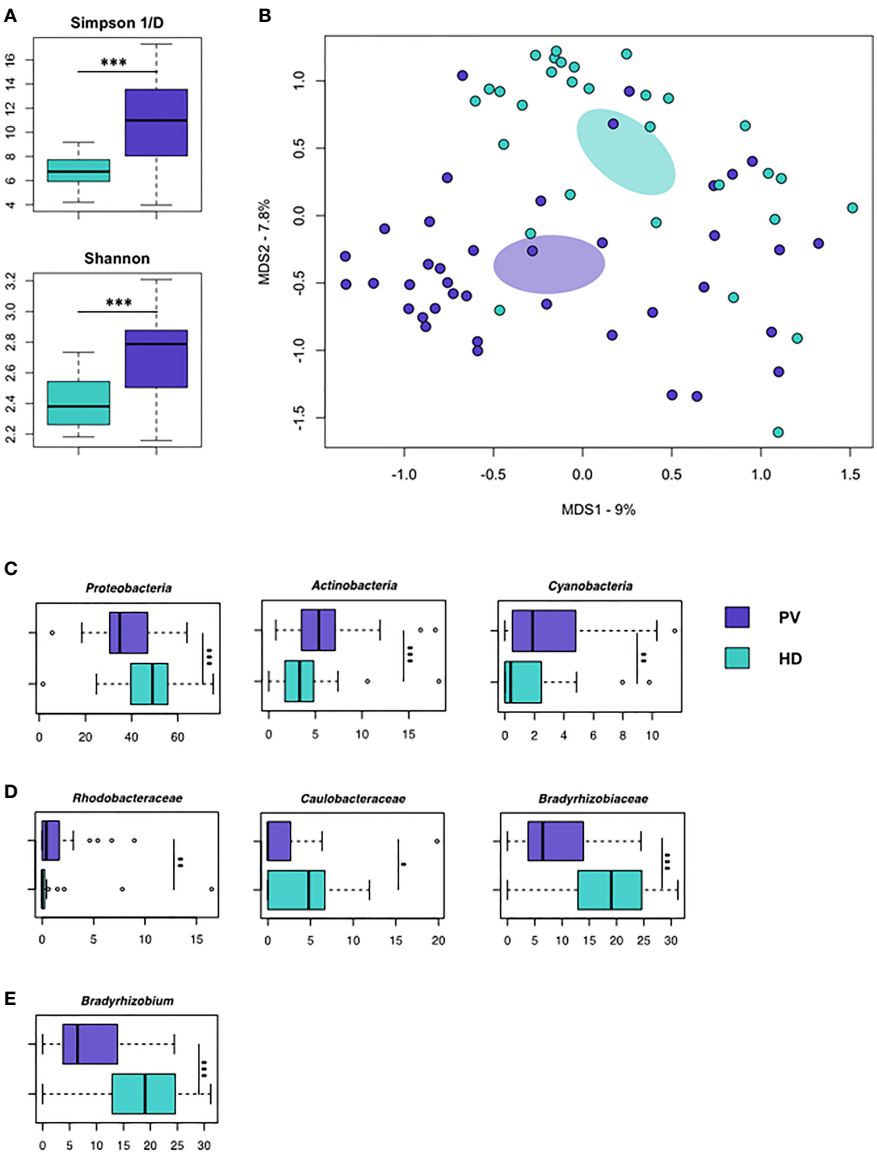


FIGURE 2 | Microbial DNA cargo of isolated EVs in PV patients and HD. **(A)** Alpha diversity estimated according to inverse Simpson (top) and Shannon (bottom) indices. PV patients show significantly higher biodiversity than HD ($p < 0.001$, Wilcoxon rank-sum test). **(B)** Principal Coordinates Analysis (PCoA) based on Jaccard similarity between the genus-level profiles of EVs from PV patients and HD. Significant segregation between groups was found ($p = 0.001$, permutation test with pseudo-*F* ratio). **(C–E)** Boxplots showing the relative abundance distribution of phyla, families and genera that were significantly differentially represented between HD and PV patients (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; Wilcoxon rank-sum test). Only bacterial taxa with mean relative abundance ≥1% in at least one of the comparison groups are shown.

Characterization of Fecal Microbiome

Fecal samples from PV patients and HD were also profiled by 16S rRNA gene-based NGS. A total of 2,296,462 sequence reads, with an average of 33,772 (± 11,228, SD) reads per sample, were obtained and analyzed. The comparison of the microbiome profiles at the phylum, family and genus level did not reveal any significant difference between PV patients and HD, demonstrating an overall eubiotic intestinal microbial ecosystem in PV (**Figures S4A, B**).

DISCUSSION

Prior reports showed that serum microparticles originating from platelets, erythrocytes, granulocytes, and endothelial cells of PV patients are elevated compared with healthy controls (32). In addition, it has been described that patients with MPNs that had the JAK2V617F mutation had significantly higher plasma concentrations of tissue factor–positive microparticles and erythrocyte microparticles (33). Here we aimed to identify a signature of PV by analyzing the phenotype and microbial DNA cargo of circulating EVs.

First, we observed that PV is associated with an abnormal profile of circulating EVs of megakaryocyte and platelet origin. Since EV production by megakaryocytes is based on a constitutive mechanism, but only activated platelets can produce CD62P+ EVs (34), our findings further confirm the aberrant megakaryopoiesis and platelet activation, which have been previously described in MPN, including PV patients (1, 35–37). However, this pattern of circulating MK-/PLT-EVs in PV mirrors the profile previously described for MF and ET patients (19), suggesting that abnormal MK-/PLT-EV frequency is common in MPNs, irrespective of disease and mutation status (35, 36).

Secondly, despite our analyses could not discriminate whether EVs were of bacterial or human origin, we found that PV patients had an increased proportion of LPS-associated EVs as compared to HD. These findings suggest not only an increased intestinal permeability in PV but also that, as a cargo of microbial factors such as LPS, EVs could be instrumental for blood microbial components/microbes to mediate their impact on the host inflammatory state and the subsequent activation of the innate immune responses (38). Therefore, we can speculate that in PV the increased proportion of LPS-associated EVs might represent a stimulus for the immune system by boosting key cells such as monocytes/macrophages, dendritic and T cells, thereby stimulating the release of inflammatory/fibrogenic cytokines and contributing to the maintenance of chronic inflammation. However, further investigations are necessary to explore this suggestion.

Finally, PV was associated with a microbial DNA signature of isolated EVs with higher diversity and distinct microbial composition than the healthy counterparts, including greater amounts of Rhodobacteraceae DNA and reduced proportions of DNA related to Caulobacteraceae and Bradyrhizobium. These microorganisms are well known components of soil and aqueous microbiomes and possibly represent a proxy of the personal microbiome exposome, defined as the peculiar pattern of environmental microorganisms we are exposed to.

Of note, it should be underlined that, though in our cohort most PV patients were under treatment at the time of the study and few of them were out of treatment, we were able to demonstrate an abnormal microbial DNA layout when circulating EVs were analyzed. At variance, no significant differences were observed between patients and controls when the gut microbiome was investigated, suggesting that circulating EVs in PV, irrespective of cytotoxic therapy, are biomarker of the disease.

In conclusion, PV is associated with circulating EVs harboring abnormal phenotype and dysbiosis signature with a potential role in the (inflammatory) pathogenesis of the disease. Even though further studies, possibly integrating other-omics approaches on a larger cohort, are warranted to validate our findings and delve into taxonomic, these data might be also of interest in the development of novel personalized therapeutic approaches targeting the microenvironment of PV.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA) repository, accession number PRJNA737425.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Comitato Etico Indipendente di Area Vasta Emilia Centro della Regione Emilia Romagna-IRCCS Azienda Ospedaliero Universitaria di Bologna. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FP, MaC and LC designed the study. FP, GA and NV provided clinical information and blood/fecal samples from PV patients. MaB, FR, GCo phenotypically characterized and isolated EVs. FF and EB analysed EV number/size. VP and GCe performed electron microscopy analysis. MoB performed EV/feces NGS study. MaB, MoB, ST, FP and LC wrote the manuscript. MaC, PT and MiC reviewed the manuscript. All authors contributed to interpretation of the data, read and approved the final manuscript.

FUNDING

This work was funded by AIL Bologna.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.715217/full#supplementary-material

REFERENCES

1. Woods B, Chen W, Chiu S, Marinaccio C, Fu C, Gu L, et al. Activation of JAK/STAT Signaling in Megakaryocytes Sustains Myeloproliferation. *Clin Cancer Res* (2019) 25(19):5901–12. doi: 10.1158/1078-0432.CCR-18-4089
2. Wickenhauser C, Thiele J, Lorenzen J, Schmitz B, Frimpong S, Schramm K, et al. Polycythemia Vera Megakaryocytes But Not Megakaryocytes From Normal Controls and Patients With Smokers Polyglobuly Spontaneously Express IL-6 and IL-6R and Secrete IL-6. *Leukemia* (1999) 13(3):327–34. doi: 10.1038/sj.leu.2401314
3. Tefferi A, Barbui T. Polycythemia Vera and Essential Thrombocythemia: 2019 Update on Diagnosis, Risk-Stratification and Management. *Am J Hematol* (2019) 94(1):133–43. doi: 10.1002/ajh.25303
4. Cerquozzi S, Barraco D, Lasho T, Finke C, Hanson CA, Ketterling RP, et al. Risk Factors for Arterial Versus Venous Thrombosis in Polycythemia Vera: A Single Center Experience in 587 Patients. *Blood Cancer J* (2017) 7(12):662. doi: 10.1038/s41408-017-0035-6
5. Vainchenker W, Kralovics R. Genetic Basis and Molecular Pathophysiology of Classical Myeloproliferative Neoplasms. *Blood* (2017) 129(6):667–79. doi: 10.1182/blood-2016-10-695940
6. Lussana F, Rambaldi A. Inflammation and Myeloproliferative Neoplasms. *J Autoimmun* (2017) 85:58–63. doi: 10.1016/j.jaut.2017.06.010
7. Cacemiro MDC, Cominal JG, Tognon R, Nunes NS, Simões BP, Figueiredo-Pontes LL, et al. Philadelphia-Negative Myeloproliferative Neoplasms as Disorders Marked by Cytokine Modulation. *Hematol Transfus Cell Ther* (2018) 40(2):120–31. doi: 10.1016/j.htct.2017.12.003
8. Vaidya R, Gangat N, Jimma T, Finke CM, Lasho TL, Pardanani A, et al. Plasma Cytokines in Polycythemia Vera: Phenotypic Correlates, Prognostic Relevance, and Comparison With Myelofibrosis. *Am J Hematol* (2012) 87 (11):1003–5. doi: 10.1002/ajh.23295
9. Barbui T, Carobbio A, Finazzi G, Vannucchi AM, Barosi G, Antonioli E, et al. Inflammation and Thrombosis in Essential Thrombocythemia and Polycythemia Vera: Different Role of C-Reactive Protein and Pentraxin 3. *Haematologica* (2011) 96(2):315–8. doi: 10.3324/haematol.2010.031070
10. Shah R, Patel T, Freedman JE. Circulating Extracellular Vesicles in Human Disease. *N Engl J Med* (2018) 379(10):958–66. doi: 10.1056/NEJMra1704286
11. Robbins PD, Dorronsoro A, Booker CN. Regulation of Chronic Inflammatory and Immune Processes by Extracellular Vesicles. *J Clin Invest* (2016) 126 (4):1173–80. doi: 10.1172/JCI81131
12. Forte D, Barone M, Palandri F, Catani L. The "Vesicular Intelligence" Strategy of Blood Cancers. *Genes (Basel)* (2021) 12(3):416–45. doi: 10.3390/genes12030416
13. Trino S, Lamorte D, Caivano A, De Luca L, Sgambato A, Laurenzana I. Clinical Relevance of Extracellular Vesicles in Hematological Neoplasms: From Liquid Biopsy to Cell Biopsy. *Leukemia* (2021) 35(3):661–78. doi: 10.1038/s41375-020-01104-1
14. Castillo DJ, Rifkin RF, Cowan DA, Potgieter M. The Healthy Human Blood Microbiome: Fact or Fiction? *Front Cell Infect Microbiol* (2019) 9:148. doi: 10.3389/fcimb.2019.00148
15. Ricci V, Carcione D, Messina S, Colombo GI, D'Alessandra Y. Circulating 16s RNA in Biofluids: Extracellular Vesicles as Mirrors of Human Microbiome? *Int J Mol Sci* (2020) 21(23):8959–73. doi: 10.3390/ijms21238959
16. Schwechheimer C, Kuehn MJ. Outer-Membrane Vesicles From Gram-Negative Bacteria: Biogenesis and Functions. *Nat Rev Microbiol* (2015) 13 (10):605–19. doi: 10.1038/nrmicro3525
17. Tulkens J, Vergauwen G, Van Deun J, Geeurickx E, Dhondt B, Lippens L, et al. Increased Levels of Systemic LPS-Positive Bacterial Extracellular Vesicles in Patients With Intestinal Barrier Dysfunction. *Gut* (2020) 69(1):191–3. doi: 10.1136/gutjnl-2018-317726
18. Barbui T, Thiele J, Gisslinger H, Kvasnicka HM, Vannucchi AM, Guglielmelli P, et al. The 2016 WHO Classification and Diagnostic Criteria for Myeloproliferative Neoplasms: Document Summary and in-Depth Discussion. *Blood Cancer J* (2018) 8(2):15. doi: 10.1038/s41408-018-0054-y
19. Barone M, Ricci F, Sollazzo D, Ottaviani E, Romano M, Auteri G, et al. Circulating Megakaryocyte and Platelet Microvesicles Correlate With Response to Ruxolitinib and Distinct Disease Severity in Patients With Myelofibrosis. *Br J Haematol* (2019) 185(5):987–91. doi: 10.1111/bjh.15682
20. Forte D, Barone M, Morsiani C, Simonetti G, Fabbri F, Bruno S, et al. Distinct Profile of CD34 + Cells and Plasma-Derived Extracellular Vesicles From Triple-Negative Patients With Myelofibrosis Reveals Potential Markers of Aggressive Disease. *J Exp Clin Cancer Res* (2021) 40(1):49. doi: 10.1186/s13046-020-01776-8
21. Biagi E, Franceschi C, Rampelli S, Severgnini M, Ostan R, Turroni S, et al. Gut Microbiota and Extreme Longevity. *Curr Biol* (2016) 26(11):1480–5. doi: 10.1016/j.cub.2016.04.016
22. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of General 16S Ribosomal RNA Gene PCR Primers for Classical and Next-Generation Sequencing-Based Diversity Studies. *Nucleic Acids Res* (2013) 41(1):e1. doi: 10.1093/nar/gks808
23. Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD. PANDAseq: Paired-End Assembler for Illumina Sequences. *BMC Bioinformatics* (2012) 13:31. doi: 10.1186/1471-2105-13-31
24. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2. *Nat Biotechnol* (2019) 37(8):852–7. doi: 10.1038/s41587-019-0209-9
25. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-Resolution Sample Inference From Illumina Amplicon Data. *Nat Methods* (2016) 13(7):581–3. doi: 10.1038/nmeth.3869
26. Callahan BJ, McMurdie PJ, Holmes SP. Exact Sequence Variants Should Replace Operational Taxonomic Units in Marker-Gene Data Analysis. *ISME J* (2017) 11(12):2639–43. doi: 10.1038/ismej.2017.119
27. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: A Versatile Open Source Tool for Metagenomics. *PeerJ* (2016) 4:e2584. doi: 10.7717/peerj.2584
28. Dash NR, Khoder G, Nada AM, Al Bataineh MT. Exploring the Impact of Helicobacter Pylori on Gut Microbiome Composition. *PloS One* (2019) 14(6): e0218274. doi: 10.1371/journal.pone.0218274
29. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, et al. *Vegan: Community Ecology Package. R Package Version 2.5-6.* (2019).
30. Culhane AC, Thioulouse J, Perrière G, Higgins DG. MADE4: An R Package for Multivariate Analysis of Gene Expression Data. *Bioinformatics* (2005) 21 (11):2789–90. doi: 10.1093/bioinformatics/bti394
31. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing (2019).
32. Tan X, Shi J, Fu Y, Gao C, Yang X, Li J, et al. Role of Erythrocytes and Platelets in the Hypercoagulable Status in Polycythemia Vera Through Phosphatidylserine Exposure and Microparticle Generation. *Thromb Haemost* (2013) 109(6):1025–32. doi: 10.1160/TH12-11-0811
33. Zhang W, Qi J, Zhao S, Shen W, Dai L, Han W, et al. Clinical Significance of Circulating Microparticles in Ph – Myeloproliferative Neoplasms. *Oncol Lett* (2017) 14(2):2531–6. doi: 10.3892/ol.2017.6459
34. Flaumenhaft R, Dilks JR, Richardson J, Alden E, Patel-Hett SR, Battinelli E, et al. Megakaryocyte-Derived Microparticles: Direct Visualization and Distinction From Platelet-Derived Microparticles. *Blood* (2009) 113 (5):1112–21. doi: 10.1182/blood-2008-06-163832
35. Patrono C, Rocca B, De Stefano V. Platelet Activation and Inhibition in Polycythemia Vera and Essential Thrombocythemia. *Blood* (2013) 121 (10):1701–11. doi: 10.1182/blood-2012-10-429134
36. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. *Blood* (2016) 127(20):2391–405. doi: 10.1182/blood-2016-03-643544
37. Ciurea SO, Merchant D, Mahmud N, Ishii T, Zhao Y, Hu W, et al. Pivotal Contributions of Megakaryocytes to the Biology of Idiopathic Myelofibrosis. *Blood* (2007) 110(3):986–93. doi: 10.1182/blood-2006-12-064626
38. Kuipers ME, Hokke CH, Smits HH, Nolte-'t Hoen ENM. Pathogen-Derived Extracellular Vesicle-Associated Molecules That Affect the Host Immune System: An Overview. *Front Microbiol* (2018) 9:2182. doi: 10.3389/fmicb.2018.02182

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 publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Oncology

MINI REVIEW
published: 13 December 2021
doi: 10.3389/fonc.2021.801524

Approaching First-Line Treatment in Patients With Advanced CMML: Hypomethylating Agents or Cytotoxic Treatment?

Konstantinos Liapis * *and Ioannis Kotsianidis*

Department of Hematology, Democritus University of Thrace Medical School, Alexandroupolis, Greece

Chronic myelomonocytic leukemia (CMML) is a rare clonal haematological malignancy bearing characteristics of both myelodysplastic syndromes and myeloproliferative neoplasms. It primarily affects older people (median age at diagnosis ~72 years). There are many challenges encountered in its treatment. One striking issue is the lack of strong clinical evidence from large randomized clinical trials for treating this disease. Another issue is that patients with CMML have highly variable outcomes with current treatments. Additional challenges include a wider application of current knowledge, an improved understanding of pathogenesis, development of new therapies, and management of refractory cases/disease progression. It is clear that there is still progress to be made. Here, we review the available first-line treatment options for advanced CMML. Emphasis has been placed on choosing between hypomethylating agents and cytotoxic treatments, on the basis on disease-specific and patient-specific characteristics. A proper selection between these two treatments could lead to a better quality of care for patients with CMML.

Keywords: first-line treatment, hypomethylating agents, azacitidine (5-AzaC), decitabine (DAC), cytotoxic (or anti-neoplastic) chemotherapeutic agents, hydroxyurea (hu), chronic myelomonocytic leukemia (CMML), myelodysplastic/myeloproliferative neoplasms (MDS/MPN)

OPEN ACCESS

Edited by:
Argiris Symeonidis,
University of Patras, Greece

Reviewed by:
Roberto Latagliata,
Unitelma Sapienza University, Italy

***Correspondence:**
Konstantinos Liapis
kosliapis@hotmail.com

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

Received: *25 October 2021*
Accepted: *29 November 2021*
Published: *13 December 2021*

Citation:
Liapis K and Kotsianidis I (2021) Approaching First-Line Treatment in Patients With Advanced CMML: Hypomethylating Agents or Cytotoxic Treatment? Front. Oncol. 11:801524. doi: 10.3389/fonc.2021.801524

INTRODUCTION

Chronic myelomonocytic leukemia (CMML) is a mixed myelodysplastic/myeloproliferative disorder with symptoms that encompass anemia, thrombocytopenia, and splenomegaly (1). CMML has undergone several revisions in its classification reflecting the complexity of the disease. It is subdivided, on the basis of a white-cell count (WBC) of 13×10^9/L, into dysplastic (MD) and proliferative (MP) variants (2). Outcomes of patients with MP CMML are worse than that of patients with MD CMML. The outcome is also related to the percentage of blasts in the bone marrow (BM). This value is an important part of the diagnosis and is used for a second subdivision into CMML-1 (blasts <5% in blood and <10% in BM) and CMML-2 (blasts 5-19% in blood and/or 10-19% in BM) (2).

Once diagnosed, the prime consideration is determining whether the patient needs treatment, followed by deciding the appropriate treatment. Ideally, standard management recommendations should be supported by prospective data from randomized controlled trials (RCTs). In reality, however, patients with CMML are often excluded from clinical trials (3). Thus, a major issue is the lack of strong clinical evidence from RCTs for treating CMML (4). The management of CMML, therefore, has evolved without high-quality, data-driven evidence regarding the relative benefit of different treatments.

Another issue to consider is that the outcome of treatment can be highly variable between patients, leading to divergent outcomes in different individuals (1, 4). Such variability among patients is multifactorial. Several prognostic scoring systems have been developed in recent years and the biological factors underlying this variability have been investigated (5–9). For example, the CMML-specific Prognostic Scoring System (CPSS) uses four variables (FAB and WHO CMML subtypes; erythrocyte transfusion dependence; and cytogenetic findings) to classify patents into four risk groups (low; intermediate-1; intermediate-2; and high risk) (5). Without doubt, molecular biology has changed the way we manage patients with acute myeloid leukemia (AML) and other myeloid malignancies (10–12), but in the case of CMML, full implementation of clinical genomics still has a long way to go and the molecular discoveries have not yet been translated into changes in clinical practice. The clinician who consults patients with CMML is faced with the problem of therapeutic uncertainty and biologic variation.

WHEN TO INITIATE TREATMENT IN CMML

Not all patients need to be treated immediately. Asymptomatic patients without cytopenia or signs of myeloproliferation (e.g. palpable splenomegaly), as well as those without excess blasts (<2% blasts in peripheral-blood and <5% in BM), have a relatively stable and more indolent course and a lower probability of transformation to AML. According to the CMML treatment guidelines (published by the European Hematology Association and the European LeukemiaNet), these patients may be observed without treatment until evidence of disease progression or clinical symptoms develop (13). This is a desired approach to avoid treatment complications and the concomitant deterioration in the quality of life (QOL) in patients with asymptomatic, lower-risk CMML. However, this approach is also rooted in the principle that most treatment in CMML is largely directed towards symptom management.

Therapy should be started when CMML is symptomatic or progressive (13). More particularly, treatment is initiated for hemoglobin <10 g/dL, BM blasts >5%, platelets $<100\times10^9$/L, progressive leukocytosis ($>30\times10^9$/L), extramedullary involvement (skin lesions, pleural/pericardial effusions, lymphadenopathy), constitutional symptoms (weight loss, fever), and symptomatic splenomegaly or splenomegaly that is palpable ≥5 cm below the left costal margin (4). The key to improving the care of patients with higher risk CMML lies in providing effective therapeutic options that modify the disease course. Their management has two main objectives: to provide effective long-term disease control while maintaining patient's QOL, and to prevent clonal evolution and transition to AML.

USE OF HYPOMETHYLATING AGENTS IN CMML

The hypomethylating agents (HMAs) 5-azacitidine (AZA) and decitabine (DEC) induce hypomethylation by inhibiting DNA methyltransferase and are widely used in patients with myelodysplastic syndromes (MDS) and AML (14, 15). Although AZA has been approved in the USA for all patients with CMML since 2004 and DEC since 2006, the EU did not approve DEC and restricted the licence of AZA to patients with dysplastic CMML-2 in 2008 (16). Therefore, many patients with CMML in Europe do not receive treatment with HMAs and, when they do, it is often through off-label prescribing. Nonetheless, a growing body of evidence shows that HMAs may play an important role in the treatment of MP CMML (**Table 1**) (17–25). HMAs can effectively reduce leukocytosis, improve splenomegaly and extramedullary lesions. Subari and colleagues found that HMAs reduced the palpable spleen size to 50% of the baseline measurement in 45% of their patients (26). For such treatment, the overall response rate (ORR) is ~50% (30-60%) and complete response (CR) rate ~17% (10-20%). Most patients achieve a response after 3 cycles of treatment and median overall survival (OS) is ~29 months (12-37 months) (4, 13). Given that considerable time may be required for response, hydroxyurea (1g/day) may be used in patients with proliferative features during the first 3 cycles, until response is attained.

The recent publication of the results of a large multicenter trial showed that patients with higher risk disease i.e. MP CMML, blasts ≥10%, and higher risk CMML according to the CPSS, have significantly better outcomes with HMAs compared with hydroxyurea or chemotherapy, whereas patients with lower-risk disease i.e. MD CMML with <10% blasts and lower risk CPSS, do not benefit from HMA treatment (27). Although this study provides the only direct, real-life comparison of HMAs with other available treatments, its retrospective and multicenter nature raises concerns about the presence of treatment-selection bias that cannot be overcome with Cox's models. Also, it should be noted that the vast majority of the patients (>80%) were treated with AZA and less than 1% of patients underwent allogeneic hematopoietic-cell transplantation (HCT). Nevertheless, the study provides data that could help clinicians to address real-world questions about care options in CMML.

It is important to note that responses to HMAs are generally not durable, do not reduce mutant allele burdens, and prognosis after loss of response is dismal (median OS ~6 months) (28–30). Half the patients with primary or secondary HMA failure transform to AML (31). However, it is equally important to point out the major impact of CR on OS — that is, patients with CMML who achieve CR after HMA treatment have markedly enhanced remission duration and prolonged OS (32).

TABLE 1 | Phase II studies of hypomethylating agents in chronic myelomonocytic leukemia.

Study	Number of patients	Regimen	Response rate	Median survival (months)	Progression to AML	Reference
Costa et al.	38	Azacitidine 75 mg/m^2/day for 7 days or 100 mg/m^2/day for 5 days every 4 weeks	ORR: 39% CR: 11% PR: 3% HI: 25%	12	NR	(17)
Adès et al.	76	Azacitidine 75 mg/m^2 for 5-7 days every 28 days	ORR: 43% CR: 17% PR: 1% Marrow CR: 8% HI: 17%	29	31% after 1.2 years from azacitidine initiation	(18)
Wong et al.	11	Azacitidine 75 mg/m^2 for 7 days every 28 days	ORR: 55% CR: 9% Marrow CR: 27% PR: 9% HI: 9%	17	18%	(19)
Fianchi et al.	31	Azacitidine 50-75 mg/m^2 for 7 days in 22 patients, and 100 mg flat dose for 5-7 days in 9 patients	ORR: 54% CR: 45% PR: 3% HI: 6%	37	16% after 12.7 months	(20)
Drummond et al.	32	Azacitidine 75 mg/m^2 for 7 days, every 28 days	ORR: 17% CR: 7% Marrow CR: 7% PR: 0% HI:3%	16	33% after 13 months	(21)
Tantravahi et al.	11	Azacitidine 75 mg/m2 for 7 days, every 28 days	ORR: 45% CR: 27% Marrow CR: 18% SD: 36% PD: 9%	30	18% at 2 years	(22)
Wijermans et al.	31	Decitabine 15 mg/m^2 3 times per day on 3 consecutive days, with a total dose of 135 mg/m^2 per course, every 6 weeks	ORR: 35% CR: 10% PR: 16% HI: 19%	15	NR	(23)
Braun et al.	39	Decitabine 20 mg/m^2 for 5 days every 28 days	ORR: 38% CR: 10% PR: 20% HI: 8%	18	NR	(24)
Santini et al.	43	Decitabine 20 mg/m^2 for 5 days, every 28 days	ORR: 47.6% CR: 16% Marrow CR: 19% PR: 2.4% HI: 9.5%	17	57.5% after 51.5 months	(25)

ORR, overall response rate; CR, complete remission; PR, partial remission; HI, hematologic improvement; SD, stable disease; PD, progressive disease; NR, not reported.

WHICH PATIENTS ARE LIKELY TO RESPOND TO HYPOMETHYLATING AGENTS?

The ability to identify individuals who are unlikely to receive therapeutic benefit could facilitate a personalized approach to treatment selection and inform transplant strategies. Although clinical factors are inadequate for predicting responses to HMAs in individual patients, new molecular techniques have identified certain mutational profiles as potential biomarkers, but with conflicting results. In a retrospective series of 174 patients with CMML, patients with *TET2* mutations without *ASXL1* mutation (~25% of patients) had the highest ORR (66% versus 47% for all other genotypes) and CR rate (32% versus 11% for all other genotypes) to HMA treatment. Mutations in *ASXL1*, *RUNX1* and *CBL* were associated with low ORR and poor OS (33). By contrast, the study by Costa and co-workers indicated that *ASXL1* and *TET2* mutations did not predict response to AZA (17). Clearly, such questions can be answered only by means of dedicated, large-scale cohort studies. Several methodological barriers must be overcome to safely use mutational profiles as predictive biomarkers, as has been recently shown during the development of the new Molecular International Prognostic Scoring System (IPSS-M) for MDS (34). The identification of

predictors to HMA resistance is the topic of intense research, but it must be emphasized that, in the present context, no established biomarker exists for prediction of response to HMA treatment.

CYTOTOXIC CHEMOTHERAPY FOR CMML

Treatment should be tailored to the biology of the disease and CMML is known to be relatively resistant to cytotoxic drugs (13). Treatment regimens similar to those used to treat newly-diagnosed AML have been used in CMML. Intensive combinations of anthracycline–cytarabine, cytarabine–topotecan, or regimens including clofarabine have moderate efficacy in CMML (13, 35–37). Overall, the results of such treatments have been disappointing with a remission rate of 40%, short remission duration, and relapse rate of 90%. With the advent of HMAs, AML-type chemotherapy is used less frequently in CMML. Yet CR rates with the use of HMAs are lower (~17%) than rates achieved with induction chemotherapy (~40%). Despite major improvements in supportive care, there is a substantial risk of early death (up to 25%) and the potential for serious harm in older patients (>65 years) after induction chemotherapy, which means that the risks and benefits of treatment must be weighed carefully when formulating a treatment plan. All considered, chemotherapy remains an option for patients aged <65-70 years with minimal impairment of function who have advanced-stage or rapidly evolving disease with ≥10% BM blasts (i.e. CMML-2). The rationale behind the use of cytotoxic chemotherapy is to reduce BM blasts and aim for CR before HCT. Cytotoxic chemotherapy may also be considered for CMML with *NPM1* mutation. In particular, cases with a high *NPM1* mutational burden are associated with a higher probability of rapid transition to AML (at a median of 5 months) with myelomonocytic (M4) or monocytic (M5) differentiation and a poorer outcome, even when treated with chemotherapy (38, 39). *NPM1* mutations are uncommon in CMML occurring in <5% of cases and, if found, the alternative diagnosis of AML-M4/M5 with mutated *NPM1* should always be borne in mind (2). In contrast, the presence of *FLT3-ITD* (also occurring in <5% of cases) does not necessarily herald the onset of AML transformation (13).

ALLOGENEIC HEMATOPOIETIC-CELL TRANSPLANTATION

This approach remains the only curative treatment of higher-risk CMML. However, it can generally be offered only to a few patients with CMML—younger patients may be offered myeloablative HCT and older patients can be offered reduced-intensity HCT from an HLA-identical donor. The conventional upper age limit for HCT is around 70 years.

Several studies have retrospectively analyzed the results of allogeneic HCT in CMML. It emerges that, at the moment, outcomes of HCT in CMML are worse than in MDS: the response rates in CMML have ranged from 17% to 50%, and treatment-related mortality (TRM) rates have ranged from 12% to 52% (40–44). The largest study of HCT to date including 513 patients found a relapse rate of 32%, non-relapse mortality (NRM) 41%, disease-free survival 27%, and OS 33% at 4 years. Disease status was the main risk factor for TRM, and the achievement of CR pre-transplant was the only predictor of survival (45).

Since the achievement of CR prior to HCT is the most important prognostic factor for a favorable outcome (45), the members of an expert panel have recommended treatment before HCT, particularly in cases with BM blasts ≥10% and/or intermediate-2 or high risk CPSS (46). In view of the lack of evidence from RCTs, the best treatment for reducing tumor burden before HCT remains a controversial issue (13, 47, 48). AZA may allow for similar outcomes after HCT as compared with induction chemotherapy (49, 50). However, the CR rate with chemotherapy is higher than HMAs (excluding patients with *TET2+/ASXL1-* mutations), suggesting that chemotherapy may be suitable in selected, younger patients with high blast count. Transplantation should preferably be performed early after diagnosis and after establishing the best possible response.

CYTOREDUCTION WITH HYDROXYUREA

Single-agent, low-dose chemotherapy (e.g low-dose cytarabine, etoposide, 6-mercaptopurine) gives poor results in CMML (51–54). However, hydroxyurea remains an important component of CMML treatment despite its lack of disease-modifying activity. Since the classic study by Wattel and co-workers in 1996 (54), hydroxyurea is used for the control of leukocytosis, organomegaly, visceral involvement and hypercatabolic symptoms associated with advanced-stage, MP CMML. In this study, advanced-stage CMML was defined as presence of either extramedullary disease, excluding enlargement of the spleen and liver, or ≥2 of the following criteria: BM blasts >5%, neutrophils $>16\times10^9$/L, hemoglobin <10 g/dL, platelets $<100\times10^9$/L, and splenomegaly >5 cm below costal margin. The usual dose is 1 g/day (doubled in case of visceral involvement), escalated up to 3 g/day in the absence of response after 2 weeks of treatment, and adjusted to maintain WBC 4-10×10^9/L (54). Hydroxyurea yields only partial responses, has low efficacy on visceral involvement, may lead to worsening of anemia, and survival is generally poor (median OS ~24 months) (54). Prognostic factors for lower response rates and poor OS include unfavorable karyotype (monosomy 7 or complex) and low hemoglobin level (54). In a small, retrospective series of patients with MP CMML, previous hydroxyurea exposure appeared to have a modest negative impact on the response rate to HMA treatment (55), but this finding requires prospective confirmation.

DACOTA TRIAL: A SIMPLE, PHASE 3 RCT WITH COMPLEX QUESTIONS

The DACOTA trial, a multicenter prospective phase 3 RCT of DEC versus hydroxyurea, included 170 patients with advanced-stage, MP CMML between October 2014 and September 2019

(84 in the DEC group; 86 in the hydroxyurea group). The data presented at ASH showed that although DEC was associated with a higher ORR as compared with hydroxyurea (56% versus 30%), no significant differences between the two groups were noted with respect to OS and event-free survival (EFS) (56). Why, then, patients that have a higher response rate with DEC do not have a superior OS or EFS? One reason might be that one third of patients with hydroxyurea subsequently received HMAs after exiting the study because of disease progression. Another possibility is that the benefit of DEC may be confined to a subset of patients with MP CMML——e.g., patients without *ASXL1* mutations and/or patients with higher risk CPSS. Or, perhaps more likely, the better disease control achieved with DEC was offset by more frequent complications and treatment delays. If so, relying on supportive care with prophylactic antimicrobial agents could be a wise decision in these cases. To put the study in perspective, the main conclusion is that, although hydroxyurea remains a valid option, HMAs, given their higher response rate, have the potential to serve as a bridge to allogeneic HCT in patients with advanced-stage, MP CMML.

CONCLUSION

First-Line Treatment in Patients With CMML: Hypomethylating Agents or Cytotoxic Treatment?

Although our understanding of the pathophysiology of CMML has improved remarkably, its treatment remains an unmet clinical need. At present, HCT is the only treatment that can induce long-term remission in CMML. Such therapy, however, is not applicable for most patients, since the median age at diagnosis is 72 years (1). Many patients have comorbidities and impaired functional status, which can lead to poor post-transplantation outcomes. In addition, the substantial risks of death and complications associated with this procedure may not justify its use in patients at lower risk. We consider HCT a treatment option for younger and fitter patients who have a poor prognosis or severe symptoms. Improving the efficacy and safety of HCT would allow many more patients to be cured.

Regardless of whether HCT is scheduled, AZA is our preferred treatment option to reduce the leukemic burden and overcome transfusion dependence in patients who need treatment, including patients with MP CMML. Yet we may consider induction chemotherapy for younger patients with adequate organ function and performance status who have advanced-stage or rapidly evolving disease with ≥10% BM blasts. Although not approved by the EU, AZA has been widely used for MP CMML for the past 10-15 years. It appears that HMAs have a disease-modifying activity in CMML, but, as is the case of MDS and AML, their effect is temporary and combinations with novel agents targeting different pathways is urgently needed in order to enhance remission duration and prolong OS (57). Current efforts in clinical research focus on the discovery of HMA combination therapies that are intended to provide an improvement in efficacy over HMA monotherapy. An important new drug in combination with HMA is venetoclax, a highly-selective, potent, oral BCL-2 inhibitor. Ongoing clinical trials are evaluating the combination of AZA with venetoclax in higher risk CMML (NCT04160052, NCT03404193). However, a recent study showed that leukemic monocytic cells often develop resistance to venetoclax due to biological properties intrinsic to monocytic differentiation, as they can shift antiapoptotic proteins from BCL-2 to MCL-1, promoting cell survival (58). Additionally, *RAS* pathway mutations, found in ~11% at diagnosis (4) but ~35% at progression/relapse (59), may confer resistance to venetoclax (57, 60). The combination of HMAs with other new molecular-targeting agents and monoclonal antibodies is also under investigation in higher-risk CMML (57).

In appropriate circumstances, cytoreduction with hydroxyurea still has a role in the care of patients with CMML. The decision for such treatment should be based on the patient's age, symptoms, comorbidity, and disease status. In our experience, hydroxyurea should be the choice for older patients with advanced-stage, MP CMML without severe anemia, thrombocytopenia, excess marrow blasts, unfavorable cytogenetics, or higher-risk CPSS. The presence of *NPM1* mutation (~5% of patients) calls for reconsideration of the diagnosis of CMML, as AML-M4 can masquerade initially as CMML, and intensive chemotherapy could be used in younger, fitter patients or combination treatment with azacitidine plus venetoclax in older individuals (13, 60).

In approaching first-line treatment in patients with CMML, we might ask another, broader and more unsettling question: how to balance clinical practice between what is known, not known, and uncertain in our knowledge.

AUTHOR CONTRIBUTIONS

KL searched the literature and wrote the manuscript. IK over-reviewed the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Patnaik MM, Tefferi A. Chronic Myelomonocytic Leukemia: 2020 Update on Diagnosis, Risk Stratification and Management. *Am J Hematol* (2020) 95:97–115. doi: 10.1002/ajh.25684
2. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press (2017).
3. The Lancet Haematology. Forging a Path for Chronic Myelomonocytic Leukaemia. *Lancet Haematol* (2019) 6:e229. doi: 10.1016/S2352-3026(19)30069-9
4. Kwon J. Diagnosis and Treatment of Chronic Myelomonocytic Leukemia. *Blood Res* (2021) 56:S5–S16. doi: 10.5045/br.2021.2020321
5. Such E, Germing U, Malcovati L, Cervera J, Kuendgen A, Della Porta MG, et al. Development and Validation of a Prognostic Scoring System for Patients With Chronic Myelomonocytic Leukemia. *Blood* (2013) 121:3005–15. doi: 10.1182/blood-2012-08-452938

6. Itzykson R, Kosmider O, Renneville A, Gelsi-Boyer V, Meggendorfer M, Morabito M, et al. Prognostic Score Including Gene Mutations in Chronic Myelomonocytic Leukemia. *J Clin Oncol* (2013) 31:2428–36. doi: 10.1200/JCO.2012.47.3314
7. Patnaik MM, Padron E, LaBorde RR, Lasho TL, Finke CM, Hanson CA, et al. Mayo Prognostic Model for WHO-Defined Chronic Myelomonocytic Leukemia: ASXL1 and Spliceosome Component Mutations and Outcomes. *Leukemia* (2013) 27:1504–10. doi: 10.1038/leu.2013.88
8. Diamantopoulos PT, Kotsianidis I, Symeonidis A, Pappa V, Galanopoulos A, Gogos D, et al. Chronic Myelomonocytic Leukemia Treated With 5-Azacytidine - Results From the Hellenic 5-Azacytidine Registry: Proposal of a New Risk Stratification System. *Leuk Lymphoma* (2019) 60:1721–30. doi: 10.1080/10428194.2018.1540783
9. Elena C, Gallì A, Such E, Meggendorfer M, Germing U, Rizzo E, et al. Integrating Clinical Features and Genetic Lesions in the Risk Assessment of Patients With Chronic Myelomonocytic Leukemia. *Blood* (2016) 128:1408–17. doi: 10.1182/blood-2016-05-714030
10. 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
11. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and Management of AML in Adults: 2017 ELN Recommendations From an International Expert Panel. *Blood* (2017) 129:424–47. doi: 10.1182/blood-2016-08-733196
12. Grinfeld J, Nangalia J, Baxter EJ, Wedge DC, Angelopoulos N, Cantrill R, et al. Classification and Personalized Prognosis in Myeloproliferative Neoplasms. *N Engl J Med* (2018) 379:1416–30. doi: 10.1056/NEJMoa1716614
13. Itzykson R, Fenaux P, Bowen D, Cross NCP, Cortes J, De Witte T, et al. Diagnosis and Treatment of Chronic Myelomonocytic Leukemias in Adults: Recommendations From the European Hematology Association and the European LeukemiaNet. *Hemasphere* (2018) 2:e150. doi: 10.1097/HS9.0000000000000150
14. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, et al. Efficacy of Azacitidine Compared With That of Conventional Care Regimens in the Treatment of Higher-Risk Myelodysplastic Syndromes: A Randomised, Open-Label, Phase III Study. *Lancet Oncol* (2009) 10:223–32. doi: 10.1016/S1470-2045(09)70003-8
15. Dombret H, Seymour JF, Butrym A, Wierzbowska A, Selleslag D, Jang JH, et al. International Phase 3 Study of Azacitidine vs Conventional Care Regimens in Older Patients With Newly Diagnosed AML With >30% Blasts. *Blood* (2015) 126:291–9. doi: 10.1182/blood-2015-01-621664
16. *Vidaza (Azacitidine) [Package Insert]*. Uxbridge, UK: Celgene Ltd (2019).
17. Costa R, Abdulhaq H, Haq B, Shadduck RK, Latsko J, Zenati M, et al. Activity of Azacitidine in Chronic Myelomonocytic Leukemia. *Cancer* (2011) 117:2690–6. doi: 10.1002/cncr.25759
18. Adès L, Sekeres MA, Wolfromm A, Teichman ML, Tiu RV, Itzykson R, et al. Predictive Factors of Response and Survival Among Chronic Myelomonocytic Leukemia Patients Treated With Azacitidine. *Leuk Res* (2013) 37:609–13. doi: 10.1016/j.leukres.2013.01.004
19. Wong E, Seymour JF, Kenealy M, Westerman D, Herbert K, Dickinson M. Treatment of Chronic Myelomonocytic Leukemia With Azacitidine. *Leuk Lymphoma* (2013) 54:878–80. doi: 10.3109/10428194.2012.730615
20. Fianchi L, Criscuolo M, Breccia M, Maurillo L, Salvi F, Musto P, et al. High Rate of Remissions in Chronic Myelomonocytic Leukemia Treated With 5-Azacytidine: Results of an Italian Retrospective Study. *Leuk Lymphoma* (2013) 54:658–61. doi: 10.3109/10428194.2012.719617
21. Drummond MW, Pocock C, Boissinot M, Mills J, Brown J, Cauchy P, et al. A Multi-Centre Phase 2 Study of Azacitidine in Chronic Myelomonocytic Leukaemia. *Leukemia* (2014) 28:1570–2. doi: 10.1038/leu.2014.85
22. Tantravahi SK, Szankasi P, Khorashad JS, Dao KH, Kovacsovics T, Kelley TW, et al. A Phase II Study of the Efficacy, Safety, and Determinants of Response to 5-Azacitidine (Vidaza®) in Patients With Chronic Myelomonocytic Leukemia. *Leuk Lymphoma* (2016) 57:2441–4. doi: 10.3109/10428194.2016.1138295
23. Wijermans PW, Rüter B, Baer MR, Slack JL, Saba HI, Lübbert M. Efficacy of Decitabine in the Treatment of Patients With Chronic Myelomonocytic Leukemia (CMML). *Leuk Res* (2008) 32:587–91. doi: 10.1016/j.leukres.2007.08.004
24. Braun T, Itzykson R, Renneville A, de Renzis B, Dreyfus F, Laribi K, et al. Molecular Predictors of Response to Decitabine in Advanced Chronic Myelomonocytic Leukemia: A Phase 2 Trial. *Blood* (2011) 118:3824–31. doi: 10.1182/blood-2011-05-352039
25. Santini V, Allione B, Zini G, Gioia D, Lunghi M, Poloni A, et al. A Phase II, Multicentre Trial of Decitabine in Higher-Risk Chronic Myelomonocytic Leukemia. *Leukemia* (2018) 32:413–8. doi: 10.1038/leu.2017
26. Subari S, Patnaik M, Alfakara D, Zblewski D, Hook C, Hashmi S, et al. Hypomethylating Agents Are Effective in Shrinking Splenomegaly in Patients With Chronic Myelomonocytic Leukemia. *Leuk Lymphoma* (2016) 57:1714–5. doi: 10.3109/10428194.2015.1105371
27. Pleyer L, Leisch M, Kourakli A, Padron E, Maciejewski JP, Xicoy Cirici B, et al. Outcomes of Patients With Chronic Myelomonocytic Leukaemia Treated With non-Curative Therapies: A Retrospective Cohort Study. *Lancet Haematol* (2021) 8:e135–48. doi: 10.1016/S2352-3026(20)30374-4
28. Alfonso A, Montalban-Bravo G, Takahashi K, Jabbour EJ, Kadia T, Ravandi F, et al. Natural History of Chronic Myelomonocytic Leukemia Treated With Hypomethylating Agents. *Am J Hematol* (2017) 92:599–606. doi: 10.1002/ajh.24735
29. Merlevede J, Droin N, Qin T, Meldi K, Yoshida K, Morabito M, et al. Mutation Allele Burden Remains Unchanged in Chronic Myelomonocytic Leukaemia Responding to Hypomethylating Agents. *Nat Commun* (2016) 7:10767. doi: 10.1038/ncomms10767
30. Kotsianidis I, Papageorgiou SG, Pappa V, Galanopoulos AG, Viniou NA, Vassilakopoulos TP, et al. Azacytidine Failure Revisited: An Appraisal Based on Real-Life Data From the MDS Registry of the Hellenic Myelodysplastic Syndrome Study Group (HMDS). *Mediterr J Hematol Infect Dis* (2019) 11:e2019045. doi: 10.4084/MJHID.2019.045
31. Niscola P, Tendas A, Scaramucci L, Giovannini M, Piccioni D, de Fabritiis P. Acute Myeloid Leukemia Arising From Chronic Myelomonocytic Leukemia During Hypomethylating Therapy. *Blood Res* (2014) 49:65–6. doi: 10.5045/br.2014.49.1.65
32. Papoutselis M, Douvali E, Papadopoulos V, Spanoudakis E, Margaritis D, Tsatalas C, et al. Has Introduction of Azacytidine in Everyday Clinical Practice Improved Survival in Late-Stage Myelodysplastic Syndrome? A Single Center Experience. *Leuk Res* (2014) 38:161–5. doi: 10.1016/j.leukres.2013.10.015
33. Duchmann M, Yalniz FF, Sanna A, Sallman D, Coombs CC, Renneville A, et al. Prognostic Role of Gene Mutations in Chronic Myelomonocytic Leukemia Patients Treated With Hypomethylating Agents. *EBioMedicine* (2018) 31:174–81. doi: 10.1016/j.ebiom.2018.04.018
34. Bernard E, Tuechler H, Greenberg PL, Hasserjian RP, Arango Ossa J, Nannya Y, et al. The American Society of Hematology——63rd Annual Meeting and Exposition. Molecular International Prognosis Scoring System for Myelodysplastic Syndromes. *Blood* (2021).
35. Tricot G, Boogaerts MA. The Role of Aggressive Chemotherapy in the Treatment of the Myelodysplastic Syndromes. *Br J Haematol* (1986) 63:477–83. doi: 10.1111/j.1365-2141.1986.tb07524.x
36. Beran M, Estey E, O'Brien S, Cortes J, Koller CA, Giles FJ, et al. Topotecan and Cytarabine Is an Active Combination Regimen in Myelodysplastic Syndromes and Chronic Myelomonocytic Leukemia. *J Clin Oncol* (1999) 17:2819–30. doi: 10.1200/JCO.1999.17.9.2819
37. Ghanem H, Garcia-Manero G, Faderl S, Ravandi F, Cortes J, Katragadda L, et al. Outcomes of Patients With Myelodysplatic Syndrome and Chronic Myelomonocytic Leukemia Post Clofarabine Failure. *Ther Adv Hematol* (2014) 5:29–34. doi: 10.1177/2040620713519742
38. Peng J, Zuo Z, Fu B, Oki Y, Tang G, Goswami M, et al. Chronic Myelomonocytic Leukemia With Nucleophosmin (NPM1) Mutation. *Eur J Haematol* (2016) 96:65–71. doi: 10.1111/ejh.12549
39. Vallapureddy R, Lasho TL, Hoversten K, Finke CM, Ketterling R, Hanson C, et al. Nucleophosmin 1 (NPM1) Mutations in Chronic Myelomonocytic Leukemia and Their Prognostic Relevance. *Am J Hematol* (2017) 92:E614–8. doi: 10.1002/ajh.24861
40. Kröger N, Zabelina T, Guardiola P, Runde V, Sierra J, Van Biezen A, et al. Allogeneic Stem Cell Transplantation of Adult Chronic Myelomonocytic Leukaemia. A Report on Behalf of the Chronic Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). *Br J Haematol* (2002) 118:67–73. doi: 10.1046/j.1365-2141.2002.03552.x

41. Elliott MA, Tefferi A, Hogan WJ, Letendre L, Gastineau DA, Ansell SM, et al. Allogeneic Stem Cell Transplantation and Donor Lymphocyte Infusions for Chronic Myelomonocytic Leukemia. *Bone Marrow Transplant* (2006) 37:1003–8. doi: 10.1038/sj.bmt.1705369
42. Ocheni S, Kröger N, Zabelina T, Zander AR, Bacher U. Outcome of Allo-SCT for Chronic Myelomonocytic Leukemia. *Bone Marrow Transplant* (2009) 43:659–61. doi: 10.1038/bmt.2008.366
43. Krishnamurthy P, Lim ZY, Nagi W, Kenyon M, Mijovic A, Ireland R, et al. Allogeneic Haematopoietic SCT for Chronic Myelomonocytic Leukaemia: A Single-Centre Experience. *Bone Marrow Transpl* (2010) 45:1502–07. doi: 10.1038/bmt.2009.375
44. Park S, Labopin M, Yakoub-Agha I, Delaunay J, Dhedin N, Deconinck E, et al. Allogeneic Stem Cell Transplantation for Chronic Myelomonocytic Leukemia: A Report From the Societe Francaise De Greffe De Moelle Et De Therapie Cellulaire. *Eur J Haematol* (2013) 90:355–64. doi: 10.1111/ejh.12073
45. Symeonidis A, van Biezen A, de Wreede L, Piciocchi A, Finke J, Beelen D, et al. Achievement of Complete Remission Predicts Outcome of Allogeneic Haematopoietic Stem Cell Transplantation in Patients With Chronic Myelomonocytic Leukaemia. A Study of the Chronic Malignancies Working Party of the European Group for Blood and Marrow Transplantation. *Br J Haematol* (2015) 171:239–46. doi: 10.1111/bjh.13576
46. de Witte T, Bowen D, Robin M, Malcovati L, Niederwieser D, Yakoub-Agha I, et al. Allogeneic Hematopoietic Stem Cell Transplantation for MDS and CMML: Recommendations From an International Expert Panel. *Blood* (2017) 129:1753–62. doi: 10.1182/blood-2016-06-724500
47. Solary E, Itzykson R. How I Treat Chronic Myelomonocytic Leukemia. *Blood* (2017) 130:126–36. doi: 10.1182/blood-2017-04-736421
48. Robin M, Fenaux P. Hypomethylating Agents as Bridging Therapy Before Allogeneic Hematopoietic Stem Cell Transplantation in Patients With Chronic Myelomonocytic Leukemia? *Biol Blood Marrow Transplant* (2016) 22:1–2. doi: 10.1016/j.bbmt.2015.11.004
49. Potter VT, Iacobelli S, van Biezen A, Maertens J, Bourhis JH, Passweg JR, et al. Comparison of Intensive Chemotherapy and Hypomethylating Agents Before Allogeneic Stem Cell Transplantation for Advanced Myelodysplastic Syndromes: A Study of the Myelodysplastic Syndrome Subcommittee of the Chronic Malignancies Working Party of the European Society for Blood and Marrow Transplant Research. *Biol Blood Marrow Transpl* (2016) 22:1615–20. doi: 10.1016/j.bbmt.2016.05.026
50. Kongtim P, Popat U, Jimenez A, Gaballa S, El Fakih R, Rondon G, et al. Treatment With Hypomethylating Agents Before Allogeneic Stem Cell Transplant Improves Progression-Free Survival for Patients With Chronic Myelomonocytic Leukemia. *Biol Blood Marrow Transpl* (2016) 22:47–53. doi: 10.1016/j.bbmt.2015.08.031
51. Fenaux P, Jouet JP, Bauters F. Low-Dose Cytosine Arabinoside in Adult Chronic Myelomonocytic Leukemia. *J Clin Oncol* (1987) 5:1129–30.
52. Cheson BD, Jasperse DM, Simon R, Friedman M. A Critical Appraisal of Low-Dose Cytosine Arabinoside in Patients With Acute Non-Lymphocytic Leukemia and Myelodysplastic Syndromes. *J Clin Oncol* (1986) 4:1857–64. doi: 10.1200/JCO.1986.4.12.1857
53. Oscier D, Worsley A, Hamblin T, Mufti G. Treatment of CMML With Low Dose Etoposide. *Br J Haematol* (1989) 72:468–71. doi: 10.1111/j.1365-2141.1989.tb07735.x
54. Wattel E, Guerci A, Hecquet B, Economopoulos T, Copplestone A, Mahé B, et al. A Randomized Trial of Hydroxyurea Versus VP16 in Adult Chronic Myelomonocytic Leukemia. Groupe Francais Des Myelodysplasies and European CMML Group. *Blood* (1996) 88:2480–7. doi: 10.1182/blood.V88.7.2480.bloodjournal8872480
55. Pleyer L, Germing U, Sperr WR, Linkesch W, Burgstaller S, Stauder R, et al. Azacitidine in CMML: Matched-Pair Analyses of Daily-Life Patients Reveal Modest Effects on Clinical Course and Survival. *Leuk Res* (2014) 38:475–83. doi: 10.1016/j.leukres.2014.01.006
56. Itzykson R, Santini V, Chaffaut C, Adès L, Thepot S, Giagounidis A, et al. The American Society of Hematology——62nd Annual Meeting and Exposition. Decitabine Versus Hydroxyurea for Advanced Proliferative CMML: Results of the EMSCO Randomized Phase 3 DACOTA Trial. *Blood* (2020) 136:53–4. doi: 10.1182/blood-2020-138680
57. Renneville A, Patnaik MM, Chan O, Padron E, Solary E. Increasing Recognition and Emerging Therapies Argue for Dedicated Clinical Trials in Chronic Myelomonocytic Leukemia. *Leukemia* (2021) 35:2739–51. doi: 10.1038/s41375-021-01330-1
58. Pei S, Pollyea DA, Gustafson A, Stevens BM, Minhajuddin M, Fu R, et al. Monocytic Subclones Confer Resistance to Venetoclax-Based Therapy in Patients With Acute Myeloid Leukemia. *Cancer Discov* (2020) 10:536–51. doi: 10.1158/2159-8290.CD-19-0710
59. Carr RM, Vorobyev D, Lasho T, Marks DL, Tolosa EJ, Vedder A, et al. RAS Mutations Drive Proliferative Chronic Myelomonocytic Leukemia *via* a KMT2A-PLK1 Axis. *Nat Commun* (2021) 12:2901. doi: 10.1038/s41467-021-23186-w
60. Montalban-Bravo G, Hammond D, DiNardo CD, Konopleva M, Borthakur G, Short NJ, et al. Activity of Venetoclax-Based Therapy in Chronic Myelomonocytic Leukemia. *Leukemia* (2021) 35:1494–99. doi: 10.1038/s41375-021-01240-2

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Oncology

REVIEW
published: 14 April 2022
doi: 10.3389/fonc.2022.891961

Chronic Neutrophilic Leukemia: A Comprehensive Review of Clinical Characteristics, Genetic Landscape and Management

Thomas P. Thomopoulos[1], *Argiris Symeonidis*[2], *Alexandra Kourakli*[2], *Sotirios G. Papageorgiou*[1] *and Vasiliki Pappa*[1]*

[1] Second Department of Internal Medicine, Attikon Hospital, Research Institute, National and Kapodistrian University of Athens, Athens, Greece, [2] Department of Internal Medicine, University Hospital of Patras, Rio, Greece

Chronic neutrophilic leukemia (CNL) represents a rare disease, that has been classified among the BCR/ABL-negative myeloproliferative neoplasms. The disease is characterized by marked leukocytosis with absolute neutrophilia and its clinical presentation may vary from asymptomatic to highly symptomatic with massive splenomegaly and constitutional symptoms. CNL prognosis remains relatively poor, as most patients succumb to disease complications or transform to acute myeloid leukemia. Recent studies have demonstrated that *CSF3R* mutations drive the disease, albeit the presence of other secondary mutations perplex the genetic landscape of the disease. Notably, the presence of *CSF3R* mutations has been adopted as a criterion for diagnosis of CNL. Despite the vigorous research, the management of the disease remains suboptimal. Allogeneic stem cell transplantation represents the only treatment that could lead to cure; however, it is accompanied by high rates of treatment-related mortality. Recently, ruxolitinib has shown significant responses in patients with CNL; however, emergence of resistance might perturbate long-term management of the disease. The aim of this review is to summarize the clinical course and laboratory findings of CNL, highlight its pathogenesis and complex genetic landscape, and provide the context for the appropriate management of patients with CNL.

Keywords: chronic neutrophilic leukemia, myeloproliferative neoplasm, CSF3R, ruxolitinib, allogeneic HSCT

OPEN ACCESS

Edited by:
Simona Soverini,
University of Bologna, Italy

Reviewed by:
Ibrahim C. Haznedaroglu,
Hacettepe University Hospital, Turkey
Rosa Ayala,
Research Institute Hospital 12 de Octubre, Spain

***Correspondence:**
Vasiliki Pappa
vas_pappa@yahoo.com

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

Received: *08 March 2022*
Accepted: *28 March 2022*
Published: *14 April 2022*

Citation:
Thomopoulos TP, Symeonidis A, Kourakli A, Papageorgiou SG and Pappa V (2022) Chronic Neutrophilic Leukemia: A Comprehensive Review of Clinical Characteristics, Genetic Landscape and Management. Front. Oncol. 12:891961. doi: 10.3389/fonc.2022.891961

INTRODUCTION

Chronic neutrophilic leukemia (CNL) is a rare disease, with an annual incidence of about 1 new case per million people, a median age at diagnosis of about 65 years and a slight male preponderance. The disease was initially described in 1920 by Tuohy who reported the case of a 58-year-old female patient with persistent polymorphonuclear hyperleukocytosis and massive splenomegaly, who subsequently succumbed to the disease (1). Due to the rarity of the disease and the absence of clear diagnostic criteria, some early described cases are ill-defined; however, an additional well-documented case from France was published in 1932 (2), followed by a paucity of publications

for almost 25 years, until the report of a case describing the disease as a myeloproliferative neoplasm (MPN) (3). After the identification of Philadelphia chromosome as a hallmark of chronic myelogenous leukemia (CML), Tanzer et al. were the first to report a patient without Philadelphia chromosome or increased marrow fibrosis but high Leucocyte- or Neutrophil-Alkaline Phosphatase (LAP or NAP) score. Notably, they used the term "Chronic Neutrophilic Leukemia" to describe the disease (4). Additional case reports or small series of patients were published thereafter that have delineated this syndrome, although in some of them the authors have described the disease as a variant of CML (5–12).

CNL is a clonal hematopoietic stem cell disorder, and this has been documented early, as soon as clonality assays with X-linked restriction fragment length polymorphisms (RFLP) and/or X-inactivation patterns were applied (13, 14). The clonal nature of the disease is also evident by the detection of cytogenetic abnormalities (15–17), although in most cases, patients with CNL have a normal karyotype. From the epidemiological aspect of view the precise etiology of the disease remains unknown and there is only one report, associating CNL with a previous exposure to thorotrast, in a patient who, eventually evolved to acute myeloid leukemia (AML) (18). Moreover, when applying strict molecular criteria for disease characterization, it appears that the median age at diagnosis might be younger (19). Recently, mutations of the *CSF3R* gene have been shown to constitute the driver genetic event for the manifestation of this disease (20), which according to the latest version of the WHO classification of Hematopoietic Neoplasms, is classified as a chronic myeloproliferative neoplasm (21) However, there has been a vigorous debate whether CNL should be classified instead among the myelodysplastic syndromes (MDS) or the overlap myelodysplastic/myeloproliferative (MDS/MPN) neoplasms, similarly to chronic myelomonocytic leukemia (CMML), as it might share several clinical, hematological and cytogenetic features, reminiscent of MDS or MDS/MPN. Recently, criteria for the precise diagnosis of this disease have been proposed by the latest revision of the WHO classification, although some variation in clinical parameters might exist.

CLINICAL MANIFESTATIONS

CNL has substantial clinical and prognostic heterogeneity, which might correspond to the different driver mutations of the *CSF3R* gene. Most patients may go through an asymptomatic or minimally symptomatic period, characterized only by neutrophilic leukocytosis, with or without mild splenomegaly, lasting for several months or even years, before evolving to symptomatic disease. In many cases the disease may be discovered incidentally as unexplained persistent neutrophilia, after excluding any other potential causes as infections or inflammatory and neoplastic disorders, which occasionally can induce extreme hyperleukocytosis (22, 23). Less commonly, patients may complain for fatigue and restlessness, weight loss, night sweats, pruritus, and mild bone pain or may exhibit various hemorrhagic manifestations and recurrent episodes of gout (24). Fever is usually absent or minimal but persistent low-grade fever may be a prominent feature of advanced disease.

Bleeding diathesis, occasionally unrelated or disproportional to the degree of thrombocytopenia or splenomegaly/hypersplenism, is also a prominent feature of CNL, potentially reflecting platelet qualitative abnormalities (5, 7, 25, 26). Severe hemorrhagic manifestations, including recurrent episodes of epistaxis, blood oozing from the site of bone marrow aspiration, gastrointestinal tract hemorrhages and even intracranial hemorrhages have been reported throughout the disease course and may represent a common cause of death in these patients (5, 25, 26). One study of three patients with CNL, demonstrated prolonged bleeding time and abnormal platelet aggregation response to collagen, epinephrine and ADP, whereas in one patient, platelet adhesiveness and adenine content were found decreased (27). Moreover, abnormalities of the vascular compartment might contribute to the hemorrhagic manifestations. There have been several reports of cutaneous leucocytoclastic vasculitis or acute febrile neutrophilic dermatosis (Sweet's syndrome), occurring in patients with CNL. Therefore, various types of erythematous and/or hemorrhagic skin lesions, occasionally affecting palms and soles, represent a common manifestation of CNL. In some of these cases it is unclear whether the described skin lesions represent an allergic reaction, vasculitis or frank skin infiltration by the leukemic neutrophils, but whatever might be the case, severity of skin lesions runs in parallel with disease activity and severity (28–32).

Despite leukocytosis/neutrophilia patients with CNL are prone to develop severe, life-threatening or even fatal infections from both, common and opportunistic pathogens, such as mycobacteria and various fungi. This could be explained by the defective function of neutrophils as described in the following section. Patients may present with a severe infection, sometimes inducing septic shock (33, 34) or infections may occur frequently, during the course of the disease, and ultimately, they may represent one of the main causes of death (35, 36). It is therefore, noteworthy that in such cases, suspicion for the diagnosis of CNL may be obscured by the concurrent presence of a severe infection and neutrophilic leukocytosis often marked, which might be attributed to the infection.

Hepato-splenomegaly is commonly present. Splenomegaly is found in about 40% of the patients at the time of initial diagnosis and in the majority of them is mild to moderate; however, as disease slightly and steadily progresses, splenomegaly may become severe in almost all patients (10–12). Several cases of massive splenomegaly have been described, rarely requiring palliative splenectomy. When disease is rapidly evolving and white blood cells (WBC) are fast increasing, splenomegaly might become painful. Hepatomegaly is less common and less prominent, whereas lymphadenopathy has occasionally been reported but it is unusual. In some cases, lymph node biopsy has clearly revealed neutrophilic infiltration (37), whereas in some autopsy studies, infiltration by immature neutrophils and by other hematopoietic cells were identified in retroperitoneal lymph nodes, spleen, liver and the kidneys, a finding supporting the proliferative nature of the disease (37, 38). Although, vein thrombotic episodes in association with CNL have been rarely

reported (39, 40), a recent description of the clinical course of the disease in 19 patients, reported a history of thrombosis in about half of the patients (41).

LABORATORY FINDINGS

The peripheral blood smear is characterized by persistent mature neutrophilic leukocytosis, with about 80-95% of the enumerating cells being neutrophils or bands. Left shift is absent or minimal and only occasional myelocytes-metamyelocytes are observed. Notably, CNL is characterized by the absence of monocytosis, eosinophilia or basophilia. In some cases, leukocytosis/neutrophilia might be severe, exceeding 100 x 10^9/L and reaching up to 500 x 10^9/L. Neutrophils appear morphologically normal by both, light and electron microscopy and in the majority of cases contain abundant primary and secondary granules (8). Presence of Döhle bodies and neutrophil inclusions have been reported rarely; however, dysgranulopoiesis is not consistent with the diagnosis of CNL (21). Notably, reported cases of CNL presenting with dysplastic morphological features, particularly in more advanced or long-standing cases, including abnormal chromatin clumping, poor granulation, cytoplasmic vacuolation, microtubular inclusions (42), ringed-shape nuclei (42, 43), are now considered to represent cases of atypical CML rather than genuine CNL cases

Neutrophils and granulocytes of patients with CNL usually have an activated phenotype, but they might also exhibit several functional abnormalities and are less viable in stress conditions. The enzymatic equipment of neutrophils is normal, as this can be revealed by the appropriate cytochemical stains. Myeloperoxidase is strongly positive as is also LAP, whose elevated score has been used to distinguish this disease from BCR/ABL positive CML, in which LAP is very low to completely absent (6, 7, 11, 12). Some studies have reported reduced lysozyme and β-glycuronidase content in the neutrophils (8, 37) but these enzymes are highly released from clonal neutrophils, following stimulation. Neutrophil motility, deformability and chemotaxis have been found abnormal and the respiratory burst is usually impaired, with reduced superoxide anion production in response to various stimuli, as well as decreased cytosolic C kinase activity (44, 45). Other studies have demonstrated normal phagocytosis and bactericidal activity and normal nitroblue tetrazolium reduction assays suggesting that these neutrophils are found in an abnormally activated status (46). Bactericidal activity, however, has also been reported to be decreased and the same patients exhibited decreased granulocytic clonogenic activity from both, peripheral blood and bone marrow, compared to normal subjects (47). In one study, several functional neutrophils tests were found normal, however neutrophils of CNL patients produced significantly lower amounts of leukotriene B4 (48). In another multifunctional study the authors found reduced serum G-CSF and GM-CSF levels and inadequate *in vitro* neutrophil stimulation, induced by these cytokines. They also demonstrated impaired STAT3 and MAP kinase downstream signal, despite the intact expression of both G-CSF- and GM-CSF receptor and they suggest a potential dysregulation of the intracellular part of the receptor(s), although they were unable to show any mutation of this domain (49).

Mild to moderate anemia might be present, in most cases normochromic-normocytic sharing the features of the anemia of chronic diseases (12), whereas cases with macrocytic anemia have also been reported, despite the very high serum B12 levels, reflecting underlying dyserythropoiesis, as shown by the report of impaired erythroblastic colony formation in patients with CNL (7).

Platelet count may be normal, reduced or less commonly, increased. Morphologically, platelets may appear normal, but giant platelets and platelets with poor granulation, reminiscent of storage pool disorders have been observed (11, 12). Deteriorating thrombocytopenia might be a feature of disease transformation towards AML. Rarely, nucleated red blood cells can also be identified. In a recent short report, numerous ringed sideroblasts were found in the marrow of a patient with CNL (50).

Bone marrow findings are more reminiscent of a myeloproliferative neoplasm, with almost 100% cellularity and fat disappearance, extreme granulocytic hyperplasia without maturation arrest and absence of monocytosis, monocyte precursors and basophilia (11, 12). The myeloid/erythroid ratio is usually higher than 10, but blast cells are not increased, unless the disease has entered an accelerated phase and evolves towards AML (51). Megakaryocytic hyperplasia is also common, and in most cases megakaryocytes appear morphologically normal and mainly mature and hyper-lobulated, i.e. with higher ploidy as compared to MDS or typical BCR/ABL-positive CML (27); however, megakaryocyte dysplasia is absent or minimal. Moreover, minimal fibrosis might be present in the bone marrow of patients with CNL but should not exceed a grade of 1+ (21). As a result of increased cellular turnover, pseudo-Gaucher cells may be found in the marrow (52).

The main findings of patients' biochemical profile consist of elevated serum LDH and uric acid levels, reflecting the increased hematopoietic cell turnover. Serum B12 and transcobalamin-I and -III, that are released in circulation by the mature cells of granulopoietic lineage, are usually elevated. Serum alkaline phosphatase and γ-glutamyl-transpeptidase might also be found increased, reflecting liver infiltration by hematopoietic cells. Markers of acute phase reaction are usually normal at the time of initial diagnosis but may become abnormal in advanced stages, associated with marked leukocytosis or in cases of disease progression. Serum G-CSF levels are not routinely estimated, but if performed, they are found suppressed. In milder cases of CNL differential diagnosis should exclude a leukemoid reaction attributed to various underlying diseases and conditions, and other types of myeloproliferative disorders. A practical diagnostic approach together with the diagnostic criteria of CNL are shown in **Table 1**.

DIFFERENTIAL DIAGNOSIS

In order to establish the diagnosis of CNL, other cases of reactive leukocytosis should be excluded. Occasionally CNL has been

TABLE 1 | Diagnostic work up and diagnostic criteria for CNL.

Work up	Diagnostic criteria
Confirm persistent leukocytosis/ neutrophilia	Repeated blood counts should confirm WBC >25 x 10^9/L in repeated evaluations
Exclude all reactive or secondary causes of neutrophilia	Complete survey must not reveal any cause of secondary neutrophilia and if revealed, a *CSF3R* mutation should also be present
Evaluate peripheral blood findings and morphology	>80% of the WBC should be neutrophils or bands, with less mature forms <10% and blasts <1%
Evaluate the clinical and biochemical profile	Hepatosplenomegaly is common, and serum LDH, uric acid, B12, and liver cholestatic enzymes are usually increased
Evaluate cytochemical profile	LAP score is elevated or markedly elevated
Evaluate bone marrow findings and morphology	Marrow cellularity is highly increased with granulocytic hyperplasia without evident dysplasia or excess of blasts
Evaluate bone marrow histology and immunophenotype	A clear myeloproliferative syndrome pattern without an increase of monocytes, eosinophils, basophils or mast cells
Exclude BCR/ABL positive Chronic Myelogenous Leukemia	PCR for BCR/ABL transcripts should be negative and karyotype should not exhibit Ph chromosome
Exclude BCR/ABL negative myeloproliferative neoplasms	Diagnostic criteria for polycythemia vera, primary myelofibrosis and essential thrombocythemia should not be confirmed
Exclude chronic myelomonocytic leukemia	The absolute monocyte count in the blood should be <1 x 10^9/L
Exclude a classical myelodysplastic syndrome	Dysplastic changes should be absent
Exclude atypical BCR/ABL-negative chronic myelogenous leukemia	Dysplastic changes should be absent. Multilineage dysplastic changes and >10% immature cells in the PB are prominent in aCML. *CSF3R* mutation should be demonstrated
Perform direct molecular characterization of the disease	There should be a mutation in the *CSF3R* gene, but with NGS additional mutations may also be revealed
Investigate for presence of commonly coexisted conditions	Extramedullary infiltration, vasculitic syndromes or plasma cell dyscrasias might be present. *CSF3R* mutations necessary to be confirmed

reported to coexist with lymphoid neoplasms (53), but the most striking association has been with various plasma cell dyscrasias, such as monoclonal gammopathy of undetermined significance (MGUS) (39, 54–58), and mainly multiple myeloma. There have been several cases of patients reported to have concurrently or consecutively, these two, apparently different hematological dyscrasias (28, 59–71). However, most of these cases represent a neutrophilic leukemoid reaction, potentially mediated by cytokines produced by the clonal plasma cells. Notably, these cases cannot fulfill the diagnostic criteria for CNL (72). Nonetheless, in the presence of a plasma cell dyscrasia, clonality must be demonstrated by cytogenetic or molecular in order to establish the diagnosis of CNL the diagnosis of CNL (21).

As per the 2016 WHO classification, other MPN and MDS/MPN should also be excluded. Classical MPN, namely polycythemia vera, essential thrombocytosis, and primary myelofibrosis can be easily excluded by the absence of the characteristic morphological and molecular features of the latter. Similarly, the absence of *BCR/ABL* fusion gene invariably precludes the diagnosis of CML. Regarding chronic myelomonocytic leukemia, presence of persistent absolute monocytosis and dysplastic features distinguish this entity from CNL (21). Undoubtedly, the most challenging aspect in differential diagnosis is to distinguish CNL from atypical chronic myeloid leukemia (aCML). Presence of prominent dysplasia in >10% of cells, as well as, a more prominent immature granulocytosis favor the diagnosis of aCML. Notably, presence of *CSF3R* mutations cannot be used for differential diagnosis between aCML and CNL, as there have been several cases of aCML harboring *CSF3R* mutations (73).

PATHOGENESIS

Gene Mutations

CSF3R

The granulocyte colony stimulating factor (G-CSF), a cytokine primarily produced by endothelial cells, fibroblasts and macrophages, is the major regulator of both basal and emergency granulopoiesis. G-CSF is crucial for commitment of myeloid cells towards granulocytic differentiation; concomitantly, G-CSF accelerates maturation of metamyelocytes into mature neutrophils, prolongs the survival of neutrophils and their progenitors, and enhances neutrophil function (74).

G-CSF exerts its actions by binding to the receptor CSF3R on myeloid cells, which consists of a single polypeptide chain with great homology to other cytokine receptors. CSF3R extracellular domain, comprising of an immunoglobulin-like (IgG) domain followed by a cytokine receptor homology domain (CRH) and three fibronectin III (FNIII) domains, plays a crucial role in receptor activation. The intracellular domain contains two proximal motifs termed Box 1 and Box 2 that are critical for signal transduction as they bind to JAK, whereas the distal C-terminal domain drives differentiation and transduction of phagocytic signals in mature neutrophils. C-terminal domain also contains Box 3, which acts as a negative regulator of G-CSF signaling (75). A graphical representation of the CSF3R structure is provided in **Figure 1**.

Binding of G-CSF to CSF3R induces homodimerization of the receptor leading to the activation of downstream pathways. The proximal domain activates mostly the JAK/STAT pathway

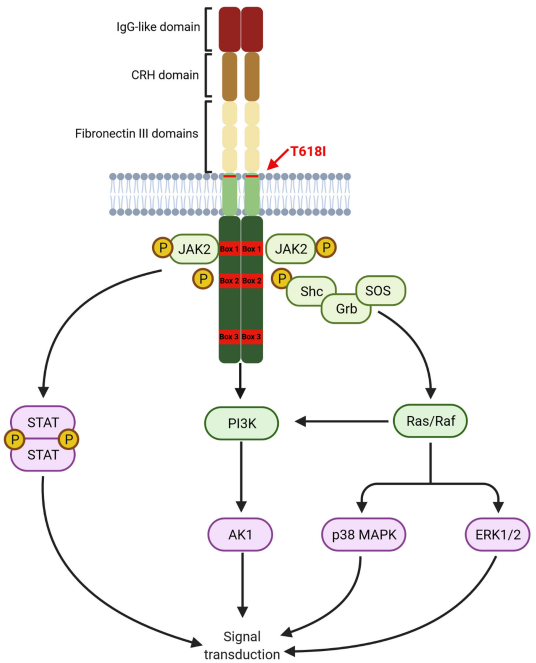


FIGURE 1 | Structure of CSF3R. Proximal transmembrane T618I mutation induced a G-CSF independent activation of receptor homodimers leading to constant signal transduction primarily through the JAK/STAT pathway. Other pathways, namely PI3K/AKT and ERK1/2 are also constantly activated (*Created with BioRender.com*).

leading to proliferation and differentiation. The PI3K/AKT pathway is also activated by a LYN kinase, also interacting with the proximal domain, leading to increased survival. Other pathways, particularly the MAPK/ERK are activated by interaction with more distal residues in the intracellular domain.

Given the importance of G-CSF in granulopoiesis, *CSF3R* mutations have been associated with the pathogenesis of various diseases. Extracellular domain missense mutations conferring refractoriness to G-CSF have been identified in severe congenital neutropenia (SCN) and chronic idiopathic neutropenia (CIN) (76). Nonsense or frameshift mutations in the extracellular domain, also found in SCN patients, promote ligand-independent binding to the full-length CSF3R that appears to suppress G-CSF-mediated signaling (77). On the other hand, intracellular domain mutations, mostly of nonsense nature, result in a truncated receptor with normal affinity for G-CSF, but a higher proliferation and lower differentiation potential in response to G-CSF. These mutations are almost exclusively found in patients with SCN who develop MDS or AML.

Proximal transmembrane mutations, namely T618I and T640N, promote the ligand-independent activation of pre-existing dimers of CSF3R and lead to low level, sustained activation of all downstream pathways, that cannot be terminated by internalization of the activated CSF3R, that usually follows the activation of wild-type CSF3R (78). These mutations are found to be particularly enriched in patients with CNL.

In their pivotal trial in 2013, Maxson et al. demonstrated that 8 of 9 CNL patients (89%) harbor *CSF3R* mutations. Notably, all of them had proximal transmembrane mutation, most commonly T618I, whereas an additional truncating intracellular mutation was found in three patients as a compound mutation (20, 79). The results of Maxson et al. have been subsequently validated by Pardanani et al. who demonstrated a *CSF3R* T618I mutation in 83% of patients with WHO-defined CNL. Most strikingly, this mutation was virtually absent from cases of atypical CML or other myeloproliferative neoplasms, highlighting that *CSF3R* T618I could serve as a highly sensitive and specific marker for CNL (80). The oncogenic potential of this mutation has been demonstrated in a murine model, as transplantation of hematopoietic cells harboring the T618I mutation sufficed for the development of a lethal myeloproliferative neoplasm resembling CNL (81). On the other hand, truncating mutations seem not to be oncogenic; however, they might act synergistically with proximal mutations to drive leukemogenesis, potentially through enhanced MAPK signaling, as shown by *in vitro* studies by Rohrabaugh et al. Notably, although compound mutations might confer resistance to JAK or SRC kinase inhibition by ruxolitinib and dasatinib respectively, cell lines harboring compound mutations remain sensitive to MEK inhibitors, such as trametinib.

Other Pathogenic Mutations

Several recent studies have tried to elucidate the complex genetic landscape of CNL. In most cases, additional mutations in genes commonly affected in myeloid malignancies have been observed; however, their frequency has been highly variable among cohorts. The most commonly affected genes are those involved in epigenetic and transcriptional regulation (*ASXL1*, *TET2*), in the assembly of the spliceosome (*SRSF2*, *U2AF1*), as well as mutations in genes such as *SETBP1*. Notably, mutations in genes involved in the cell-signaling pathways, such as *JAK2* and *NRAS* are scarcely found (82). The frequency of gene mutations in CNL is graphically represented in **Figure 2**.

In the largest cohort of 39 CNL cases, mutations of *ASXL1* were found in 77% of cases. This gene, which is involved in histone modification, was notably the most commonly affected gene in CNL, with frameshift or nonsense mutations. Further, *ASXL1* mutations demonstrated variable variant allelic frequencies (VAF), indicating that this genetic event might occur early in the founder clone, or acquired in later subclones in other cases. Phenotypically, patients with *ASXL1* mutations tended to be of older age and presented with higher WBC count, as well as increased needs for platelet transfusions; a trend for worse overall survival was also noted (83). In line with this finding, Elliot et al. demonstrated that *ASXL1* mutations, present in 57% of cases, along with thrombocytopenia, were independent prognostic factors for adverse overall survival (84). *TET2* mutations, have been observed less frequently in CNL (~21% of cases). In most cases these lesions might be acquired at later stages of clonal evolution. Regarding the disease phenotype, the presence of *TET2* mutations correlated with low platelet count, low neutrophil percentage, high monocyte percentage, and bone

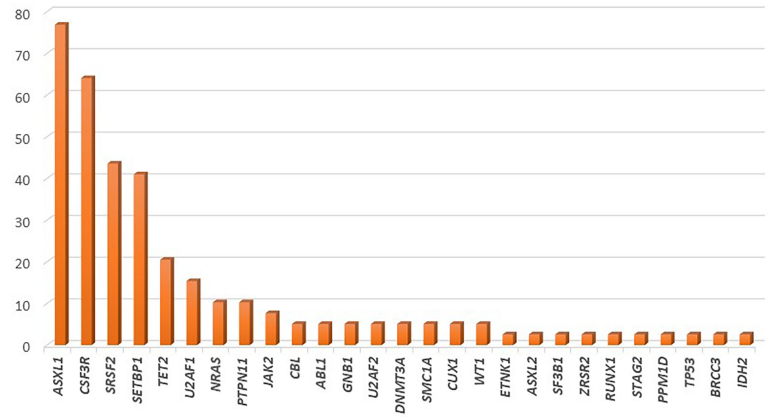


FIGURE 2 | Frequency of gene mutations in chronic neutrophilic leukemia, as demonstrated by application of next generation sequencing in a cohort of 39 patients.

marrow dysplasia (83). Of special interest is the fact that no cases with concomitant mutations of *TET2* and *SRSF2* have been observed in CNL, as the $TET2^{mut}/SRSF2^{mut}$ genotype is highly specific of CMML (85). Furthermore, *EZH2* mutations have been found relatively frequently in CNL cases, even though their significance remains unclear.

Mutations affecting the spliceosome have also been reported with variable frequency in patients with CNL. *SRSF2* and *U2AF1*, mutated in up to 44% and 15% of patients respectively, are the most commonly affected genes within this group. Similarly to *ASXL1*, these mutations might be acquired in variable timepoints along the clonal evolution of CNL (83). The contribution of these mutations in disease phenotype and prognosis has yet to be assessed.

SETBP1 has been found to be mutated in approximately 41% of CNL cases. Notably, a high co-occurrence of *SETBP1* mutations with *CSF3R* T618I has been consistently reported, reaching 67% in more recent studies (86, 87). A trend towards inferior overall survival for patients with CNL harboring *SETBP1* mutations has been reported; however, a meta-analysis of the three available studies that assessed the prognostic significance of this gene in CNL demonstrated no association with overall survival (88). Interestingly, *SETBP1* mutations have been associated with a more myeloproliferative phenotype with increased hemoglobin and platelet counts.

Models of Clonal Evolution

CNL demonstrates high genetic heterogeneity with frequent co-occurrence of mutations in different genes with variable VAF. Indeed, in the study of Zhang et al. the median number of mutations per patient was 3.6, indicating that more than three pathways were simultaneously affected in most patients (83). In an attempt to explain the genetic variability of CNL, Maxson et al. suggested that CNL pathogenesis might be a multistep process involving sequential genetic events giving rise to different subclones. At least two models of clonal evolution have been suggested (82). In the first model, a healthy hematopoietic stem cell acquires a mutation associated with epigenetic modification (*ASXL1, TET2*) or spliceosome assembly (*SRSF2, U2A1*), giving rise to a clonal hematopoiesis of indeterminate potential (CHIP) which remains asymptomatic or presents with minimal myelodysplasia. Acquisition of a signaling mutation, most commonly *CSF3R* T618I, offers a neutrophil differentiation bias leading to overt CNL. In the other model, the *CSF3R* T618I arises as a founder mutation in healthy stem cells, causing a highly myeloproliferative phenotype. Owing to the high replication potential, the cells rapidly acquire additional mutations in epigenetic/splicing genes, adding dysplastic features (82). These models are summarized in **Figure 3**.

Cytogenetic Abnormalities

Most patients with CNL present with normal karyotype at diagnosis. In a series of 40 patients, cytogenetic abnormalities were reported in 32.5% patients. These abnormalities were detected at diagnosis in 20% of patients, or emerged as clonal evolution in the remaining patients. Given their scarcity, no definite conclusions can be drawn regarding the occurrence of cytogenetic abnormalities in CNL; however, deletion of 20q, 11q, 12p, and trisomy 21 might represent nonrandom abnormalities, as they present with increased frequency in other myeloid malignancies (89).

PROGNOSIS

Overall survival of patients with CNL has been shown to be rather short. In a population-based study combining data from the Surveillance, Epidemiology, and End Results (SEER) program and the National Cancer Database (NCDB), an OS of 1.8 years in SEER and 2.2 years in NCDB was found (90). Similarly, in a series of 40 WHO-defined CNL, median overall survival was 23.5 months; causes of death included intracranial hemorrhage, progressive disease, infections, and leukemic transformation (89). Transformation to AML might occur in 16% of patients at a median of 21 months. Interestingly, several cases of transformation to CMML have been reported (41).

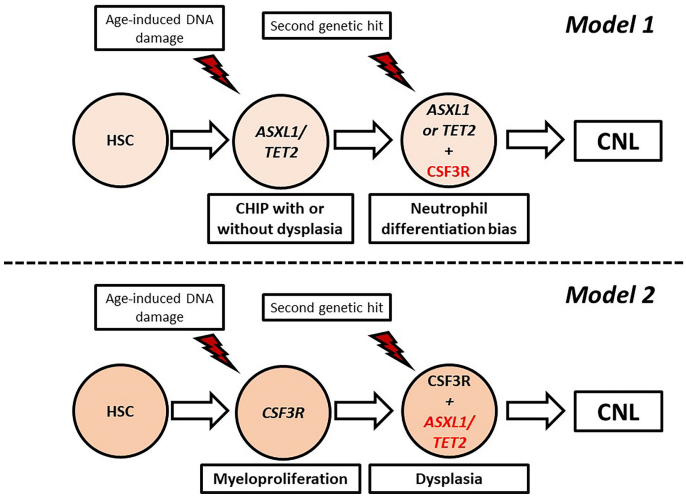


FIGURE 3 | Proposed models of clonal evolution in chronic neutrophilic leukemia. CHIP, Clonal hematopoiesis of indeterminate potential; HSC, hematopoietic stem cell.

As CNL represents a rare disease, data pertaining to prognostic factors are relatively scarce. Cui et al. demonstrated that WBC count >50 x 10^9/L represent a negative prognostic factor for OS (86). As mentioned above, Elliot et al. showed that *ASXL1* mutation and thrombocytopenia were independent prognostic factors of inferior OS (84). Most recently, presence of *NRAS*, *ASXL1*, *GATA2*, and *DNMT3A* mutations correlated with a trend of shorter OS, whereas *CBL* mutations predicted a more favorable OS (83). A prognostic scoring system has been suggested by Szuber et al. from Mayo Clinic, incorporating three variables, namely platelet count $<160 \times 10^9$/L, leukocyte count $>60 \times 10^9$/L, and presence of *ASXL1* mutation. Thrombocytopenia has been assigned with two points, whereas the other variables with one point each. A two-tier stratification of patients into low-risk (0–1 points) and high-risk (2–4 points), yielded a statistically significant difference in OS (not reached for low-risk vs. 22.4 months for high-risk) (41).

MANAGEMENT

Because of its exceptional rarity, there is no standard of care for CNL. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only therapeutic approach with curative intent. Conventional treatments mainly aim to alleviate the disease burden, albeit they do not seem to affect the natural history of the disease and overall survival. Therefore, the development of potentially disease-modifying treatments constitutes a major unmet need. Currently, the available treatment options include pharmacological agents (hydroxyurea, interferons, JAK inhibitors, and other tyrosine kinase inhibitors) and allo-HSCT. A proposed algorithm of management of CNL is depicted in **Figure 4**.

Hydroxyurea

Hydroxyurea, an oral cytoreductive agent, has traditionally been used in first-line treatment of CNL. Approximately 75% of

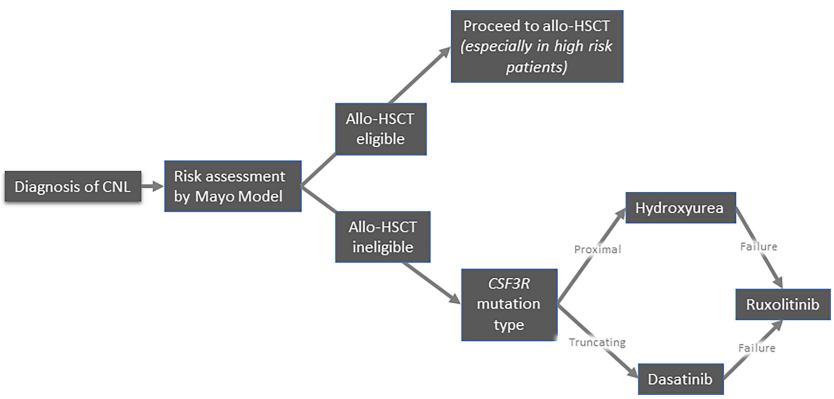


FIGURE 4 | Suggested algorithm for treatment of patients with chronic neutrophilic leukemia.

patients are expected to demonstrate clinical response in terms of decrease in leukocytosis and/or splenomegaly; however, the responses are transient, as all patients are expected to demonstrate disease progression or transformation to AML within a median of 12 months (89).In this context, hydroxyurea should be considered palliative treatment, as it fails to halt clonal evolution and therefore disease progression.

Interferon

Interferon-alpha (IFN-a) represents another therapeutic approach in CNL, based on published case reports; however, no clinical trial establishing its effectiveness has been reported. Although IFN-a might lead to long-term remission, even in patients primarily refractory to hydroxyurea, the impact of IFN-a to CNL clonal evolution has not been assessed. Recently, Yassin et al. reported the case of a woman with CNL achieving hematological response with pegylated interferon alpha-2a, albeit no data on duration of response have been disclosed (91). Despite the absence of solid evidence for the efficacy of IFN-a, it remains a valid option for CNL treatment.

Intensive Chemotherapy

Conventional chemotherapy with anthracycline and cytarabine (3 + 7) might represent a treatment option for patients transforming to AML; however, overall survival is relatively poor, as expected for secondary AML (92). Regarding CNL in chronic phase, intensive chemotherapy is not recommended as it is accompanied by high treatment-related mortality and the majority of patients are refractory to this approach (11, 89).

Ruxolitinib

The discovery of the key role of *CSF3R* in CNL and the JAK/STAT signaling pathway in CNL pathobiology has impelled the investigation of ruxolitinib, a JAK1/2 inhibitor, for the treatment of CNL. Preclinical studies demonstrated that ruxolitinib could decrease WBC count and splenomegaly in mice with *CSF3R*T618I mutated CNL; however, initial case reports demonstrated variable responses to ruxolitinib (93–96). Similarly, Szuber et al. in a case series of 19 patients, noted that ruxolitinib, received by four patients, was associated with a temporary response in half of the patients (41).

A phase II trial of ruxolitinib including 21 patients with CNL has been published recently. Overall response rate (ORR) was 58% for CNL patients. Among them, 4 complete remissions and 9 partial remissions were noted. Further, ORR was restricted to 8% in the group with wild-type *CSF3R*, while no association between response and cytogenetics, number of mutations, and *ASXL1* or *SETBP1* mutations was observed. Median OS for all patients was 18.8 months. By response, median survival for non-responders and responders was 15.6 and 23.1 months respectively. Most importantly, ruxolitinib was shown to decrease the *CSF3R* T618I allelic burden in responders, particularly for those achieving CR (mean absolute VAF change: −0.26 for CR, −0.05 for PR).

The effect of compound mutations of *CSF3R* on sensitivity to ruxolitinib has been an object of debate. *In vitro* studies have demonstrated the resistance of cell lines harboring compound mutation to ruxolitinib; however, Gunawan et al. have reported on a case with compound *CSF3R* mutations that had a substantial yet short-lived response to ruxolitinib (94). Most recently, Hinze et al. reported a case of a compound-mutated CNL achieving long-term remission with ruxolitinib (97). The co-occurrence of *CSF3R* and *SETBP1* mutations have shown to confer resistance to ruxolitinib; however, in the aforementioned phase II trial, no association between *SETBP1* mutation and response to ruxolitinib was noted (98).

The mechanisms underlying clonal evolutions in patients with CNL undergoing treatment with ruxolitinib might be multifaceted. Stoner et al. evaluated seven patients with CNL demonstrating molecular or clinical progression under treatment with ruxolitinib. VAF reduction of *CSF3R* mutation was noted in 3 cases, whereas two patients demonstrated no changes in allelic burden overtime. Primary resistance to ruxolitinib could be attributed to co-occurring *NOTCH2* and *SRSF2* mutations in the founder clone of one case. Notably, subclones harboring *STAT3*, *STAG2*, and *RUNX1* mutations emerged in three cases. Acting downstream of JAK kinases, *STAT3* could bypass the inhibitory effects of ruxolitinib, whereas co-operation of *RUNX1* with *CSF3R* mutations might be involved in CNL disease progression and AML transformation. On the contrary, the role of the *STAG2* mutations in disease progression has not been elucidated yet (99).

Fedratinib, another JAK inhibitor is being evaluated in a phase II trial for patients with CNL (NCT05177211).

Other Tyrosine Kinase Inhibitors

The rationale for the use of dasatinib in CNL stems from the demonstration of the *in vitro* sensitivity of CNL lines harboring truncating *CSF3R* mutations to SRC kinase inhibition (20); however, no data on the *in vivo* efficacy of dasatinib are available. On the contrary, a recent case report demonstrated that co-occurrence of truncating and proximal *CSF3R* mutations confer resistance to dasatinib in a patient with CNL (97). In this context, a short trial with dasatinib could be offered in patients harboring truncating mutations with close monitoring for disease progression.

Preclinical data have suggested that compound mutations of *CSF3R* might exert their leukemogenic potential through enhancement of MAPK signaling. In support of this, trametinib, a MEK1/2 inhibitor, has demonstrated *in vitro* activity in compound-mutant CNL models; however, this agent has not been tested in clinical practice.

Allogeneic Stem Cell Transplantation

Given the lack of therapeutic agents that could provide long-term disease control, allo-HSCT represents the only therapeutic option with curative potential; however, the published evidence remains scarce, consisting mostly of case reports and small case series. Elliot et al. reported of five patients with CNL who underwent allo-HSCT. Four of them achieved CR and remained disease-free up to six years from transplantation (89). In another case-series of 19 patients, two patients in blast transformation underwent allo-HSCT. One patient had a favorable outcome, remaining disease-free 40 months post-transplant, whereas the other patient died from transplantation-related complications (41).Ruan et al. in a population-based study reported that 2% of CNL patients underwent allo-HSCT. Notably, all of them were alive at five years post-treatment (90). Although

these studies are informative of the curative role of allo-HSCT, they do not provide details pertaining to the transplantation procedures.

A retrospective nationwide study in Japan aimed to provide a more comprehensive overview of allo-HSCT in this rare myeloid neoplasm. Between 2003 and 2014, five patients with CNL underwent allo-HSCT. Three patients received hydroxyurea, one received dasatinib, and one was offered intensive chemotherapy, albeit none of the patients demonstrated response to treatment prior to transplantation. Notably, none of them received ruxolitinib. Four patients received transplantation from matched-unrelated donors, whereas one patient received transplantation from an HLA-haploidentical sibling donor. All but one patients received a myeloablative conditioning regimen. Neutrophil engraftment was achieved in all but one patient who died due to bleeding. Two of the patients who engrafted achieved CR that was retained without signs of relapse at day +362 and +441, whereas one patients, although in CR, died from sinusoidal obstruction syndrome at day +56; one patients showed no response to transplant and succumbed to the disease 76 days post-transplant.

CONCLUSION

Chronic neutrophilic leukemia represents an extremely rare myeloproliferative neoplasm that has been poorly characterized for over a century, owing to the wide variability of the clinical presentation and laboratory findings of patients. Recently, there has been an increase in the understanding of the molecular pathogenesis of the disease, as mutations in the *CSF3R* gene are believed to be the driver mutations in most cases, albeit other mutations are required for the development of overt disease. The prognosis of the disease remains poor even for patients that proceed to allo-HSCT, which represents the only therapeutic option with curative intent. Studies on other therapeutic modalities such as ruxolitinib, have demonstrated satisfactory response rates; however, clonal evolution and emergence of resistance to these agents might limit their efficacy. Therefore, there is an unmet need for the optimal treatment of patients with CNL, highlighting the need for more studies enrolling patients with WHO-defined CNL, that could disentangle the complex genetic landscape of the disease and provide novel potential targets for development of more potent therapeutic agents.

AUTHOR CONTRIBUTIONS

Conceptualization, investigation, data curation, writing-original draft preparation: TPT, AS, AK, SGP, and VP. Writing—review and editing: TPT, and VP. Supervision: VP All authors have read, reviewed, edited and agreed to the published version of the manuscript.

REFERENCES

1. Tuohy EL. A Case of Splenomegaly With Polymorphonuclear Neutrophil Hyperleukocytosis. *Am J Med Sci* (1920) 160:18 24. doi: 10.1097/00000441-192007000-00003
2. Emile-Weil P, See G. La Leucémie Myélogène À Polynucléaires Neutrophiles. *Presse Méd* (1932) 40:1071–4.
3. Exton-Smith AN, Chazan AA. Myeloproliferative Syndrome Presenting as Neutrophilic Leukaemia. *Proc R Soc Med* (1957) 50(7):510–2. doi: 10.1177/003591575705000711
4. Tanzer J, Harel P, Boiron M, Bernard J. Cytochemical and Cytogenetic Findings in a Case of Chronic Neutriphilic Leukaemia of Mature Cell Type. *Lancet* (1964) 1(7329):387–8. doi: 10.1016/s0140-6736(64)92142-7
5. You W, Weisbrot IM. Chronic Neutrophilic Leukemia. Report of Two Cases and Review of the Literature. *Am J Clin Pathol* (1979) 72(2):233–42. doi: 10.1093/ajcp/72.2.233
6. Grinblat J, Mammon Z, Lewitus Z, Joshua H. Chronic Myelogenous Leukemia With Elevated Leukocyte Alkaline Phosphatase, Positive Indirect Coombs' Test, Neutrophilic Leukocytosis and Unusual Cytogenetical Findings. *Acta Haematol* (1977) 57(5):298–304. doi: 10.1159/000207894
7. Bareford D, Jacobs P. Chronic Neutrophilic Leukaemia. *Am J Clin Pathol* (1980) 73(6):837. doi: 10.1093/ajcp/73.6.837
8. Dotten DA, Pruzanski W, Wong D. Functional Characterization of the Cells in Chronic Neutrophilic Leukemia. *Am J Hematol* (1982) 12(2):157–65. doi: 10.1002/ajh.2830120208
9. Zittoun R, Réa D, Ngoc LH, Ramond S. Chronic Neutrophilic Leukemia. A Study of Four Cases. *Ann Hematol* (1994) 68(2):55–60. doi: 10.1007/bf01715131
10. Elliott MA, Dewald GW, Tefferi A, Hanson CA. Chronic Neutrophilic Leukemia (Cnl): A Clinical, Pathologic and Cytogenetic Study. *Leukemia* (2001) 15(1):35–40. doi: 10.1038/sj.leu.2401993
11. Böhm J, Schaefer HE. Chronic Neutrophilic Leukaemia: 14 New Cases of an Uncommon Myeloproliferative Disease. *J Clin Pathol* (2002) 55(11):862–4. doi: 10.1136/jcp.55.11.862
12. Elliott MA. Chronic Neutrophilic Leukemia and Chronic Myelomonocytic Leukemia: Who Defined. *Best Pract Res Clin Haematol* (2006) 19(3):571–93. doi: 10.1016/j.beha.2005.07.012
13. Kwong YL, Cheng G. Clonal Nature of Chronic Neutrophilic Leukemia. *Blood* (1993) 82(3):1035–6. doi: 10.1182/blood.V82.3.1035b.1035b
14. Standen GR, Steers FJ, Jones L. Clonality of Chronic Neutrophilic Leukaemia Associated With Myeloma: Analysis Using the X-Linked Probe M27 Beta. *J Clin Pathol* (1993) 46(4):297–8. doi: 10.1136/jcp.46.4.297
15. Lorente JA, Peña JM, Ferro T, Garcia-Alegria J, Gonzalez JJ, Barbado J, et al. A Case of Chronic Neutrophilic Leukemia With Original Chromosomal Abnormalities. *Eur J Haematol* (1988) 41(3):285–8. doi: 10.1111/j.1600-0609.1988.tb01194.x
16. Orazi A, Cattoretti G, Sozzi G. A Case of Chronic Neutrophilic Leukemia With Trisomy 8. *Acta Haematol* (1989) 81(3):148–51. doi: 10.1159/000205548
17. Di Donato C, Croci G, Lazzari S, Scarduelli L, Vignoli R, Buia M, et al. Chronic Neutrophilic Leukemia: Description of a New Case With Karyotypic Abnormalities. *Am J Clin Pathol* (1986) 85(3):369–71. doi: 10.1093/ajcp/85.3.369
18. Boggs DR, Kaplan SS. Cytobiologic and Clinical Aspects in a Patient With Chronic Neutrophilic Leukemia After Thorotrast Exposure. *Am J Med* (1986) 81(5):905–10. doi: 10.1016/0002-9343(86)90367-0
19. Ouyang Y, Qiao C, Chen Y, Zhang SJ. Clinical Significance of Csf3r, Srsf2 and Setbp1 Mutations in Chronic Neutrophilic Leukemia and Chronic Myelomonocytic Leukemia. *Oncotarget* (2017) 8(13):20834–41. doi: 10.18632/oncotarget.15355
20. Maxson JE, Gotlib J, Pollyea DA, Fleischman AG, Agarwal A, Eide CA, et al. Oncogenic Csf3r Mutations in Chronic Neutrophilic Leukemia and Atypical Cml. *N Engl J Med* (2013) 368(19):1781–90. doi: 10.1056/NEJMoa1214514
21. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. *Blood* (2016) 127(20):2391–405. doi: 10.1182/blood-2016-03-643544
22. Tilak V, Rai M, Singh VP, Rai AK, Raman R. Hepatocellular Carcinoma Presenting as Neutrophilic Leukaemoid Reaction–a Rare Entity. *J Indian Med Assoc* (2007) 105(8):462, 4–5.

23. Mittal N, Gupta R, Rahman K, Singh P, Panda I, Nityanand S. A Case of Paraneoplastic Hyperleukocytosis Closely Mimicking Chronic Neutrophilic Leukemia. *J Cancer Res Ther* (2020) 16(1):186–8. doi: 10.4103/0973-1482.199434
24. Kakkar N, Joseph John M. Chronic Neutrophilic Leukemia Presenting as Secondary Gout: Report of a Rare Myeloproliferative Disorder. *Indian J Cancer* (2020) 57(2):201–4. doi: 10.4103/ijc.IJC_560_18
25. Hossfeld DK, Lokhorst HW, Garbrecht M. Neutrophilic Leukemia Accompanied by Hemorrhagic Diathesis: Report of Two Cases. *Blut* (1987) 54(2):109–13. doi: 10.1007/bf00321039
26. Silva PR, Ferreira C, Bizarro S, Cerveira N, Torres L, Moreira I, et al. Diagnosis, Complications and Management of Chronic Neutrophilic Leukaemia: A Case Report. *Oncol Lett* (2015) 9(6):2657–60. doi: 10.3892/ol.2015.3148
27. Mori H, Ikegami T, Shimizu T, Harada H, Miyoshi Y, Okada S, et al. [Changes in the Megakaryocyte-Platelet System in Chronic Neutrophilic Leukemia]. *Rinsho Ketsueki* (1991) 32(12):1574–6.
28. Gan GG, Pasagna JF, Eow GI, Nadarajan VS. Chronic Neutrophilic Leukaemia. *Singapore Med J* (2007) 48(3):e74-6.
29. Castanet J, Lacour JP, Garnier G, Perrin C, Taillan B, Fuzibet JG, et al. Neutrophilic Dermatosis Associated With Chronic Neutrophilic Leukemia. *J Am Acad Dermatol* (1993) 29(2 Pt 2):290–2. doi: 10.1016/0190-9622(93)70178-v
30. Kaur S, Bery A, Garg B, Sood N. Sweet's Syndrome Associated With Chronic Neutrophilic Leukemia. *Indian J Dermatol Venereol Leprol* (2015) 81(2):203–6. doi: 10.4103/0378-6323.152304
31. Kwon O, Park J, Chung H, Park KD. Leukemia Cutis in Chronic Neutrophilic Leukemia Associated With Colony Stimulating Factor 3 Receptor Mutation: Clinical Severity Paralleled With Hematologic Abnormality. *Ann Dermatol* (2019) 31(6):673–7. doi: 10.5021/ad.2019.31.6.673
32. Hobbs LK, Carr PC, Gru AA, Flowers RH. Case and Review: Cutaneous Involvement by Chronic Neutrophilic Leukemia Vs Sweet Syndrome- A Diagnostic Dilemma. *J Cutan Pathol* (2021) 48(5):644–9. doi: 10.1111/cup.13925
33. Mendiola VL, Qian YW, Jana B. Septic Shock Predisposed by an Underlying Chronic Neutrophilic Leukemia With an Atypical Presentation; A Case Report. *Case Rep Oncol* (2018) 11(3):871–9. doi: 10.1159/000495458
34. Sugino K, Gocho K, Ota H, Kobayashi M, Sano G, Isobe K, et al. Miliary Tuberculosis Associated With Chronic Neutrophilic Leukemia. *Intern Med* (2009) 48(15):1283–7. doi: 10.2169/internalmedicine.48.2080
35. Delgado Beltrán P, Rubio-Félix D, García Zueco JC, Capablo JL, Alvarez R. [Chronic Neutrophilic Leukemia (Cnl) and Meningeal Tuberculosis (Tbc): Chance or Cause?]. *Sangre (Barc)* (1995) 40(3):235–7.
36. Yasui H, Adachi Y, Ishii Y, Kato Y, Imai K. Mucormycosis as an Etiology of Cerebral Hemorrhage in Patients With Chronic Neutrophilic Leukemia. *Am J Med* (2003) 115(8):674–6. doi: 10.1016/s0002-9343(03)00426-1
37. Feremans W, Marcelis L, Ardichvili D. Chronic Neutrophilic Leukaemia With Enlarged Lymph Nodes and Lysozyme Deficiency. *J Clin Pathol* (1983) 36(3):324–8. doi: 10.1136/jcp.36.3.324
38. Nakamine H, Hirano K, Tsujimoto M, Nishino E, Takenaka T, Maeda J, et al. Lymph Node Involvement in Chronic Neutrophilic Leukemia. An Immunohistochemical Study. *Virchows Arch A Pathol Anat Histopathol* (1988) 412(3):241–5. doi: 10.1007/bf00737148
39. Watanabe A, Yoshida Y, Yamamoto H, Kimura S, Nagashima H, Kitajima K. A Case of Chronic Neutrophilic Leukemia With Paraproteinemia (Igg Type Lambda and Iga Type K). *Jpn J Med* (1984) 23(1):39–44. doi: 10.2169/internalmedicine1962.23.39
40. Luo Q, Shen J, Yang Y, Tang H, Shi M, Liu J, et al. Csf3r T618i, Asxl1 G942 Fs and Stat5b N642h Trimutation Co-Contribute to a Rare Chronic Neutrophilic Leukaemia Manifested by Rapidly Progressive Leucocytosis, Severe Infections, Persistent Fever and Deep Venous Thrombosis. *Br J Haematol* (2018) 180(6):892–4. doi: 10.1111/bjh.14456
41. Szuber N, Finke CM, Lasho TL, Elliott MA, Hanson CA, Pardanani A, et al. Csf3r-Mutated Chronic Neutrophilic Leukemia: Long-Term Outcome in 19 Consecutive Patients and Risk Model for Survival. *Blood Cancer J* (2018) 8(2):21. doi: 10.1038/s41408-018-0058-7
42. Takemori N, Hirai K, Onodera R, Saito N, Namiki M. Electron Microscopic Findings of Parallel Tubular Granules in a Case of Chronic Neutrophilic Leukaemia. *J Clin Pathol* (1994) 47(4):367–9. doi: 10.1136/jcp.47.4.367
43. Kanoh T, Saigo K, Yamagishi M. Neutrophils With Ring-Shaped Nuclei in Chronic Neutrophilic Leukemia. *Am J Clin Pathol* (1986) 86(6):748–51. doi: 10.1093/ajcp/86.6.748
44. Kaplan SS, Berkow RL, Joyce RA, Basford RE, Barton JC. Neutrophil Function in Chronic Neutrophilic Leukemia: Defective Respiratory Burst in Response to Phorbol Esters. *Acta Haematol* (1992) 87(1-2):16–21. doi: 10.1159/000204707
45. Okada S, Miyoshi Y, Takizawa Y, Hagiwara S, Mori H, Niikura H, et al. [Neutrophil Dysfunction in Chronic Neutrophilic Leukemia Without Rearrangements of Bcr and Immunoglobulin Heavy Chain Genes]. *Rinsho Ketsueki* (1989) 30(10):1881–5.
46. Ohtsuki T, Katsura Y, Mizukami H, Matsu-ura Y, Kimura F, Ohnishi M, et al. Elevated Neutrophil Function in Chronic Neutrophilic Leukemia. *Am J Hematol* (1992) 41(1):50–6. doi: 10.1002/ajh.2830410110
47. Mehrotra PK, Winfield DA, Fergusson LH. Cellular Abnormalities and Reduced Colony-Forming Cells in Chronic Neutrophilic Leukaemia. *Acta Haematol* (1985) 73(1):47–50. doi: 10.1159/000206273
48. Oogushi K, Shimamoto Y, Hamasaki Y, Koga H, Sano M, Yamaguchi M, et al. The Discrepancy Between Chemotaxis and Leukotriene B4 Production in a Patient With Chronic Neutrophilic Leukemia. *Jpn J Med* (1989) 28(6):717–21. doi: 10.2169/internalmedicine1962.28.717
49. Uemura Y, Taguchi T, Kubota T, Saito T, Bandobashi K, Yokoyama A. Neutrophil Function and Cytokine-Specific Signaling in Chronic Neutrophilic Leukemia. *Int J Lab Hematol* (2009) 31(1):36–47. doi: 10.1111/j.1751-553X.2007.01000.x
50. Feng Y, Feng W. Numerous Ring Sideroblasts in Chronic Neutrophilic Leukemia. *Blood* (2021) 138(18):1783. doi: 10.1182/blood.2021013310
51. Yin B, Chen X, Gao F, Li J, Wang HW. Analysis of Gene Mutation Characteristics in Patients With Chronic Neutrophilic Leukaemia. *Hematology* (2019) 24(1):538–43. doi: 10.1080/16078454.2019.1642554
52. Tang DJ, Yu ZF, Song JZ, Chen MC, Wang ZW. Gaucher-Like Cells in Chronic Neutrophilic Leukemia. A Case Report. *Chin Med J (Engl)* (1987) 100(12):988–9. doi: 10.5555/cmj.0366-6999.100.12.p988.01
53. Ito K, Usuki K, Iki S, Urabe A. Chronic Neutrophilic Leukemia Associated With Chronic Lymphocytic Leukemia. *Int J Hematol* (1998) 68(1):87–94. doi: 10.1016/s0925-5710(98)00031-0
54. Vorobiof DA, Benjamin J, Kaplan H, Dvilansky A. Chronic Granulocytic Leukemia, Neutrophilic Type, With Paraproteinemia (Iga Type K). *Acta Haematol* (1978) 60(5):316–20. doi: 10.1159/000207729
55. Naparstek Y, Zlotnick A, Polliack A. Coexistent Chronic Myeloid Leukemia and Iga Monoclonal Gammopathy: Report of a Case and Review of the Literature. *Am J Med Sci* (1980) 279(2):111–5. doi: 10.1097/00000441-198003000-00005
56. Kubo A, Kawanami M, Matsuyama E, Tamura T, Kanoh T. [Chronic Neutrophilic Leukemia Associated With Monoclonal Gammopathy (Iga, Kappa Type)]. *Rinsho Ketsueki* (1989) 30(6):858–62.
57. Florensa L, Woessner S, Vicente P, Roig Martí A, Solé F, Pérez A. Chronic Neutrophilic Leukemia Associated With Monoclonal Gammopathy of Undetermined Significance. A Multimethod Study. *Ann Hematol* (1993) 67(3):129–31. doi: 10.1007/bf01701736
58. Gao JP, Zhai LJ, Gao XH, Min FL. Chronic Neutrophilic Leukemia Complicated With Monoclonal Gammopathy of Undetermined Significance: A Case Report and Literature Review. *J Clin Lab Anal* (2022) 00:e24287. doi: 10.1002/jcla.24287
59. Franchi F, Seminara P, Giunchi G. Chronic Neutrophilic Leukemia and Myeloma. Report on Long Survival. *Tumori* (1984) 70(1):105–7. doi: 10.1177/030089168407000117
60. Lewis MJ, Oelbaum MH, Coleman M, Allen S. An Association Between Chronic Neutrophilic Leukaemia and Multiple Myeloma With a Study of Cobalamin-Binding Proteins. *Br J Haematol* (1986) 63(1):173–80. doi: 10.1111/j.1365-2141.1986.tb07507.x
61. Zoumbos NC, Chrysanthopoulos C, Starakis J, Kapatais-Zoumbos K. Kappa Light Chain Myeloma Developing in a Patient With Chronic Neutrophilic Leukaemia. *Br J Haematol* (1987) 65(4):504–5. doi: 10.1111/j.1365-2141.1987.tb04164.x
62. Rovira M, Cervantes F, Nomdedeu B, Rozman C. Chronic Neutrophilic Leukaemia Preceding for Seven Years the Development of Multiple Myeloma. *Acta Haematol* (1990) 83(2):94–5. doi: 10.1159/000205176

63. Standen GR, Jasani B, Wagstaff M, Wardrop CA. Chronic Neutrophilic Leukemia and Multiple Myeloma. An Association With Lambda Light Chain Expression. *Cancer* (1990) 66(1):162–6. doi: 10.1002/1097-0142(19900701)66:1<162::aid-cncr2820660129>3.0.co;2-z
64. Hidalgo C, Camacho J, Fernández J, Hernández A, Poveda F, Peña JM. [Chronic Neutrophilic Leukemia: Apropos of 2 Cases and Review of the Literature]. *Med Clin (Barc)* (1990) 95(11):421–3.
65. Troussard X, Lebrun E, Macro M, Galateau F, Reman O, Leporrier M. [Neutrophilic Leukemia and Multiple Myeloma. 2 Cases]. *Ann Med Interne (Paris)* (1992) 143(2):136–9.
66. Masini L, Salvarani C, Macchioni P, Baldi G, Rossi F, Croci G, et al. Chronic Neutrophilic Leukemia (Cnl) With Karyotypic Abnormalities Associated With Plasma Cell Dyscrasia: A Case Report. *Haematologica* (1992) 77(3):277–9.
67. Diéguez JC, Fernández Jurado A, Amián A, Rodríguez JN, Martino ML, Cañavate M, et al. [Chronic Neutrophilic Leukemia Associated With Myeloma. Simultaneous Presentation]. *Sangre (Barc)* (1992) 37(5):403–6.
68. Cehreli C, Undar B, Akkoc N, Onvural B, Altungoz O. Coexistence of Chronic Neutrophilic Leukemia With Light Chain Myeloma. *Acta Haematol* (1994) 91(1):32–4. doi: 10.1159/000204241
69. Dinçol G, Nalçaci M, Doğan O, Aktan M, Küçükkaya R, Ağan M, et al. Coexistence of Chronic Neutrophilic Leukemia With Multiple Myeloma. *Leuk Lymphoma* (2002) 43(3):649–51. doi: 10.1080/10428190290012218
70. Taiwo E, Wang H, Lewis R. Treatment of Coexisting Chronic Neutrophilic Leukemia and Light Chain Multiple Myeloma With Hydroxyurea, Bortezomib, and Dexamethasone. *Case Rep Hematol* (2014) 2014:869395. doi: 10.1155/2014/869395
71. Shi J, Ni Y, Li J, Qiu H, Miao K. Concurrent Chronic Neutrophilic Leukemia Blast Crisis and Multiple Myeloma: A Case Report and Literature Review. *Oncol Lett* (2015) 9(5):2208–10. doi: 10.3892/ol.2015.3043
72. Bain BJ, Ahmad S. Chronic Neutrophilic Leukaemia and Plasma Cell-Related Neutrophilic Leukaemoid Reactions. *Br J Haematol* (2015) 171(3):400–10. doi: 10.1111/bjh.13600
73. Dao KH, Tyner JW. What's Different About Atypical Cml and Chronic Neutrophilic Leukemia? *Hematol Am Soc Hematol Educ Program* (2015) 2015:264–71. doi: 10.1182/asheducation-2015.1.264
74. Roberts AW. G-Csf: A Key Regulator of Neutrophil Production, But That's Not All! *Growth Factors* (2005) 23(1):33–41. doi: 10.1080/08977190500055836
75. Mehta HM, Corey SJ. G-Csf, the Guardian of Granulopoiesis. *Semin Immunol* (2021) 54:101515. doi: 10.1016/j.smim.2021.101515
76. Liongue C, Ward AC. Granulocyte Colony-Stimulating Factor Receptor Mutations in Myeloid Malignancy. *Front Oncol* (2014) 4:93. doi: 10.3389/fonc.2014.00093
77. Druhan LJ, Ai J, Massullo P, Kindwall-Keller T, Ranalli MA, Avalos BR. Novel Mechanism of G-Csf Refractoriness in Patients With Severe Congenital Neutropenia. *Blood* (2005) 105(2):584–91. doi: 10.1182/blood-2004-07-2613
78. Mehta HM, Glaubach T, Long A, Lu H, Przychodzen B, Makishima H, et al. Granulocyte Colony-Stimulating Factor Receptor T595i (T618i) Mutation Confers Ligand Independence and Enhanced Signaling. *Leukemia* (2013) 27(12):2407–10. doi: 10.1038/leu.2013.164
79. Gotlib J, Maxson JE, George TI, Tyner JW. The New Genetics of Chronic Neutrophilic Leukemia and Atypical Cml: Implications for Diagnosis and Treatment. *Blood* (2013) 122(10):1707–11. doi: 10.1182/blood-2013-05-500959
80. Pardanani A, Lasho TL, Laborde RR, Elliott M, Hanson CA, Knudson RA, et al. Csf3r T618i Is a Highly Prevalent and Specific Mutation in Chronic Neutrophilic Leukemia. *Leukemia* (2013) 27(9):1870–3. doi: 10.1038/leu.2013.122
81. Fleischman AG, Maxson JE, Luty SB, Agarwal A, Royer LR, Abel ML, et al. The Csf3r T618i Mutation Causes a Lethal Neutrophilic Neoplasia in Mice That Is Responsive to Therapeutic Jak Inhibition. *Blood* (2013) 122(22):3628–31. doi: 10.1182/blood-2013-06-509976
82. Maxson JE, Tyner JW. Genomics of Chronic Neutrophilic Leukemia. *Blood* (2017) 129(6):715–22. doi: 10.1182/blood-2016-10-695981
83. Zhang H, Wilmot B, Bottomly D, Dao KT, Stevens E, Eide CA, et al. Genomic Landscape of Neutrophilic Leukemias of Ambiguous Diagnosis. *Blood* (2019) 134(11):867–79. doi: 10.1182/blood.2019000611
84. Elliott MA, Pardanani A, Hanson CA, Lasho TL, Finke CM, Belachew AA, et al. Asxl1 Mutations Are Frequent and Prognostically Detrimental in Csf3r-Mutated Chronic Neutrophilic Leukemia. *Am J Hematol* (2015) 90(7):653–6. doi: 10.1002/ajh.24031
85. Thomopoulos TP, Bouhla A, Papageorgiou SG, Pappa V. Chronic Myelomonocytic Leukemia - A Review. *Expert Rev Hematol* (2021) 14(1):59–77. doi: 10.1080/17474086.2021.1860004
86. Cui YJ, Jiang Q, Liu JQ, Li B, Xu ZF, Qin TJ, et al. The Clinical Characteristics, Gene Mutations and Prognosis of Chronic Neutrophilic Leukemia. *Zhonghua Xue Ye Xue Za Zhi* (2017) 38(1):28–32. doi: 10.3760/cma.j.issn.0253-2727.2017.01.006
87. Cui Y, Li B, Gale RP, Jiang Q, Xu Z, Qin T, et al. Csf3r, Setbp1 and Calr Mutations in Chronic Neutrophilic Leukemia. *J Hematol Oncol* (2014) 7:77. doi: 10.1186/s13045-014-0077-1
88. Shou LH, Cao D, Dong XH, Fang Q, Wu Y, Zhang Y, et al. Prognostic Significance of Setbp1 Mutations in Myelodysplastic Syndromes, Chronic Myelomonocytic Leukemia, and Chronic Neutrophilic Leukemia: A Meta-Analysis. *PloS One* (2017) 12(2):e0171608. doi: 10.1371/journal.pone.0171608
89. Elliott MA, Hanson CA, Dewald GW, Smoley SA, Lasho TL, Tefferi A. Who-Defined Chronic Neutrophilic Leukemia: A Long-Term Analysis of 12 Cases and a Critical Review of the Literature. *Leukemia* (2005) 19(2):313–7. doi: 10.1038/sj.leu.2403562
90. Ruan GJ, Smith CJ, Day C, Harmsen WS, Zblewski DL, Alkhateeb H, et al. A Population-Based Study of Chronic Neutrophilic Leukemia in the United States. *Blood Cancer J* (2020) 10(6):68. doi: 10.1038/s41408-020-0334-1
91. Yassin MA, Kohla S, Al-Sabbagh A, Soliman AT, Yousif A, Moustafa A, et al. A Case of Chronic Neutrophilic Leukemia Successfully Treated With Pegylated Interferon Alpha-2a. *Clin Med Insights Case Rep* (2015) 8:33–6. doi: 10.4137/CCRep.S22820
92. Martínez-Cuadrón D, Megías-Vericat JE, Serrano J, Martínez-Sánchez P, Rodríguez-Arbolí E, Gil C, et al. Treatment Patterns and Outcomes of 2310 Patients With Secondary Acute Myeloid Leukemia: A Pethema Registry Study. *Blood Adv* (2022) 6(4):1278–95. doi: 10.1182/bloodadvances.2021005335
93. Lasho TL, Mims A, Elliott MA, Finke C, Pardanani A, Tefferi A. Chronic Neutrophilic Leukemia With Concurrent Csf3r and Setbp1 Mutations: Single Colony Clonality Studies, *In Vitro* Sensitivity to Jak Inhibitors and Lack of Treatment Response to Ruxolitinib. *Leukemia* (2014) 28(6):1363–5. doi: 10.1038/leu.2014.39
94. Gunawan AS, McLornan DP, Wilkins B, Waghorn K, Hoade Y, Cross NCP, et al. Ruxolitinib, a Potent Jak1/Jak2 Inhibitor, Induces Temporary Reductions in the Allelic Burden of Concurrent Csf3r Mutations in Chronic Neutrophilic Leukemia. *Haematologica* (2017) 102(6):e238–40. doi: 10.3324/haematol.2017.163790
95. Nooruddin Z, Miltgen N, Wei Q, Schowinsky J, Pan Z, Tobin J, et al. Changes in Allele Frequencies of Csf3r and Setbp1 Mutations and Evidence of Clonal Evolution in a Chronic Neutrophilic Leukemia Patient Treated With Ruxolitinib. *Haematologica* (2017) 102(5):e207–9. doi: 10.3324/haematol.2016.163089
96. Stahl M, Xu ML, Steensma DP, Rampal R, Much M, Zeidan AM. Clinical Response to Ruxolitinib in Csf3r T618-Mutated Chronic Neutrophilic Leukemia. *Ann Hematol* (2016) 95(7):1197–200. doi: 10.1007/s00277-016-2664-4
97. Hinze A, Rinke J, Hochhaus A, Ernst T. Durable Remission With Ruxolitinib in a Chronic Neutrophilic Leukemia Patient Harboring a Truncation and Membrane Proximal Csf3r Compound Mutation. *Ann Hematol* (2021) 100(2):581–4. doi: 10.1007/s00277-020-04152-w
98. Dao KT, Gotlib J, Deininger MMN, Oh ST, Cortes JE, Collins RH Jr, et al. Efficacy of Ruxolitinib in Patients With Chronic Neutrophilic Leukemia and Atypical Chronic Myeloid Leukemia. *J Clin Oncol* (2020) 38(10):1006–18. doi: 10.1200/jco.19.00895
99. Stoner RC, Press RD, Maxson JE, Tyner JW, Dao KT. Insights on Mechanisms of Clonal Evolution in Chronic Neutrophilic Leukemia on Ruxolitinib Therapy. *Leukemia* (2020) 34(6):1684–8. doi: 10.1038/s41375-019-0688-1

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.

this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Oncology

REVIEW
published: 05 August 2022
doi: 10.3389/fonc.2022.884723

Allogeneic Hematopoietic Stem Cell Transplantation for Mixed or Overlap Myelodysplastic/Myeloproliferative Disorders

Argiris Symeonidis[1*], *Spiros Chondropoulos*[2], *Evgenia Verigou*[3], *Vasileios Lazaris*[3], *Alexandra Kourakli*[3] *and Panagiotis Tsirigotis*[4]

[1] University of Patras Medical School, Hematology Division, Patras, Greece, [2] University General Hospital Attikon, Athens, Greece, [3] Hematology Division, General University Hospital of Patras, Rion of Patras, Greece, [4] Department of Medicine, School of Health Sciences, National and Kapodistrian University of Athens, Athens, Greece

OPEN ACCESS

Edited by:
Marcos De Lima,
The Ohio State University,
United States

Reviewed by:
Francesco Onida,
IRCCS Ca 'Granda Foundation Maggiore Policlinico Hospital, Italy
Depei Wu,
The First Affiliated Hospital of Soochow University, China

***Correspondence:**
Argiris Symeonidis
argiris.symeonidis@yahoo.gr
orcid.org/0000-0002-0543-046X

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

Received: 26 February 2022
Accepted: 23 May 2022
Published: 05 August 2022

Citation:
Symeonidis A, Chondropoulos S, Verigou E, Lazaris V, Kourakli A and Tsirigotis P (2022) Allogeneic Hematopoietic Stem Cell Transplantation for Mixed or Overlap Myelodysplastic/Myeloproliferative Disorders. Front. Oncol. 12:884723. doi: 10.3389/fonc.2022.884723

Chronic myelomonocytic leukemia (CMML) and the remaining, less frequent hybrid, mixed, or overlap myelodysplastic syndromes/myeloproliferative neoplasms (MDSs/MPNs) are difficult to treat neoplastic hematological disorders, exhibiting substantial clinical and prognostic heterogeneity, for which clear therapeutic guidelines or effective treatment options are still missing. CMML has an overall survival ranging from a few months to several years. Although patients with proliferative or dysplastic features may benefit from hydroxyurea and hypomethylating agent treatment, respectively, none of these treatments can establish long-term remission and prevent the inevitable transformation to acute leukemia. Novel targeted treatment approaches are emerging but are still under investigation. Therefore, currently, allogeneic stem cell transplantation (allo-SCT) remains the only treatment modality with a curative potential, but its widespread application is limited, due to significant morbidity and mortality associated with the procedure, especially in the elderly and in patients with comorbidities. Recognition of patient eligibility for allo-SCT is crucial, and the procedure should be addressed to patients with a good performance status without severe comorbidities and mainly to those in intermediate- to high-risk category, with a suitable stem cell donor available. The issues of best timing for performing transplantation, patient and donor eligibility, the type of conditioning regimen, and the outcomes after various allo-SCT procedures are the topics of this review.

Keywords: allogeneic stem cell transplantation, chronic myelomonocytic leukemia (CMML), atypical chronic myelogenous leukemia (aCML), Juvenile Myelomonocytic Leukemia (JMML), chronic neutrophilic leukemia (CNL), outcome, prognosis

INTRODUCTION

Myelodysplastic syndromes/myeloproliferative neoplasms (MDSs/MPNs) represent a difficult-to-treat group of clonal hematopoietic stem cell disorders, without specific molecular signatures, exhibiting both, myelodysplastic and myeloproliferative features. According to the most recent revision of the World Health Organization (WHO) classification, entities classified in this category include chronic myelomonocytic leukemia (CMML), atypical bcr/abl-negative chronic myeloid leukemia (aCML), juvenile myelomonocytic leukemia (JMML), and myelodysplastic syndrome/myeloproliferative neoplasm unclassifiable (MDS/MPN-U) (1, 2). Chronic neutrophilic leukemia (CNL), although currently classified among Myeloproliferative Neoplasms (MPNs), sometimes shares several dysplastic features, and it has been postulated that this disease might stand closer to MDS/MPN (3, 4).

For the most common entity, CMML, several prognostic systems have been proposed to best stratify patient life expectation, according to disease aggressiveness. French-American-British (FAB) Classification has defined the threshold of 13×10^9/l white blood cells (WBCs) to distinguish the dysplastic from the proliferative subtype, but this cutoff value cannot reflect the biological differences of the two subtypes. Cytogenetic risk, appears not to be comparable to that of classical MDS (5). Prognostically relevant is the WHO 2016 classification, based on the percentage of peripheral blood (PB) and/or bone marrow (BM) blasts, as CMML-0 (BM blasts 0%–4%), CMML-1 (5%–9%), and CMML-2 (10%–19%), although often clear differences between CMML-0 and CMML-1 may not be found. However, difficulties might emerge in the correct characterization of marrow blasts, since several immature monocytoid cells could be considered blasts, as also promonocytes should be considered as blasts, together with myeloblasts and monoblasts. Thus, this concept should always be kept in mind when assessing patient risk according to the WHO subclassification (CMML-0 vs. CMML-1 vs. CMML-2) and/or according to scoring systems such as the CMML Prognostic Scoring System(CPSS) including BM blast percentage among prognostic factors to be considered (6). Other systems, such as the MD Anderson Prognostic Score (MDAPS) and the Mayo prognostic model, rely on clinical, morphological, and laboratory parameters (7, 8) because either they did not test the importance of genetic markers (7) or the tested markers were not proven to be prognostically important (8). Molecular information has been incorporated within the Group Francophone (GFM) (9), the Mayo, and the CMML Prognostic Scoring System molecular (CPSS-mol) (10) prognostic systems, in the latter together with cytogenetics as genetic risk grouping. In a comparative study between the CPSS, the MDAPS, and the Mayo prognostic system, CPSS was found superior, and the authors further improved it by adding platelet count, thus creating the CPSS-P (11). However, all of these tools are applicable at baseline, and not to patients proceeding to allogeneic stem cell transplantation (allo-SCT). BM fibrosis may occur in some CMML patients, who more frequently exhibit Janus Kinase 2 (JAK2) gene mutations. Patients with fibrotic CMML have a dismal outcome and should be distinguished from patients with primary myelofibrosis and monocytosis (12). Finally, therapy-related CMML appears to be pathogenetically distinct, has worse prognosis than primary disease, and has also been suggested to be distinguished (13, 14).

OVERVIEW OF TREATMENT APPROACHES FOR CHRONIC MYELOMONOCYTIC LEUKEMIA AND THE OTHER MYELODYSPLASTIC SYNDROMES/ MYELOPROLIFERATIVE NEOPLASMS

Treatment options for these diseases vary from supportive care to allo-SCT. This variability clearly reflects the extreme heterogeneity of prognosis, according to disease and patient characteristics at diagnosis. Until recently, clear treatment guidelines were lacking, although excellent reviews and treatment recommendations have been published (15–18). The lack of evidence-based recommendations is mainly attributed to the absence of large, multicenter, prospective, randomized trials investigating prespecified treatment outcomes. A potential explanation is the high degree of clinical, laboratory, molecular, and prognostic heterogeneity of these diseases. The use of hypomethylating agents (HMAs) as initial treatment becomes more and more popular; however, results are less favorable than those achieved by patients with classical MDS, fewer patients achieve complete remission (CR), and responses are shorter (19, 20). Recent molecular analyses have shown that ASXL1 and RAS mutations are associated with poorer response to HMA treatment, whereas TET2 mutations represent a favorable factor for response (21).

Oral cytoreductive treatment, usually hydroxyurea, is temporarily effective and is administered to patients with the proliferative subtype or with extramedullary organ involvement. Single-agent chemotherapy, most commonly low-dose subcutaneous or intravenous cytarabine, may be given to patients with uncontrolled monocytosis and/or increased marrow blasts. However, responses are short, and the majority of patients soon become refractory, developing multidrug resistance. Combination chemotherapy, consisting of cytarabine plus an anthracycline or a topoisomerase inhibitor, may be more effective, induces longer remissions, but is poorly tolerated by the usually advanced-aged or unfit patients. Combination chemotherapy-induced CR is usually of short duration, as compared to remissions induced in patients with *de novo* AML.

The only treatment with curative potential remains allo-SCT, which should be provided to all patients with prognostically unfavorable, rapidly evolving, or symptomatic CMML, or other MDSs/MPNs, who have an available stem cell donor. Ideally, this should be performed early, before disease progression, because in the latter case, non-relapse mortality (NRM) and relapse rate (RR) are higher and worse than those in AML evolved from classical MDS (22). There are several barriers, preventing the broad application of allo-SCT, including patient-related issues (advanced age, poor performance status, comorbidities) and disease-related issues (unstable disease, delayed postchemotherapy marrow reconstitution, infectious complications, and organ impairment).

Several studies aim to rationalize the therapeutic decision for allo-SCT in these diseases. According to the Mayo Clinic experience, among 406 patients, 70 underwent allo-SCT, and median leukemia-free survival (LFS) by the application of propensity score-matched analysis was clearly better in the transplanted than that in the non-transplanted group (40 vs. 20 months) as was overall survival (OS; 40 vs. 21 months) (23). Furthermore, in a multicenter analysis of 261 patients, of whom 119 underwent allo-SCT, after a prolonged median follow-up of 6.1 years, transplanted patients had significantly better median OS (4.3 vs. 2.3 years) (24).

EXPERIENCE FROM ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES / MYELOPROLIFERATIVE NEOPLASMS

A. Chronic Myelomonocytic Leukemia

The existing experience is restricted to retrospective analyses from various transplant groups. However, in many retrospective studies, results of allo-SCT in patients with CMML are pooled together with the results of patients with other myeloid malignancies (other MDSs/MPNs, MDS, AML, MPN, etc.), and only exceptionally the outcomes of CMML patients are mentioned separately. Most recently, however, some retrospective studies have focused on the outcomes of CMML and occasionally on other MDSs/MPNs.

In early reports from the MD Anderson Cancer Center (MDACC) on 20 patients (8 with CMML), probabilities for disease-free survival (DFS) and OS at 2 years were 37% and 47%, respectively (25), while from the Mayo Clinic, among 17 transplanted patients <60 years, 7 were relapsed and, in 6 of them, 1–3 courses of donor leukocyte infusions (DLIs) were offered. Despite the high treatment-related mortality (TRM) of 41% at 3 years, 3 patients remained alive, indicating a graft-versus-leukemia (GVL) effect (26). In support of a GVL effect was the first analysis of the European Blood and Marrow Transplantation Registry (EBMT) on 50 patients, who, despite a 52% TRM at 5 years, demonstrated a lower probability of relapse when grade II–IV acute graft-versus-host disease (GVHD) developed (24% vs. 54%) and higher RR when patients received T cell-depleted allografts (27). Among 148 patients, who received a reduced-intensity conditioning (RIC), after a median of 47 months, relapse-free-survival (RFS), OS, and RR at 3 years were 27%, 27%, and 41%, respectively. For 7 CMML patients, RFS and OS were both 43% (28). Initial experience from Hamburg on 12 patients was also positive, with a low TRM, 50% DFS, and 75% OS at 4 years (29). At the King's College, 18 CMML patients received an RIC and T cell-depleted allografts. The probabilities of OS, RR, and TRM at 3 years were 31%, 47%, and 31%, respectively (30). Among 21 CMML patients transplanted in the Fred Hutchinson before 2000, 9 achieved sustained Disease-Free Survival (DFS) after a median of 7 years (31), and in a later analysis of 43 patients, NRM, RR, RFS, and OS at 4 years were 35%, 23%, 41%, and 41%, respectively (32). In the Mayo Clinic, out of 43 patients (35 with CMML, 17 on AML status), after a median of 21 months, NRM, RR, and OS were 25%, 29%, and 55%, respectively, for patients transplanted in the chronic phase and 34%, 40%, and 47%, respectively, for those transplanted following AML transformation (33). Similar results were confirmed on 70 patients, of whom 46 were transplanted in the chronic phase and 24 after AML transformation. Median OS was better for patients transplanted in the chronic phase (67 vs. 16 months), and Kaplan-Meier (K-M) estimates for OS at 5 years was 51%—one of the best ever reported (23).

In the French retrospective study of 73 CMML patients, OS, NRM, Event-Free Survival (EFS), and RR at 3 years were 32%, 36%, 29%, and 35%, respectively. NRM was lower in female patients, those transplanted after 2004, and in patients without palpable splenomegaly or pretransplant infections (34). Another collaborative analysis of 85 CMML patients, including 14 with therapy-related disease, reported 25% RR at 3 years, and the use of myeloablative conditioning was associated with better outcomes, compared to RIC (35). In the analysis from MDACC on 83 patients, the 12-month TRM was 31%, and patients who were bridged with HMA had lower RR at 3 years, compared to those receiving AML-type induction chemotherapy (22% vs. 35%), resulting in significantly longer PFS (43% vs. 27%) (36). A German group report on 45 patients, mainly with CMML, observed a low 3-year NRM of 26.7%, while OS at 5 years was 51%. The presence of mutations was used as a marker of minimal residual disease, and their persistence 6 months posttransplant was associated with significantly higher RR (37). The Nordic group applied a *post-hoc* analysis on 51 CMML patients, with a median follow-up of 5.5 years, and identified clonal mutations in 48 of them. ASXL1, TET2, RUNX1, SRSF2, and RAS were the most frequently mutated genes. Transplantation outcomes were better than those previously reported, with a 5-year OS of 46.5%, NRM of 30%, and RR of 25% (38).

The impact of the donor was investigated on 159 Japanese patients. OS, NRM, and RR at 3 years were 33%, 28%, and 39%, respectively, and the best OS was obtained by [(MRDs), 50.4%], followed by matched-unrelated donors (MUDs, 31.4%), umbilical cord blood (UCB, 15.4%; TRM >75%), and mismatched-unrelated donors (MMUDs, 16.7%) (39). The Fred Hutchinson group described outcomes on 129 patients with the longest median follow-up (9.3 years). Estimated probabilities for relapse, DFS, and OS at 10 years were 32%, 29%, and 30%, respectively, whereas NRM was 32% (40).

Many studies have focused on the conditioning regimen, and majority of them do not report any impact, with the exception of one small study on 10 patients, in which myeloablative conditioning was associated with longer EFS (41). The same is reported by the Heidelberg group on 44 patients, in whom intermediate total body irradiation (TBI) dose (6–8 Gy) combined with mofetil mycophenolate posttransplant immunosuppression was associated with longer LFS in the elderly and less fit patients, compared to alkylator-based conditioning (42). A treosulfan-fludarabine regimen, although administered to older patients with comorbidities, was accompanied by better OS than standard myeloablative or RIC regimens (43). The addition of 2 Gy TBI over a standard treosulfan-fludarabine regimen was investigated on 51 patients

with MDS and 49 with CMML. The TBI regimen showed superiority and was associated with longer PFS, whereas NRM was only 9% (44). In another prospective study on 77 patients (13 with CMML), a three-level dose-escalation TBI at non-myeloablative doses (300–450 cGy) was tested. RR, NRM, PFS, and OS at 5 years were 31%, 43%, 35%, and 38%, respectively (45). Total lymphoid irradiation (TLI) and anti-thymocyte globulin (ATG) were used in Stanford for 61 patients, and NRM at 3 years was only 11%, whereas PFS and OS were 35% and 41%, respectively. The authors recommend this regimen for patients with more advanced age (46).

The International Blood and Marrow Transplantation Registry (IBMTR) and the EBMT have published the largest retrospective studies. In the first, 209 patients were transplanted between 2001 and 2012, 35% of them receiving a graft from MRD and 27% exhibiting >5% marrow blasts. The type of bridging treatment (HMA vs. chemotherapy) and the type of conditioning (myeloablative vs. RIC) had no impact on outcome. TRM, RR, DFS, and OS at 5 years were 28%, 52%, 20%, and 30%, respectively (47). In the second, 513 patients who received a related (55.5%) or an unrelated graft (44.5%) following myeloablative conditioning (48.5%) or RIC (44%) were transplanted until December 31, 2009. Disease status at transplantation was CR in 24% and no CR in 67%. NRM, RR, DFS, and OS at 4 years were 41%, 32%, 27%, and 33%, respectively (48).

There is only one study describing encouraging outcomes with haploidentical transplantation on 19 CMML patients. The incidence of acute and chronic GVHD was acceptable, the 3-year TRM was 27%, and RR was only 11%, whereas LFS and OS were 57% and 64%, respectively. The authors suggest that this type of allo-SCT might exert a stronger GVL effect, and hence, RR may be low (49). A summary of the published reports on CMML, with the main patient and transplantation features and outcomes, is presented in **Table 1**.

B. Juvenile Myelomonocytic Leukemia

JMML is a rare pediatric leukemia affecting 1.2 children per million annually, with a median age at diagnosis of 2 years and a clear male predominance. It has an aggressive clinical course with a median OS of 10–12 months (51). Main features include splenomegaly, lung and gastrointestinal system monocytic infiltration, a leukoerythroblastic peripheral smear with absolute monocytosis, elevated fetal hemoglobin (HbF), and a hypercellular BM with increased blast percentage but <20% (52). Nearly all JMML cases (90%–95%) harbor either somatic mutations of the RAS pathway genes (PTPN11, KRAS, NRAS) or germline mutations of NF1 and CBL, which are involved in two congenital development disorders, namely, neurofibromatosis and Cbl protooncogene – E3 ubiquitin protein kinase (CBL) syndrome (53). Noonan syndrome caused by germline mutations of PTPN11, NRAS, KRAS, BRAF, SOS1, and RAF1 may exhibit a JMML-like disorder that is usually self-limited (54). Age >2 years at diagnosis, platelets <33 x 10^9/L, and HbF >10% have been identified as main predictors of poor survival (55).

In essentially all cases of JMML, allo-SCT is strongly indicated and ideally should be performed immediately after diagnosis. Cytoreductive strategies usually involve azacytidine or AML-type chemotherapy, while occasionally, splenectomy is performed for symptom alleviation. Since the patient population is composed of children, TBI is not usually included in the conditioning, but busulfan-based regimens are used. The European Working Group on MDS (EWOG-MDS) provides a recommendation for a three-alkylator regimen consisting of busulfan, cyclophosphamide, and melphalan (56). MRD or MUD should be the first option, while one-locus MMUD or UCB transplantation is a reasonable alternative. Recently, a large study from China compared 27 patients transplanted with an MRD or MUD (Cohort-1), with 20 patients who underwent allo-SCT by using an haploidentical or an MMUD with 2 or 3 HLA disparities (Cohort-2). With a median follow-up of 26 months, OS, DFS, and NRM were 66%, 55%, and 11%, respectively, in the entire group, but the cumulative RR was significantly increased in Cohort-1 as compared with Cohort-2 (56% vs. 5%, $P \leq 0.001$). Nevertheless, haploidentical allo-SCT might represent a solution for patients with a rapidly evolving disease for whom an MRD or an MUD is not available (57).

Age at diagnosis >2 years, NF1 or PTPN11 mutation, and high DNA methylation define a patient group with an RR of >50%, raising the issue of immunosuppression intensity and posttransplant prophylaxis (58). Thus, EWOG-MDS recommends keeping immunosuppression with cyclosporine-A at low levels (~80 g/L) and tapering early (from day +40 in the absence of grade II–IV GVHD). Donor chimerism should be tested at very short intervals (even weekly in high-risk patients), since the reappearance of small autologous cell populations mandates immediate withdrawal of immunosuppression (59). Prevention of relapse by preemptive administration of azacytidine or DLI is a frequently applied strategy. Novel approaches such as MAP kinase–ERK kinase (MEK) inhibitors (trametinib) or bcl2 inhibitors (venetoclax) in combination with azacytidine and anti-CD47 monoclonal antibodies are currently evaluated in the context of clinical trials (60). **Table 2** presents the results of allo-SCT in patients with JMML (56, 57, 61–63).

C. Atypical Chronic Myelogenous Leukemia and Unclassified Myelodysplastic Syndrome/ Myeloproliferative Neoplasm

aCML mainly affects elderly patients of male predominance and is characterized by inherent propensity for AML transformation. This is a difficult-to-treat disease with the available conventional treatment options with a median OS of about 2 years from initial diagnosis. In a group of 73 patients, age >65 years, anemia (<10 g/dl) and severe leukocytosis (>50 × 10^9/l) at diagnosis were recognized as significant adverse prognostic factors and have been used to construct a simple prognostic system, greatly affecting survival (64). In two cohorts of 55 and 65 patients from Italy and the United States, prognosis was generally poor and median OS was 25 and 12 months, respectively. AML transformation occurred in >30% of patients between 12 and 18 months from initial diagnosis [65, 66]. In a retrospective analysis of 65 patients from MDACC, intensive chemotherapy was poorly tolerated and was associated with significantly decreased OS, as compared to HMA, hydroxyurea, or ruxolitinib treatment. Recently, in a new multivariate analysis on 65 patients, older

TABLE 1 | Published retrospective studies reporting transplantation outcomes on CMML.

Year	Group	1st Author/ Reference	All pts	CMML pts	Other pts	Med Age	♂/♀	Disease status at SCT (N,%)	RIC (%)	Related Donor n (%)	Med F-up (mo)	aGVHD N (%)	cGVHD N (%)	NRM (%)	RR (%)	DFS/RFS (%)	OS (%)
2000	Fred Hutch	Zhang (31)	21	21	0	47	14/7: 2.0	CP: 9 (43) EB: 12 (57)	0	12 (57)	83.0	15/21 (71)	14/16 (87)	33	25	3-yr 39	5-yr 43
2002	EBMT	Kröger (25)	50	50	0	44	29/21: 1.4	CP: 32 (64) BP: 18 (36)	N/A	38 (76)	40.0	28/48 (59)	7/27 (26)	52	49	5-yr 18	5-yr 21
2004	MDACC	Mittal (25)	20	8	7	51	15/5: 3.0	Not reported	0	15 (75)	17.5	9/20 (45)	12/20 (60)	30	31	2-yr 37	2-yr 47
2005	Fred Hutch	Kerbauy (32)	43	43	0	48	25/18: 1.4	CMML1: 32 CMML2: 11	2 (5)	18 (42)	69.0	N/A	21/39 (54)	35	23	4-yr 41	4-yr 41
2006	Mayo Clinic	Elliot (26)	17	17	0	50	11/9: 1.2	CP: 9 (53) BP: 8 (47)	1 (6)	14 (82)	34.5	12/16 (75)	6/15 (40)	41	41	3-yr 18	3-yr 18
2009	Hamburg	Ocheni (29)	12	12	0	56	5/7: 0.7	CR: 2 (17) no-CR: 10 (83)	6 (50)	2 (17)	26.0	10/12 (83)	5/10 (50)	25	17	2-yr 67	2-yr 75
2010	King's College	Krishnamurty (30)	18	18	0	54	12/6: 2.0	CR: 8 (44) no-CR: 10 (56)	15 (83)	7 (39)	16.0	8/18 (44)	3/14 (21)	22	47	3-yr 31	3-yr 31
2011	Fred Hutch	Eissa (35)	85	85	0	52	52/33: 1.6	CMML1: 57 CMML2: 26	15 (18)	34 (40)	62.0	58/81 (72)	37/84 (44)	33	27	10yr 38	10yr 40
2013	French	Park S (34)	73	73	0	53	NR	CR: 23 (31) no-CR: 50 (69)	43 (59)	41 (56)	23.0	28/68 (41)	25/71 (35)	36	35	3-yr 35	3-yr 36
2013	German	Fu (37)	45	39	6	57	25/20: 1.3	Not reported	30 (67)	10 (22)	46.0	28/45 (62)	22/40 (55)	27	41	5-yr 34	5-yr 46
2013	Korean	Lim (41)	10	7	3	43	9/1: 9.0	CR: 1 CP: 2, no-CR: 7(70)	5 (50)	5 (50)	47.5	2/10 (20)	4/9 (44)	10	40	5-yr 47	5-yr 42
2015	EBMT	Symeonidis (48)	513	513	0	53	343/ 170: 2	CR: 122(26) no-CR: 344(74)	226 (40)	276 (54)	43.0	155/470 (33)	123/374 (33)	41	32	4-yr 27	4-yr 33
2016	MDACC	Kongtim (36)	83	83	0	57	58/25: 2.3	CR: 24 (29) no-CR: 59 (71)	19 (23)	30 (36)	48.0	27/75 (36)	27/72 (37)	31	33	3-yr 34	3-yr 35
2017	Mayo Clinic	Sharma (33)	43	35	8	55	24/12: 2.0	CP: 18 (51) EB: 17 (49)	21 (49)	19 (54)	21.0	26/35 (74)	22/32 (69)	25-34	29-40	NR	55 vs 47
2017	IBMTR	Duong Liu (47)	209	209	0	57	146/63: 2.3	CR: 136 (65) no-CR: 73 (35)	99 (48)	73 (35)	51.0	76/209 (36)	98/209 (47)	28	52	5-yr 20	5-yr 30
2018	Japanese	Itonaga (39)	159	159	0	54	119/40: 3	CR: 25 (16) no-CR: 134 (84)	67 (42)	51 (32)	NR	NR	NR	28	39	NR	3-yr 33
2020	Fred Hutch	Woo (40)	129	129	0	55	85/44: 1.9	CMML0-1: 52 2-AML: 75	21(19)	38 (29)	88.0	93/126 (74)	57/126(45)	31	32	3-yr 37	3-yr 38
2020	Heidelberg	Radujkovic (42)	44	44	0	61	27/17: 1.6	CR: 14 (32) no-CR: 30 (68)	7 (16)	10 (23)	39.0	6/36 (17)	8/34 (23)	16	44	3-yr 38	3-yr 56
2020	Mayo Clinic	Pophali (23)	70	70	0	58	46/24: 1.8	CP: 46 (66) BP 24 (34)	37 (54)	28 (42)	70.0	29/63 (46)	41/63 (65)	29	27	NR	5-yr 44
2020	Chinese	Sun (49)	19	19	0	41	10/9: 1.1	CR: 3 (16) no-CR: 16 (84)	Beijing pr	Haplo	39.5	7/17 (39)	2/12 (17)	27	11	3-yr 57	3-yr 64
2021	Nordic	Wedge (38)	64	64	0	62.5	49/15: 3.2	CR: 18 (28) no-CR: 46 (72)	23 (38)	20 (31)	65.0	34/60 (56)	-57	30	25	NR	5-yr 46
2021	German	Gagelmann (24)	119	119	0	58	83/36: 2.3	CMML0/1: 65 (55)	63 (53)	26 (22)	73.0	NR	NR	30	27	5-yr 43	5-yr 50
2021	German	Gagelmann (50)	240	240	0	59	172/68: 2.5	CMML0/1: 143 (59.6)	134 (56)	50 (21)	66.0	NR	NR	NR	NR	NR	NR

TABLE 2 | Allogeneic stem cell transplantation in patients with JMML.

Author (ref)	No	Donor	Conditioning	Disease phase	PFS	OS
Lin Y (57)	47	MRD: 11 MUD: 22 Haplo: 14	MAC: 47	Chronic: 38 Blastic: 9	54% (5-year)	66% (5-year)
Tufecki (61)	28	MRD: 18 MUD: 8 UCB: 1 Haplo: 1	MAC: 28	NR	Relapse rate 35%	56% (5-year)
Locatelli (56)	100	MRD: 48 MUD: 52	MAC: 100	Chronic: 77 Blastic: 10 Missing: 13	54% (5-year)	66% (5-year)
Locatelli (62)	110	UCB: 100	MAC: 100	Chronic: 100	44% (5-year)	52% (5-year)
Yoshida (63)	129	MRD: 44 MUD: 85	MAC: 116 RIC: 13	NR	46% (5-year)	64% (5-year)

MRD, matched related donor; MUD, matched unrelated donor; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; PFS, progression-free survival; OS, overall survival.

TABLE 3 | Allogeneic stem cell transplantation in patients with atypical CML.

Author (ref)	No	Donor	Conditioning	Disease phase	PFS	OS
Lim SN (41)	2	MRD: 2	MAC: 2	Chronic: 1 Blastic: 1	>100 months	>100 months
Onida (72)	42	MRD: 27 MUD: 15	MAC: 32 RIC: 10	Chronic: 33 Blastic: 9	36% (5-year)	51% (5-year)
Mittal (25)	20	MRD: 15 MUD: 5	MAC: 17 RIC: 3	NR	31% (18-month)	35% (18-month)
Koldehoff (73)	9	MRD: 6 MUD: 3	MAC: 8 RIC: 1	NR	NR	88% (55-month)
Koldehoff (74)	21	NR	NR	NR	NR	80% (5-year)
Itonaga (75)	14	MRD: 5 MUD: 7 UCB: 2	MAC: 13 RIC: 1	Chronic:9 Blastic: 5	NR	54% (1-year)

MRD, matched related donor; MUD, matched unrelated donor; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; PFS, progression-free survival; OS, overall survival.

TABLE 4 | Allogeneic stem cell transplantation in patients with CNL.

Author (ref)	No	Donor	Conditioning	Disease phase	PFS	OS
Itonaga (75)	5	MUD: 2 UCB: 2 Haplo: 1	MAC: 5	Chronic:5	NR	40% (1-year)
Hasle (81)	2	MRD: 2	MAC: 2	Chronic:2	NR	100% (>5-year)
Szuber (77)	2	NR	MAC: 2	Blastic: 2	NR	50% (40-month)
Goto (82)	1	MUD: 1	MAC: 1	Chronic: 1	NR	100% (3-year)
Kako (83)	1	MUD: 1	MAC: 1	Blastic: 1	Progression d+50	NR
Piliotis (84)	1	MRD: 1	MAC: 1	Chronic:1	>1 year	>1 year

MRD, matched related donor; MUD, matched unrelated donor; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; PFS, progression-free survival; OS, overall survival.

age, thrombocytopenia, increased BM blasts, and abnormal serum lactate dehydrogenase (LDH) were parameters independently associated with decreased OS. Based on these parameters, a new scoring system was generated for a more accurate prediction of survival (67). The mutational landscape of aCML mainly involves SETBP1 and ETNK1 genes, while other commonly identified mutations include ASXL1, N/K-RAS, SRSF2, and TET2 and less frequently (<10%) CBL, CSFR3, and EZH2. JAK2, CARL, and MPL mutations are extremely uncommon (68). SETBP1 mutations have been associated with severe anemia and thrombocytopenia, increased LDH, and worse OS (69, 70). Regarding allo-SCT, many questions related to the timing of transplantation, bridging therapy, donor type, and intensity of preparative regimen still remain unanswered. In a retrospective study of 60 patients with MPN or MDS/MPN in blastic phase from the French Registry, with many of them receiving intensive cytoreduction as bridging treatment, 26 were in CR before allo-SCT, while 34 underwent transplantation with active AML. Not surprisingly, the outcome was extremely poor, with OS and LFS at 3 years of 18% and 9%, respectively. Patients with active disease before transplant had only 3% probability of 3-year OS (71).

Results of allo-SCT were also analyzed by the EBMT on 42 patients, of whom 69% were in first chronic phase, 76% received myeloablative conditioning, and 64% weretransplanted from an

MRD. T-cell depletion was applied in 26% and 87% of MRD and MUD, respectively. According to the EBMT risk score (taking into account the patient's age, disease status, time interval from diagnosis to transplant, donor type, and recipient–donor sex match), 45%, 31%, and 24% of the patients had low, intermediate, and high risk, respectively. This study confirmed the curative potential of allo-SCT in patients with aCML. RFS at 5 years was 36%, NRM was 24%, RR was 40%, and OS was 51%. Age and the EBMT score were significant predictors of OS (72).

Koldehoff et al. reported on 9 patients, of whom 4 were transplanted from MRDs, 4 from MUDs, and 1 from a syngeneic donor. Eight patients received myeloablative conditioning and 8 remain alive, with one relapse of the patient who underwent syngeneic allo-SCT (73). A subsequent follow-up from the same team including 21 patients reported a 5-year OS of 80% with a median survival of 48 months (74). In the early report from MDACC, among 7 patients with aCML, after a median follow-up of 17.5 months, OS and DFS were 35% and 31%, respectively, but five patients died (25). The Japanese group reported outcomes on 19 patients, 15 with aCML and 4 with CNL, who mainly received myeloablative conditioning. One-year OS was >58% and was higher in patients with better performance status and <5% BM blasts (75). Studies reporting results of allo-SCT in patients with aCML are presented in **Table 3**.

D. Chronic Neutrophilic Leukemia

CNL is an extremely rare but aggressive disease, with an estimated annual incidence of 1 case per 10,000,000 individuals, has as a diagnostic hallmark various CSF3R mutations and a median life expectancy of about 1.8 years (76). A prognostic model has been developed in the Mayo Clinic based on data from 19 patients. Retrospective analysis from archival material revealed ASXL1 and SETBP1 gene mutations (besides CSF3R) in 47% and 32% of the patients, respectively. Median OS of the whole group was 22.4 months, and CSF3RT618I mutation (present in 14 patients or 74%) was associated with significantly inferior OS compared to truncation mutations (17.2 vs. 42.7 months). On multivariate analysis, ASXL1 mutation, thrombocytopenia ($<160 \times 10^9$/L), and hyperleukocytosis ($>60 \times 10^9$/L) were associated with decreased OS, and these 3 parameters created a risk model for prognostic stratification of the patients in high- and low-risk groups (77).

In CNL, AML progression is almost inevitable and occurs at a median of 21 months from initial diagnosis. No standard treatment recommendations exist, and current treatments, mainly consisting of hydroxyurea and interferon-alpha, do not exert any disease-modifying benefit. A recent phase II trial investigating the safety and efficacy of ruxolitinib reported an overall response rate of 35%, making ruxolitinib a promising agent that should be tested in larger patient cohorts (78). Intensive AML-type chemotherapy is usually ineffective when administered after disease progression. Therefore, allo-SCT remains the only potentially curative therapeutic option, and evidence supports early referral as an important factor for better outcome (75, 79). In the largest case series from a nationwide survey in Japan, 5 patients were transplanted between 2003 and 2014. Intention to transplant was based on either disease progression or leukocytosis and splenomegaly uncontrolled by cytoreductive treatment. All patients received myeloablative conditioning, and graft source was an MUD (2), UCB (2), and haploidentical sibling (1). One-year OS was 40%, with one patient dying from sinusoidal obstruction syndrome (d+56), one from bleeding (d+19), and one from disease progression (d+76) (75).

Hydroxyurea or ruxolitinib should be administered to all symptomatic patients or those with significant splenomegaly. Myeloablative conditioning should be administered to fit patients <65 years, while an RIC should be preferred for older or less fit patients. An MRD or MUD should be the preferred option. However, taking into account the recent progress in haplo-SCT in double cord transplants in adults and the experience on the treatment of related disorders, an alternative donor can be used in the absence of a fully matched donor. CSF3R mutation can be used as a marker of an minimal residual disease in the posttransplant period, and persistent detection can alter the adoptive immunotherapy strategy in order to prevent relapse (cyclosporine withdrawal, DLI) (80). Schematically, the treatment algorithm of CNL is shown in **Figure 4**. Studies reporting results on allo-SCT in patients with CNL are presented in **Table 4** (75, 77, 83, 84).

FACTORS WITH PROGNOSTIC IMPORTANCE FOR ALLOGENEIC STEM CELL TRANSPLANTATION IN MYELODYSPLASTIC SYNDROMES/ MYELOPROLIFERATIVE NEOPLASMS

Many studies, particularly when including several decades of patients, have investigated predictors of outcomes, either simple factors or prognostic tools, evaluating prognosis in the non-transplant setting of the disease. The first EBMT study found that manifestation of grade II–IV acute GVHD and the use of non-T cell-depleted allografts were associated with longer DFS (27). The importance of early transplantation was initially pointed out by the Fred Hutchinson group (31), and when more patients were analyzed, the only variable associated with a higher NRM and shorter OS was the Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI) (32). The use of posttransplant DLI as an early manipulation of graft failure and of chimerism loss has been applied in at least three studies, two from the Mayo Clinic and one from the King's College with some successful results reported (26, 30, 33). The latter group has found as important prognostic indicators the percentage of BM blasts (≥5% vs. <5%) and pretransplant cytogenetics (33). Similar results are reported by the more recent analysis from Fred Hutchinson on 85 patients, in which the importance of the HCT-CI was also confirmed. Additional important factors for survival were pretransplant hematocrit and age, whereas the MDAPS (7) and a female donor to female recipient were affecting RR (36).

The group of Milwaukee analyzing 86 transplanted patients with various myeloid malignancies, but none with CMML, found no impact of patient's obesity on any outcome, although obese patients (Body Mass Index (BMI) >30) had longer hospitalization periods

(85). The significance of the chronologic period in which transplantation is performed is easily realizable, since outcomes are improving over time and supportive treatment becomes more effective. Thus, in the French study, major determinants for higher NRM and lower EFS and OS were transplantation before 2004 and the presence of palpable splenomegaly. In the same analysis, female patients exhibited significantly higher RR and NRM, and higher NRM was associated with proliferative CMML and with pretransplant infections (34). The significance of splenomegaly was also stressed by a Chinese study on 25 patients of whom, those with splenomegaly had delayed neutrophil recovery and higher RR and incidence of chronic GVHD (86). In a later report from the Mayo Clinic, splenomegaly, lower HCT-CI, and allo-SCT performed ≤12 months from diagnosis were associated with a more favorable outcome. The small group of MDS/MPN-U, which was analyzed separately, exhibited somewhat better outcomes compared to the outcomes of CMML patients (33). In the EBMT study of 42 aCML patients, patient's age and the EBMT prognostic score affected OS, whether RFS was higher in MUD, compared to MRD allo-SCT (72).

Increased BM fibrosis has also been recognized as an adverse prognostic factor for DFS and OS, attributed to delayed engraftment, more common cytogenetic abnormalities, and unfavorable driver mutations according to a Chinese retrospective analysis of 239 MDS patients (87). Poor risk cytogenetics and comorbidities were predictors of worse outcome in the retrospective analysis of MDACC on a patient group with MDS and various MDSs/MPNs, exhibiting dismal prognostic factors, for whom RIC was used (88). In the most recent report of the same group focusing on CMML, the outcomes of 83 patients are described, and in multivariate analysis, <5% BM blasts before allo-SCT, the manifestation of chronic GVHD, and initial treatment with HMA were independent predictors of a favorable outcome. In particular, previous treatment with HMA was associated with lower RR and longer PFS (36).

The significance of the donor was investigated on a Japanese group of 159 CMML patients. HLA-matched sibling donor allo-SCT was associated with longer OS and lower NRM, which was highest in the recipients of umbilical cord blood grafts, attributed to delayed neutrophil engraftment (39). In the study from Heidelberg, unrelated donor allo-SCT and TBI-included conditioning were associated with better OS, whereas CPSS could nicely stratify the probability of OS. In this analysis, age was not a significant factor for OS and no clear benefit was proven for transplanted patients with lower-risk CPSS over those not transplanted. However, as in other studies, CPSS could not predict NRM (42). The impact of GVHD was investigated by two

TABLE 5 | Well-recognized prognostic factors.

At initial diagnosis	At transplantation
Age	Age
HCT Comorbidity Index	HCT Comorbidity Index
Performance status	Performance status at transplantation
RBC transfusion dependency	RBC transfusion dependency
Palpable splenomegaly	Palpable splenomegaly
Lymphadenopathy and other extramedullary disease	Lymphadenopathy and other extramedullary disease
Cytogenetic abnormalities IPSS-described	Cytogenetic abnormalities IPSS-described
Presence of constitutional symptoms	Disease status at transplantation
Gene mutations (ASXL1, RUNX1, SRSF2, JAK2, NRAS vs. TET2)	Gene mutations (ASXL1, RUNX1, SRSF2, JAK2, NRAS vs. TET2)
Trisomy 8	Number of genes mutated
FAB subtype (Dysplastic or Proliferative)	FAB subtype (Dysplastic or Proliferative)
Leukocytosis >15 x 10^9/l/Unstable leukocytosis	Leukocytosis > 15 × 10^9/l
Severity of monocytosis	Severity of monocytosis
Peripheral blood lymphocytosis	Time interval from diagnosis to transplantation
Presence of circulating blast cells	Use of HMA as bridging treatment
Anemia <10 g/dl or severe anemia <8 g/dl	Presence of circulating blast cells
Thrombocytopenia <100 × 10^9/l	Anemia <10 g/dl or severe anemia <8 g/dl
Bone Marrow blasts ≥5%	Neutropenia <1.5 × 10^9/l
Elevated serum LDH	Thrombocytopenia <100 × 10^9/l
Elevated serum ferritin levels	Bone Marrow blasts ≥5%
	Active pretransplant infections
Specific prognostic tools (CPSS, CPSS-mol, Mayo Prognostic Model, MDACC Index, etc.)	CPSS
	MD Anderson Prognostic Index
	EBMT Prognostic Score
	Increased Bone marrow fibrosis
	Myeloablative conditioning
	Use of non-T cell-depleted grafts
	Donor HLA matching
	Donor sex mismatch
	Development of acute GVHD (unfavorable)
	Development of chronic GVHD (favorable)

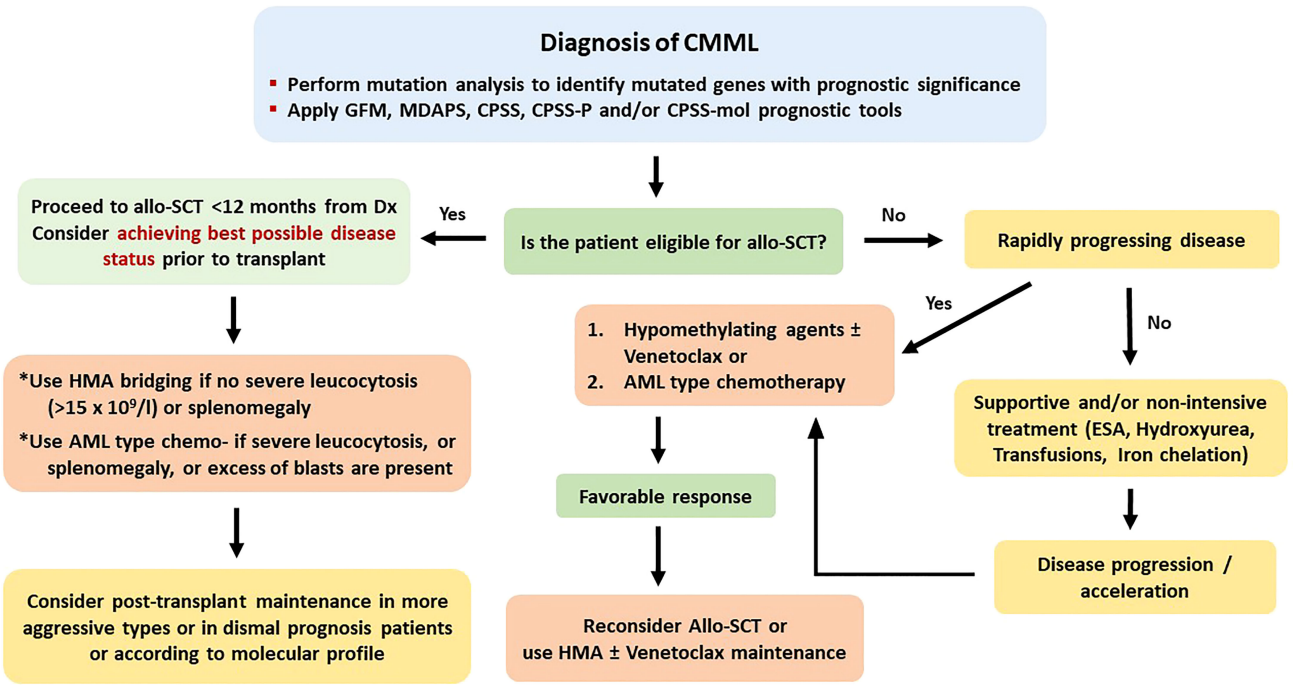


FIGURE 1 | Recommended treatment algorithm for patients with chronic myelomonocytic leukemia.

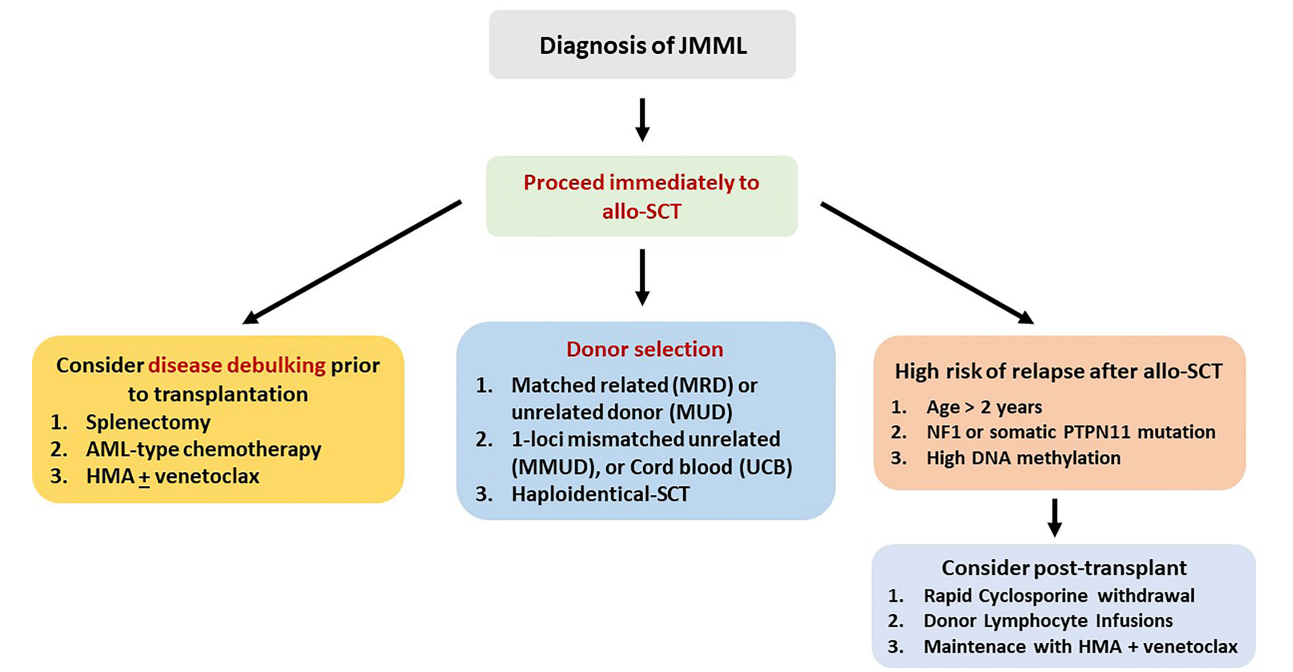


FIGURE 2 | Recommended treatment algorithm for patients with juvenile myelomonocytic leukemia. All patients should be considered candidates for allo-SCT.

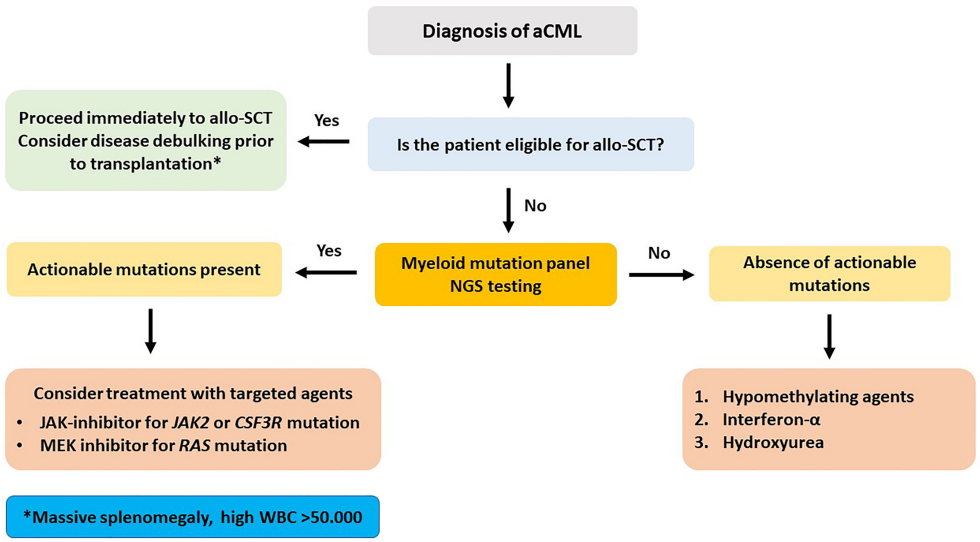


FIGURE 3 | Recommended treatment algorithm for patients with atypical chronic myelogenous leukemia. Massive splenomegaly and extreme leukocytosis at the time of initial diagnosis bear a severely dismal prognosis.

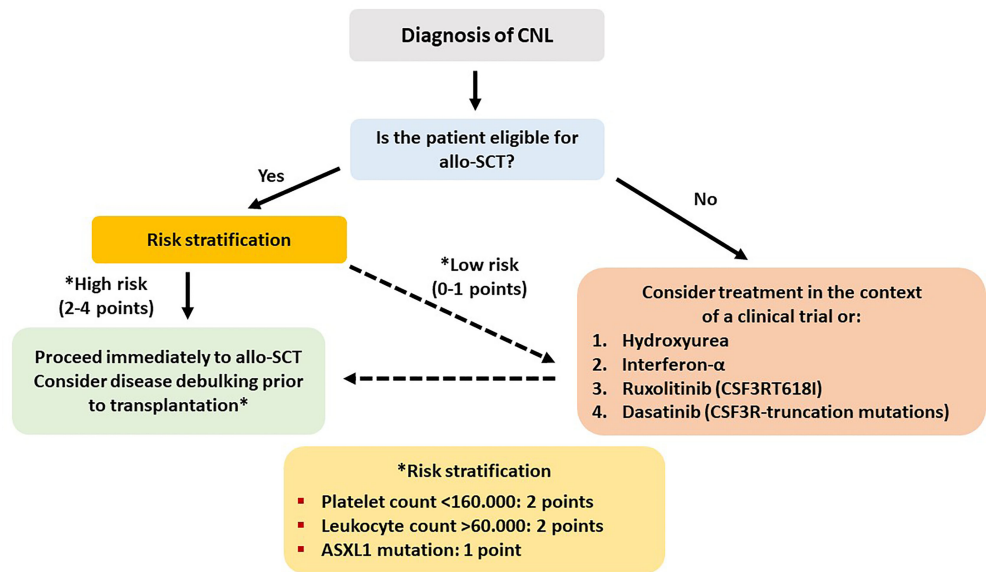


FIGURE 4 | Recommended treatment algorithm for patients with chronic neutrophilic leukemia.

Japanese studies of 115 and 141 patients, respectively. In the first, CMML and Refractory anemia with Excess of Blasts (RAEB) patients were pooled together (44 with CMML). An RIC regimen was given to 70% of the patients, and although many of them were older with poor cytogenetics, exhibited similar 4-year OS. Factors associated with poorer survival were poor cytogenetics, BM blasts ≥20% at transplantation, and absence of chronic GVHD, whereas for high-risk patients, the manifestation of chronic GVHD was associated with longer survival (89). In the second study, analysis has been focused on successfully engrafted CMML patients. Grade I acute GVHD was related to better OS and lower leukemia-related death in univariate analysis, whereas in multivariate analysis, extensive chronic GVHD was associated with significantly better OS and lower leukemia-related death in patients who were not transplanted in CR (90). In the IBMTR study, CPSS could only predict OS, which was better in the Low/

Intermediate-1 group, and patients with higher CPSS had about twice as high risk for post-relapse death. On multivariate analysis, performance status, CPSS, and the type of graft were independent predictors of DFS and OS (47). In the EBMT study, besides the impact of the period of transplantation, factors associated with a longer PFS and OS were disease status at transplantation (CR vs. no CR) and shorter interval from diagnosis to allo-SCT. Patients transplanted in CR had lower probability for NRM, and disease status at transplantation was the only significant factor for RFS and OS in multivariate analysis (48).

The significance of specific mutations was initially investigated by a German group on 45 patients who were screened for ASXL1, CBL, NRAS, and TET2 gene mutations. ASXL1 and TET2 were the most commonly mutated genes, but the type and the number of mutations had no impact on any outcome. The presence of mutations was used as a marker of minimal residual disease, and their persistence at 6 months posttransplant was associated with higher RR (37). Similar results were obtained by a Chinese study on 59 CMML patients in which the significance of post-allo-SCT minimal residual disease detected by both, flow cytometry and by PCR of the WT1 gene was investigated. Both techniques demonstrated a high level of prognostic value and could predict posttransplant relapse. The significance of the presence of WT1 mutations was not investigated (91).

The Mayo Clinic group compared 4 different prognostic scores and various clinical and genetic factors, including common mutations, on 70 transplanted and in 336 non-transplanted patients. Allo-SCT in other than chronic phase, abnormal cytogenetics, and neutropenia $<1.5 \times 10^9$/l were predictors of worse outcome. No prognostic score or any mutation had any impact on transplantation outcome. Patients with proliferative type had significantly longer survival when transplanted compared to those who were not transplanted (50 vs. 19 months), whereas a similar difference was not observed among patients with the dysplastic type (23, 24).

In contrast, the Nordic group found that TET2 mutations were associated with a favorable outcome, whereas ASXL1, RUNX1, and RAS mutations were associated with worse OS. Transfusion dependency and higher WBC count before transplant were also associated with earlier relapse, and NRAS mutations were linked to poorer survival due to increased TRM (43). In the more recent analysis of Fred Hutchinson, relapse was associated with poor cytogenetics, higher CPSS and MDACC score, and the presence of pretransplant residual disease, whereas death was associated with poor cytogenetics, pretransplant residual disease, and high HCT-CI. Clonal mutations were identified in 40.3% of the patients, and WT1 and ATRX mutations were associated with a higher RR and a shorter OS. NRAS and a high number of mutations (>10 in general or >4 epigenetic mutations) were also associated with higher RR (40).

Finally, in a recently published cooperative study on 240 CMML patients with a long median follow-up, increased percentage of BM blasts (>2%), the HCT-CI, and mutations of the ASXL1 or the KRAS genes were found to retain independent prognostic significance for OS and RFS. Collecting these factors, the authors have introduced the first prognostic tool that addresses specifically CMML patients to be transplanted (CMML-specific transplantation-specific prognostic score). The score ranges between 0 and 20 and assigns 1 point to each of the 8 comorbid conditions, described by the HCT-CI, and 4 points to each of the following three factors: pretransplant BM blasts >2 and the presence of ASXL1 or NRAS mutations. This score was superior to any previously reported, nicely predicted NRM and OS by stratifying the patients in 5 groups, with 5-year OS ranging between 19% and 81%, but has not yet been prospectively validated (50). **Table 5** summarizes the factors that have been shown to impact prognosis in CMML patients either following a non-transplant approach or undergoing allo-SCT.

DISCUSSION—CONCLUSIONS AND CURRENT RECOMMENDATIONS

CMML and other overlap MDSs/MPNs are challenging therapeutic problems for the treating physician. As a result of the substantial disease heterogeneity, he or she has to correctly identify the profile of the risk factors in each individual patient, evaluate his or her health background, and appropriately design the interventional treatment approach (15, 16, 92). For patients younger than 60 and for those older than 70 years, such approaches are rather easy to be designed, since by now the only curative intervention remains allo-SCT, which cannot be applied to very elderly and frail patients (16, 18, 92, 93). The most challenging decision for the treating physician concerns patients between 60 and 70 years and few fit patients older than 70 years. For this age range, the physician needs to discriminate higher-risk features that have been well characterized and described (10, 11). A disease mutational profile can greatly help in any case but particularly for patients of the seventh decade of their life (20). Ideally, all patients with CMML and adverse prognostic features and all patients with other MDSs/MPNs, securing an available stem cell donor should proceed to allo-SCT. Among the adverse prognostic features, WHO classification-defined CMML-1 or CMML-2, proliferative type of disease with difficult control of leukocytosis, presence of splenomegaly, extramedullary disease, constitutional symptoms, elevated serum LDH and an otherwise unexplained proinflammatory profile, manifestation of transfusion dependency, thrombocytopenia and increased marrow fibrosis, adverse cytogenetics and/or mutation profile, and a high CPSS or other relevant prognostic score are included (8–11). When eligible patients have been identified, they should thoroughly be informed as early as possible and consent for the recommended approach should be obtained.

When the basic plan has been organized, there are some "technical issues" that should be resolved. Regarding the best timing, patients exhibiting the previously mentioned profile should rapidly undergo allo-SCT after a few months of bridging treatment. The kind of this treatment (AML-type chemotherapy or HMA) appears not to play a core role, although there is emerging evidence that HMA should be preferred in CMML patients of more advanced age and cytoreductive

treatment should be the option for younger CMML patients and for those with other MDSs/MPNs (36, 92, 93). For patients with an excess (>5%) of marrow blasts, achieving a CR before transplant with the bridging treatment appears to favor a better posttransplant outcome (18, 22, 40, 43, 49). Another important "technical" issue is identification of the appropriate donor. Although data analysis from several studies has not found any significant impact of the type of donor on the outcome, it appears that this may be valid for relatively younger patients. For patients with more advanced age, the identification of a fully matched donor secures a clearly better outcome (40). A third "technical" issue concerns the use of the appropriate conditioning regimen.

For the abovementioned issues, the following basic principles can be applied. 1) Myeloablative conditioning should be preferred in younger and fit patients, while for older patients above the age of 65 years or for those with significant comorbidities, an RIC regimen appears to be more suitable. 2) A matched related or unrelated donor should be used, but in the absence of an available matched donor, haploidentical or cord blood transplantation should be considered at least for patients younger than 60 years. 3) Cytoreductive or HMA treatment should be administered in symptomatic patients and in those with splenomegaly or with CMML-1/2 before allo-SCT. For patients without an excess of marrow blasts, HMA bridging treatment might suffice. Evaluating patient risk category contributes to better predict several outcomes. To this endpoint, some of the described adverse prognostic factors might indeed reflect other already known prognostic factors. For example, in CMML, splenomegaly and leukocytosis apparently reflect a proliferative type of the disease, whereas circulating blasts and BM blasts >5% apparently fit with WHO-defined CMML-1 or CMML-2. The prognostic ability of CPSS in CMML is a debate. Some authors found it to be predictable, whereas other did not (23, 24, 35, 47). However, newer prognostic tools have been proposed, such as the CPSS-mol, the CPSS-P, and the CPSS transplant-specific, which have incorporated the prognostic importance of mutations that can predict outcome in transplanted patients, as this has been shown in several retrospective studies (23, 50, 74, 91, 93). Using these tools might help to better distinguish the transplantation risk group. The potentially ideal diagnostic and therapeutic recommendations for the four different types of myeloid neoplasia, for which this review is dedicated (CMML, aCML, JMML, and CNL), according to the authors' opinion are shown in **Figures 1**–**4**, respectively.

Probably the most important parameter for a successful transplantation is to help the patients achieve the best possible disease status before transplantation. To this point, there are newer targeted treatments besides HMA, which have not yet been tested as treatment tools. These include ruxolitinib and other JAK inhibitors, the CPX-351 complex, RAS and Hedgehog pathway inhibitors, the newer approved oral combination decitabine/cedazuridine, venetoclax, IDH1/IDH2 inhibitors, and other agents. The application of these agents might induce a better remission status before transplantation, thus rendering allo-SCT more effective. However, establishing the most appropriate drug combinations in each individual patient is a long way, which could be delineated through the use of these combinations either as a bridging treatment before transplantation or by incorporating appropriate drugs in the preparatory conditioning regimens. All of these potential new directions could only be substantiated through prospective multicenter randomized trials.

AUTHOR CONTRIBUTIONS

AS guided the article. AS and PT designed the article, wrote the main parts of the article, and critically reviewed the relevant literature. SC, VL, EV, and AK performed literature search, wrote parts of the article, and contributed to the design of the Tables and Figures. All of the authors reviewed and approved the final version of the article.

FUNDING

The University of Patras, through a personal account of the first author has covered the publication fees of this article.

REFERENCES

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. *Blood* (2016) 127(20):2391–405. doi: 10.1182/blood-2016-03-643544
2. Deininger M, Tuner JW, Solary E. Turning the Tide in Myelodysplastic/ Myeloproliferative Neoplasms. *Nat Rev Cancer* (2017) 17(7):425–40. doi: 10.1038/nrc.2017.40
3. Zoumbos NC, Symeonidis A, Kourakli-Symeonidis A. Chronic Neutrophilic Leukemia With Dysplastic Features: A New Variant of Myelodysplastic Syndromes? *Acta Hematol* (1989) 82(3):156–60. doi: 10.1159/000205367
4. Bornemann L, Schuster M, Schmitz S, Sobczak C, Bessen C, Merz SF, et al. Defective Migration and Dysmorphology of Neutrophil Granulocytes in Atypical Chronic Myeloid Leukemia Treated With Ruxolitinib. *BMC Cancer* (2020) 20(1):650. doi: 10.1186/s12885-020-07130-7
5. Such E, Cervera J, Costa D, Solé F, Vallespí T, Luño E, et al. Cytogenetic Risk Stratification in Chronic Myelomonocytic Leukemia. *Haematologica* (2011) 96(3):375–83. doi: 10.3324/haematol.2010.030957
6. Such E, Germing U, Malcovati L, Cervera J, Kuendgen A, Della Porta MG, et al. Development and Validation of a Prognostic Scoring System for Patients With Chronic Myelomonocytic Leukemia. *Blood* (2013) 121(15):3005–15. doi: 10.1182/blood-2012-08-452938
7. Onida F, Kantarjian HM, Smith TL, Ball G, Keating MJ, Estey EH, et al. Prognostic Factors and Scoring Systems in Chronic Myelomonocytic Leukemia: A Retrospective Analysis of 213 Patients. *Blood* (2002) 99 (3):840–9. doi: 10.1182/blood.v99.3.840
8. Patnaik MM, Padron E, LaBorde RR, Lasho TL, Finke CM, Hanson CA, et al. Mayo Prognostic Model for WHO-Defined Chronic Myelomonocytic Leukemia: ASXL1 and Spliceosome Component Mutations and Outcomes. *Leukemia* (2013) 27(7):1504–10. doi: 10.1038/leu.2013.88
9. Itzykson R, Kosmider O, Renneville A, Gelsi-Boyer V, Meggendorfer M, Morabito M, et al. Prognostic Score Including Gene Mutations in Chronic Myelomonocytic Leukemia. *J Clin Oncol* (2013) 31(19):2428–36. doi: 10.1200/JCO.2012.47.3314
10. Elena C, Gallì A, Such E, Meggendorfer M, Germing U, Rizzo E, et al. Integrating Clinical Features and Genetic Lesions in the Risk Assessment of Patients With Chronic Myelomonocytic Leukemia. *Blood* (2016) 128 (10):1408–17. doi: 10.1182/blood-2016-05-714030

11. Calvo X, Nomdedeu M, Santacruz R, Martínez N, Costa D, Pereira A, et al. Comparison of Three Prognostic Scoring Systems in a Series of 146 Cases of Chronic Myelomonocytic Leukemia (CMML): MD Anderson Prognostic Score (MDAPS), CMML-Specific Prognostic Scoring System (CPSS) and Mayo Prognostic Model. A Detailed Review of Prognostic Factors in CMML. *Leuk Res* (2015) 39(11):1146-53. doi: 10.1016/j.leukres.2015.05.017. S0145-2126(15)30324-6.
12. Gur HD, Loghavi S, Garcia-Manero G, Routbort M, Kanagal-Shamanna R, Quesada A, et al. Chronic Myelomonocytic Leukemia With Fibrosis Is a Distinct Disease Subset With Myeloproliferative Features and Frequent JAK2 P.V617F Mutations. *Am J Surg Pathol* (2018) 42(6):799–806. doi: 10.1097/PAS.0000000000001058
13. Subari S, Patnaik M, Alfakara D, Gangat N, Elliott M, Hogan W, et al. Patients With Therapy-Related CMML Have Shorter Median Overall Survival Than Those With *De Novo* CMML: Mayo Clinic Long-Term Follow-Up Experience. *Clin Lymphoma Myeloma Leuk* (2015) 15(9):546–9. doi: 10.1016/j.clml.2015.06.002
14. Patnaik MM, Vallapureddy R, Yalniz FF, Hanson CA, Ketterling RP, Lasho TL, et al. Therapy Related-Chronic Myelomonocytic Leukemia (CMML): Molecular, Cytogenetic, and Clinical Distinctions From *De Novo* CMML. *Am J Hematol* (2018) 93(1):65–73. doi: 10.1002/ajh.24939
15. Solary E, Itzykson R. How I Treat Chronic Myelomonocytic Leukemia. *Blood* (2017) 130(2):126–36. doi: 10.1182/blood-2017-04-736421
16. Itzykson R, Fenaux P, Bowen D, Cross NCP, Cortes J, De Witte T, et al. Diagnosis and Treatment of Chronic Myelomonocytic Leukemias in Adults. Recommendations From the European Hematology Association and the European LeukemiaNet. *Hemasphere* (2018) 2(6):e150. doi: 10.1097/HS9.0000000000000150
17. de Witte T, Bowen D, Robin M, Malcovati L, Niederwieser D, Yakoub-Agha I, et al. Allogeneic Hematopoietic Stem Cell Transplantation for MDS and CMML: Recommendations From an International Expert Panel. *Blood* (2017) 129(13):1753–62. doi: 10.1182/blood-2016-06-724500
18. Gotlib J. How I Treat Atypical Chronic Myeloid Leukemia. *Blood* (2017) 129(7):838–45. doi: 10.1182/blood-2016-08-693630
19. Coston T, Pophali P, Vallapureddy R, Lasho TL, Finke CM, Ketterling RP, et al. Suboptimal Response Rates to Hypomethylating Agent Therapy in Chronic Myelomonocytic Leukemia; a Single Institutional Study of 121 Patients. *Am J Hematol* (2019) 94(7):767–79. doi: 10.1002/ajh.25488
20. Triguero A, Xicoy B, Zamora L, Jiménez MJ, García O, Calabuig M, et al. Response to Azacitidine in Patients With Chronic Myelomonocytic Leukemia According to Overlap Myelodysplastic/ Myeloproliferative Neoplasms Criteria. *Leuk Res* (2022) 26:116. doi: 10.1016/j.leukres.2022.106836
21. Duchmann M, Yalniz FF, Sanna A, Sallman D, Coombs CC, Renneville A, et al. Prognostic Role of Gene Mutations in Chronic Myelomonocytic Leukemia Patients Treated With Hypomethylating Agents. *EBioMedicine* (2018) 31:174–81. doi: 10.1016/j.ebiom.2018.04.018
22. Kröger N, Eikema D-J, Köster L, Beelen D, de Wreede LC, Finke J, et al. Impact of Primary Disease on Outcome After Allogeneic Stem Cell Transplantation for Transformed Secondary Acute Leukaemia. *Brit J Haematol* (2019) 185(4):725–32. doi: 10.1111/bjh.15819
23. Pophali P, Matin A, Mangaonkar AA, Carr R, Binder M, Al-Kali A, et al. Prognostic Impact and Timing Considerations for Allogeneic Hematopoietic Stem Cell Transplantation in Chronic Myelomonocytic Leukemia. *Blood Cancer J* (2020) 10(11):121. doi: 10.1038/s41408-020-00387-y
24. Gagelmann N, Bogdanov R, Stölzel F, Rautenberg C, Panagiota V, Becker H, et al. Long-Term Survival Benefit After Allogeneic Hematopoietic Cell Transplantation for Chronic Myelomonocytic Leukemia. *Transplant Cell Ther* (2021) 27(1):95.e1–4. doi: 10.1016/j.bbmt.2020.10.007
25. Mittal P, Saliba RM, Giralt SA, Shahjahan M, Cohen AI, Karandish S, et al. Allogeneic Transplantation: A Therapeutic Option for Myelofibrosis, Chronic Myelomonocytic Leukemia and Philadelphia-Negative/BCR-ABL-Negative Chronic Myelogenous Leukemia. *Bone Marrow Transplant* (2004) 33(10):1005–9. doi: 10.1038/sj.bmt.1704472
26. Elliott MA, Tefferi A, Hogan WJ, Letendre L, Gastineau DA, Ansell SM, et al. Allogeneic Stem Cell Transplantation and Donor Lymphocyte Infusions for Chronic Myelomonocytic Leukemia. *Bone Marrow Transplant* (2006) 37(11):1003–8. doi: 10.1038/sj.bmt.1705369
27. Kröger N, Zabelina T, Guardiola P, Runde V, Sierra J, van Biezen A, et al. Allogeneic Stem Cell Transplantation of Adult Chronic Myelomonocytic Leukaemia. A Report on Behalf of the Chronic Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). . *Br J Haematol* (2002) 118(1):67–73. doi: 10.1046/j.1365-2141.2002.03552.x
28. Laport GG, Sandmaier BM, Storer BE, Scott BL, Stuart MJ, Lange T, et al. Reduced-Intensity Conditioning Followed by Allogeneic Hematopoietic Cell Transplantation for Adult Patients With Myelodysplastic Syndrome and Myeloproliferative Disorders. *Biol Blood Mar Transplant* (2008) 14(2):246–55. doi: 10.1016/j.bbmt.2007.11.012
29. Ocheni S, Kröger N, Zabelina T, Zander AR, Bacher U. Outcome of Allo-SCT for Chronic Myelomonocytic Leukemia. *Bone Marrow Transplant* (2009) 43(8):659–61. doi: 10.1038/bmt.2008.366
30. Krishnamurthy P, Lim ZY, Nagi W, Kenyon M, Mijovic A, Ireland R, et al. Allogeneic Haematopoietic SCT for Chronic Myelomonocytic Leukaemia: A Single-Centre Experience. *Bone Marrow Transplant* (2010) 45(10):1502–7. doi: 10.1038/bmt.2009.375
31. Zang Y-D, Deeg HJ, Gooley D, Anderson JE, Anasetti C, Sanders J, et al. Treatment of Chronic Myelomonocytic Leukaemia by Allogeneic Bone Marrow Transplantation. *Br J Haematol* (2000) 110(1):217–22. doi: 10.1046/j.1365-2141.2000.02133.x
32. Kerbauy DMB, Chyou F, Gooley T, Sorror ML, Scott B, Pagel JM, et al. Allogeneic Hematopoietic Cell Transplantation for Chronic Myelomonocytic Leukemia. *Biol Blood Marrow Transplant* (2005) 11(9):713–20. doi: 10.1016/j.bbmt.2005.05.008
33. Sharma P, Shinde SS, Damlaj M, Hefazi Rorghabeh M, Hashmi SK, Litzow MR, et al. Allogeneic Hematopoietic Stem Cell Transplant in Adult Patients With Myelodysplastic Syndrome/Myeloproliferative Neoplasm (MDS/MPN) Overlap Syndromes. *Leuk Lymphoma* (2017) 58(4):872–81. doi: 10.1080/10428194.2016.1217529
34. Park S, Labopin M, Yakoub-Agha I, Delaunay J, Dhedin N, Deconinck E, et al. Allogeneic Stem Cell Transplantation for Chronic Myelomonocytic Leukemia: A Report From the Societe Francaise De Greffe De Moelle Et De Therapie Cellulaire. *Eur J Haematol* (2013) 90(5):355–64. doi: 10.1111/ejh.12073
35. Eissa H, Gooley TA, Sorror ML, Nguyen F, Scott BL, Doney K, et al. Allogeneic Hematopoietic Cell Transplantation for Chronic Myelomonocytic Leukemia: Relapse-Free Survival Is Determined by Karyotype and Comorbidities. *Biol Blood Marrow Transplant* (2011) 17(6):908–15. doi: 10.1016/j.bbmt.2010.09.018
36. Kongtim P, Popat U, Jimenez A, Gaballa S, El Fakih R, Rondon G, et al. Treatment With Hypomethylating Agents Before Allogeneic Stem Cell Transplant Improves Progression-Free Survival for Patients With Chronic Myelomonocytic Leukemia. *Biol Blood Marrow Transplant* (2016) 22(1):47–53. doi: 10.1016/j.bbmt.2015.08.031
37. Fu Y, Schroeder T, Zabelina T, Badbaran A, Bacher U, Kobbe G, et al. Post Allogeneic Monitoring With Molecular Markers Detected by Pretransplant Next-Generation or Sanger Sequencing Predicts Clinical Relapse in Patients With Myelodysplastic/Myeloproliferative Neoplasms. *Eur J Hematol* (2013) 92(3):189–94. doi: 10.1111/ejh.12223
38. Wedge E, Werner Hansen J, Dybedal I, Creignou M, Ejerblad E, Lorenz F, et al. Allogeneic Hematopoietic Stem Cell Transplantation for Chronic Myelomonocytic Leukemia: Clinical and Molecular Genetic Prognostic Factors in a Nordic Population. *Transplant Cell Ther* (2021) 27(12):991. doi: 10.1016/j.jtct.2021.08.028
39. Itonaga H, Aoki K, Aoki J, Ishikawa T, Ishiyama K, Uchida N, et al. Prognostic Impact of Donor Source on Allogeneic Hematopoietic Stem Cell Transplantation Outcomes in Adults With Chronic Myelomonocytic Leukemia: A Nationwide Retrospective Analysis in Japan. *Biol Blood Marrow Transplant* (2018) 24(4):840–8. doi: 10.1016/j.bbmt.2017.11.016
40. Woo J, Choi DR, Storer BE, Yeung C, Halpern AB, Salit RB, et al. Impact of Clinical, Cytogenetic, and Molecular Profiles on Long-Term Survival After Transplantation in Patients With Chronic Myelomonocytic Leukemia. *Haematologica* (2020) 105(3):652–60. doi: 10.3324/haematol.2019.218677
41. Lim SN, Lee JH, Lee JH, Kim DY, Kim SD, Kang YA, et al. Allogeneic Hematopoietic Cell Transplantation in Adult Patients With Myelodysplastic/Myeloproliferative Neoplasms. *Blood Res* (2013) 48(3):178–84. doi: 10.5045/br.2013.48.3.178
42. Radujkovic A, Hegenbart U, Müller-Tidow C, Herfarth K, Dreger P, Luft T. High Leukemia-Free Survival After TBI-Based Conditioning and

Mycophenolate Mofetil-Containing Immunosuppression in Patients Allografted for Chronic Myelomonocytic Leukemia: A Single-Center Experience. *Ann Hematol* (2020) 99(4):855–66. doi: 10.1007/s00277-020-03952-4

43. Wedge E, Sengeløv H, Werner Hansen J, Smedegaard Andersen N, Schjødt I, Petersen SL, et al. Improved Outcomes After Allogenic Hematopoietic Stem Cell Transplantation With Fludarabine/Treosulfan for Patients With Myelodysplastic Syndromes. *Biol Blood Marrow Transplant* (2020) 26 (6):1091–8. doi: 10.1016/j.bbmt.2020.02.010
44. Deeg HJ, Stevens EA, Salit BR, Ermoian RP, Fang M, Gyurkocza B, et al. Transplant Conditioning With Treosulfan/Fludarabine With or Without Total Body Irradiation: A Randomized Phase II Trial in Patients With Myelodysplastic Syndrome and Acute Myeloid Leukemia. *Biol Blood Marrow Transplant* (2018) 24(5):956–63. doi: 10.1016/j.bbmt.2017.12.785
45. Monaco F, Scott BL, Chauncey TR, Petersen FB, Storer BE, Baron F, et al. Total Body Irradiation Dose Escalation Decreases Risk of Progression and Graft Rejection After Hematopoietic Cell Transplantation for Myelodysplastic Syndromes or Myeloproliferative Neoplasms. *Haematologica* (2019) 104 (6):1221–9. doi: 10.3324/haematol.2018.199398
46. Benjamin J, Chhabra S, Kohrt HE, Lavori P, Laport GG, Arai S, et al. Total Lymphoid Irradiation - Antithymocyte Globulin Conditioning and Allogeneic Transplantation for Patients With Myelodysplastic Syndromes and Myeloproliferative Neoplasms. *Biol Blood Marrow Transplant* (2014) 20 (6):837–43. doi: 10.1016/j.bbmt.2014.02.023
47. Liu HD, Ahn KW, Hu ZH, Hamadani M, Nishihori T, Wirk B, et al. Allogeneic Hematopoietic Cell Transplantation for Adult Chronic Myelomonocytic Leukemia. *Biol Blood Marrow Transplant* (2017) 23 (5):767–75. doi: 10.1016/j.bbmt.2017.01.078
48. Symeonidis A, van Biezen A, de Wreede L, Piciocchi A, Finke J, Beelen D, et al. Achievement of Complete Remission Predicts Outcome of Allogeneic Hematopoietic Stem Cell Transplantation in Patients With Chronic Myelomonocytic Leukemia (CMML). A Study of the Chronic Malignancies Working Party of the EBMT. *Br J Haematol* (2015) 171(2):239–46. doi: 10.1111/bjh.13576
49. Sun YQ, Zhao C, Wang Y, Yan CH, Zhang XH, Xu LP, et al. Haploidentical Stem Cell Transplantation in Patients With Chronic Myelomonocytic Leukemia. *Sci China Life Sci* (2020) 63(8):1261–4. doi: 10.1007/s11427-019-1606-3
50. Gagelmann N, Badbaran A, Beelen DW, Salit RB, Stölzel F, Rautenberg C, et al. A Prognostic Score Including Mutation Profile and Clinical Features for Patients With CMML Undergoing Stem Cell Transplantation. *Blood Adv* (2021) 5(6):1760–9. doi: 10.1182/bloodadvances.2020003600
51. Niemeyer CM, Arico M, Basso G, Biondi A, Cantu Rajnoldi A, Creutzig U, et al. European Working Group on Myelodysplastic Syndromes in Childhood (EWOG-MDS). Chronic Myelomonocytic Leukemia in Childhood: A Retrospective Analysis of 110 Cases. *Blood* (1997) 89(10):3534–43. doi: 10.1182/blood.V89.10.3534
52. Weinberg RS, Leibowitz D, Weinblatt ME, Kochen J, Alter BP. Juvenile Chronic Myelogenous Leukemia: The Only Example of Truly Fetal (Not Fetal-Like) Erythropoiesis. *Br J Haematol* (1990) 76(2):307–10. doi: 10.1111/j.1365-2141.1990.tb07891.x
53. Niemeyer CM. RAS Diseases in Children. *Haematologica* (2014) 99(11):1653–62. doi: 10.3324/haematol.2014.114595
54. Kratz CP, Niemeyer CM, Castleberry RP, Cetin M, Bergsträsser E, Emanuel PD, et al. The Mutational Spectrum of PTPN11 in Juvenile Myelomonocytic Leukemia and Noonan Syndrome/Myeloproliferative Disease. *Blood* (2005) 106(6):2183–5. doi: 10.1182/blood-2005-02-0531
55. Passmore SJ, Hann IM, Stiller CA, Ramani P, Swansbury GJ, Gibbons B, et al. Pediatric Myelodysplasia: A Study of 68 Children and a New Prognostic Scoring System. *Blood* (1995) 85(7):1742–50. doi: 10.1182/blood.V85.7.1742.bloodjournal8571742
56. Locatelli F, Nöllke P, Zecca M, Korthof E, Lanino E, Peters C, et al. European Working Group on Childhood MDS; European Blood and Marrow Transplantation Group. Hematopoietic Stem Cell Transplantation (HSCT) in Children With Juvenile Myelomonocytic Leukemia (JMML): Results of the EWOG-MDS/EBMT Trial. *Blood* (2005) 105(1):410–9. doi: 10.1182/blood-2004-05-1944
57. Lin Y-C, Luo C-J, Miao Y, Wang J-M, Luo C-Y, Qin X, et al. Human Leukocyte Antigen Disparities Reduce Relapse After Hematopoietic Stem Cell Transplantation in Children With Juvenile Myelomonocytic Leukemia: A Single-Center Retrospective Study From China. *Pediatr Transplant* (2020) 25: e13825. doi: 10.1111/petr.13825
58. Schönung M, Meyer J, Nöllke P, Olshen AB, Hartmann M, Murakami N, et al. International Consensus Definition of DNA Methylation Subgroups in Juvenile Myelomonocytic Leukemia. *Clin Cancer Res* (2021) 27(1):158–68. doi: 10.1158/1078-0432.CCR-20-3184
59. Inagaki J, Fukano R, Nishikawa T, Nakashima K, Sawa D, Ito N, et al. Outcomes of Immunological Interventions for Mixed Chimerism Following Allogeneic Stem Cell Transplantation in Children With Juvenile Myelomonocytic Leukemia. *Pediatr Blood Cancer* (2013) 60(1):116–20. doi: 10.1002/pbc.24259
60. Tasian SK, Casas JA, Posocco D, Gandre-Babbe S, Gagne AL, Liang G, et al. Mutation-Specific Signaling Profiles and Kinase Inhibitor Sensitivities of Juvenile Myelomonocytic Leukemia Revealed by Induced Pluripotent Stem Cells. *Leukemia* (2019) 33(1):181–90. doi: 10.1038/s41375-018-0169-y
61. Tufecki O, Kocak U, Kaya Z, Yenicesu I, Albayrak C, Albayrak D, et al. Juvenile Myelomonocytic Leukemia in Turkey: A Retrospective Analysis of 65 Patients. *Turk J Haematol* (2018) 35(1):27–34. doi: 10.4274/tjh.2017.0021
62. Locatelli F, Crotta A, Ruggeri A, Eapen M, Wagner J, Macmillan M, et al. Analysis of Risk Factors Influencing Outcomes After Cord Blood Transplantation in Children With Juvenile Myelomonocytic Leukemia: A Eurocord, EBMT, EWOGMDS, CIBMTR Study. *Blood* (2013) 122(12):2135–41. doi: 10.1182/blood-2013-03-491589
63. Yoshida N, Sakaguchi H, Yabe M, Hasegawa D, Hama A, Hasegawa D, et al. Clinical Outcomes After Allogeneic Hematopoietic Stem Cell Transplantation in Children With Juvenile Myelomonocytic Leukemia: A Report From the Japan Society for Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant* (2020) 26(5):902–10. doi: 10.1016/j.bbmt.2019.11.029
64. Onida F, Ball G, Kantarjian HM, Smith TL, Glassman A, Albitar M, et al. Characteristics and Outcome of Patients With Philadelphia Chromosome Negative, Bcr/Abl Negative Chronic Myelogenous Leukemia. *Cancer*. (2002) 95(8):1673–84. doi: 10.1002/cncr.10832
65. Breccia M, Biondo F, Latagliata R, Carmosino I, Mandelli F, Alimena G. Identification of Risk Factors in Atypical Chronic Myeloid Leukemia. *Haematologica* (2006) 91(11):1566–8.
66. Wang SA, Hasserjian RP, Fox PS, Rogers H, Geyer J, Chabot-Richards D, et al. A Typical Chronic Myeloid Leukemia is Clinically Distinct From Unclassifiable Myelodysplastic/Myeloproliferative Neoplasms. *Blood* (2014) 123(17):2645–51. doi: 10.1182/blood-2014-02-553800
67. Montalban-Bravo G, Kanagal-Shamanna R, Sasaki K, Masarova L, Naqvi K, Jabbour E, et al. Clinicopathologic Correlates and Natural History of Atypical Chronic Myeloid Leukemia. *Cancer* (2021) 127(17):3113–24. doi: 10.1002/cncr.33622
68. Crisà E, Nicolosi M, Ferri V, Favini C, Gaidano G, Patriarca A. Atypical Chronic Myeloid Leukemia: Where Are We Now? *Int J Mol Sci* (2020) 21 (18):6862. doi: 10.3390/ijms21186862
69. Piazza R, Valletta S, Winkelmann N, Redaelli S, Spinelli R, Pirola A, et al. Recurrent SETBP1 Mutations in Atypical Chronic Myeloid Leukemia. *Nat Genet* (2013) 45(1):18–24. doi: 10.1038/ng.2495
70. Meggendorfer M, Bacher U, Alpermann T, Haferlach C, Kern W, Gambacorti-Passerini C, et al. SETBP1 Mutations Occur in 9% of MDS/MPN and in 4% of MPN Cases and are Strongly Associated With Atypical CML, Monosomy 7, Isochromosome I(17)(Q10), ASXL1 and CBL Mutations. *Leukemia* (2013) 27(9):1852–60. doi: 10.1038/leu.2013.133
71. Cahu X, Chevallier P, Clavert A, Suarez F, Michallet M, Vincent L, et al. Allo-SCT for Philadelphia-Negative Myeloproliferative Neoplasms in Blast Phase: A Study From the Societe Française De Greffe De Moelle Et De Therapie Cellulaire (SFGM-Tc). *Bone Marrow Transplant* (2014) 49(6):756–60. doi: 10.1038/bmt.2014.31
72. Onida F, de Wreede L, van Biezen A, Eikema DJ, Byrne JL, Iori AP, et al. Allogeneic Stem Cell Transplantation in Patients With Atypical Chronic Myeloid Leukaemia: A Retrospective Study From the Chronic Malignancies Working Party of the EBMT. *Br J Haematol* (2017) 177:759–65. doi: 10.1111/bjh.14619
73. Koldehoff M, Beelen DW, Trenschel R, Steckel NK, Peceny R, Ditschkowski M, et al. Outcome of Hematopoietic Stem Cell Transplantation in Patients With Atypical Chronic Myeloid Leukemia. *Bone Marrow Transplant* (2004) 34:1047–50. doi: 10.1038/sj.bmt.1704686

74. Koldehoff M, Steckel NK, Hegerfeldt Y, Ditschkowski M, Beelen DW, Elmaagacli AH. Clinical Course and Molecular Features in 21 Patients With Atypical Chronic Myeloid Leukemia. *Int J Lab Hematol* (2012) 34(1):e3–5. doi: 10.1111/j.1751-553X.2011.01351.x
75. Itonaga H, Ota S, Ikeda T, Taji H, Amano I, Hasegawa Y, et al. Allogeneic Hematopoietic Stem Cell Transplantation for the Treatment of BCRABL1-Negative Atypical Chronic Myeloid Leukemia and Chronic Neutrophilic Leukemia: A Retrospective Nationwide Study in Japan. *Leuk Res* (2018) 75:50–7. doi: 10.1016/j.leukres.2018.11.003
76. Ruan GJ, Smith CJ, Day C, Harmsen WS, Zblewski DL, Alkhateeb H, et al. A Population-Based Study of Chronic Neutrophilic Leukemia in the United States. *Blood Cancer J* (2020) 10(6):68. doi: 10.1038/s41408-020-0334-1
77. Szuber N, Finke CM, Lasho TL, Elliott MA, Hanson CA, Pardanani A, et al. CSF3R-Mutated Chronic Neutrophilic Leukemia: Long-Term Outcome in 19 Consecutive Patients and Risk Model for Survival. *Blood Cancer J* (2018) 8 (2):21. doi: 10.1038/s41408-018-0058-7
78. Dao KT, Gotlib J, Deininger MMN, Oh ST, Cortes JE, Collins RHJr, et al. Efficacy of Ruxolitinib in Patients With Chronic Neutrophilic Leukemia and Atypical Chronic Myeloid Leukemia. *J Clin Oncol* (2020) 38(10):1006–18. doi: 10.1200/JCO.19.00895
79. Menezes J, Cigudosa JC. Chronic Neutrophilic Leukemia: A Clinical Perspective. *OncoTargets Ther* (2015) 8:2383–90. doi: 10.2147/OTT.S49688
80. Langabeer SE, McCarron SL, Haslam K, O'Donovan MT, Conneally E. The CSF3R T618I Mutation as a Disease-Specific Marker of Atypical CML Post Allo-SCT. *Bone Marrow Transplant* (2014) 49(6):843–4. doi: 10.1038/bmt.2014.35
81. Hasle H, Olesen G, Kerndrup G, Philip P, Jacobsen N. Chronic Neutrophil Leukaemia in Adolescenceand Young Adulthood. *Br J Haematol* (1996) 94 (4):628–30. doi: 10.1046/j.1365-2141.1996.7082329.x
82. Goto H, Hara T, Tsurumi H, Tanabashi S, Moriwaki H. Chronic Neutrophilic Leukemia With Congenital Robertsonian Translocation Successfully Treated With Allogeneic Bone Marrow Transplantation in a Young Man. *Inter Med* (2009) 48(7):563–7. doi: 10.2169/internalmedicine.48.1334
83. Kako S, Kanda Y, Sato T, Goyama S, Noda N, Shoda E, et al. Early Relapse of JAK2 V617F-Positive Chronic Neutrophilic Leukemia With Central Nervous System Infiltration After Unrelated Bone Marrow Transplantation. *Am J Hematol* (2007) 82(5):386–90. doi: 10.1002/ajh.20805
84. Piliotis E, Kutas G, Lipton JH. Allogeneic Bone Marrow Transplantation in the Management of Chronic Neutrophilic Leukemia. *Leuk Lymphoma* (2002) 43(10):2051–4. doi: 10.1080/1042819021000016087
85. Voshtina E, Szabo A, Hamadani M, Fenske TS, D'Souza A, Chhabra S, et al. Impact of Obesity on Clinical Outcomes of Elderly Patients Undergoing Allogeneic Hematopoietic Cell Transplantation for Myeloid Malignancies. *Biol Blood Mar Transplant* (2019) 25(1):e33–8. doi: 10.1016/j.bbmt.2018.08.031
86. Zhao C, Huang XS, Zhao XS, Wang Y, Yan CH, Xu LP, et al. Impact of Splenomegaly on Outcomes of Allogeneic Hematopoietic Stem Cell Transplantation in Patients With Chronic Myelomonocytic Leukemia. *Zhonghua Xue Ye Xue Za Zhi* (2020) 41(4):308–12. doi: 10.3760/cma.j.issn.0253-2727.2020.04.008
87. Wang J, Wang Q, Zhang H, He Y, Huang Y, Zhang R, et al. Moderate to Severe Marrow Fibrosis As a More Advanced Risk Factor for MDS and MDS-AML Patients With Excess of Blasts Receiving Allogeneic Hematopoietic Stem Cell Transplantation. *Transplant Cel Ther* (2021) 27(8):666. doi: 10.1016/j.jtct.2021.05.006
88. Yucel OK, Saliba RM, Rondon G, Ahmed S, Alousi A, Bashir Q, et al. Cytogenetics and Comorbidity Predict Outcomes in Older Myelodysplastic Syndrome Patients After Allogeneic Stem Cell Transplantation Using Reduced Intensity Conditioning. *Cancer* (2017) 123(14):2661–70. doi: 10.1002/cncr.30632
89. Hiramoto N, Kurosawa S, Tajima K, Okinaka K, Tada K, Kobayashi Y, et al. Positive Impact of Chronic Graft-Versus-Host Disease on the Outcome of Patients With *De Novo* Myelodysplastic Syndrome After Allogeneic Hematopoietic Cell Transplantation: A Single-Center Analysis of 115 Patients. *Eur J Haematol* (2013) 92:137–46. doi: 10.1111/ejh.12214
90. Itonaga H, Iwanaga M, Aoki K, Aoki J, Ishiyama K, Ishikawa T, et al. Impacts of Graft-Versus-Host Disease on Outcomes After Allogeneic Hematopoietic Stem Cell Transplantation for Chronic Myelomonocytic Leukemia: A Nationwide Retrospective Study. *Leuk Res* (2016) 41:48–55. doi: 10.1016/j.leukres.2015.12.009
91. Pan X, Gao M, Sun Y, Zhou Y, Wang K, Wang Y, et al. Significance of WT1 and Multiparameter Flow Cytometry Assessment in Patients With Chronic Myelomonocytic Leukemia Receiving Allogeneic Hematopoietic Stem Cell Transplantation. *Int J Lab Hematol* (2022) 44(3):510-7. doi: 10.1111/ijlh.13788
92. Liapis K, Kotsianidis I. Approaching First-Line Treatment in Patients With Advanced CMML: Hypomethylating Agents or Cytotoxic Treatment? *Front Oncol* (2021) 11:801524. doi: 10.3389/fonc.2021.801524
93. Bewersdorf JP, Zeidan AM. Risk-Adapted, Individualized Treatment Strategies of Myelodysplastic Syndromes (MDS) and Chronic Myelomonocytic Leukemia (CMML). *Cancers (Basel).* (2021) 13(7):1610. doi: 10.3390/cancers13071610

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

GLOSSARY

aCML	atypical bcr/abl-negative chronic myeloid leukemia
aGVHD	acute Graft Versus Host Disease
allo-SCT	allogeneic hematopoietic stem cell transplantation
AML	acute myelogenous leukemia
ASXL1	Additional Sex Combs Like-1 gene
ATG	anti-thymocyte globulin
ATRX	Alpha Thalassemia mental Retardation X-linked helicase
BCL2	B-Cell Lymphoma type 2 gene
BM	bone marrow
BMI	Body Mass Index
BP	Blastic Phase
BRAF	B-Raf protooncogene, serine/threonine kinase
CALR	Calreticulin
CBL	Cbl protooncogene, E3ubiquitin kinase
cGVHD	chronic Graft Versus Host Disease
CSFR3	ColonyStimulating Factor-3 Receptor
CMML	chronic myelomonocytic leukemia
CNL	chronic neutrophilic leukemia
CPSS	CMML-specific Prognostic Scoring System
CPSS-mol	CPSSmolecular
CPSS-P	CPSS including platelet count
CR	Complete Remission
DFS	disease-free survival
DLI	donor lymphocyte infusion
DNA	DeoxyRibonucleotidic Acid
EB	Excess of Blasts
EBMT	European Blood and Marrow Transplantation Registry
EFS	Event-Free Survival
ETNK1	Ethanolamine kinase 1
EWOG-MDS	European Working Group on MDS
EZH2	Enhancer of Zeste 2 Polycomb Repressive Complex 2
GVHD	graft-versus-host disease
GVL	graft-versus-leukemia
HbF	fetal hemoglobin
HCT-CI	Hematopoietic Cell Transplantation-Specific Comorbidity Index
HMA	hypomethylating agent
HLA	human leukocyte antigen
JAK2	Janus Kinase type 2
JMML	juvenile myelomonocytic leukemia
IBMTR	International Blood and Marrow Transplantation Registry
K-M	Kaplan-Meier
KRAS	KRAS protooncogene GTPase
LDH	lactate dehydrogenase
LFS	leukemia-free survival
MDACC	MD Anderson Cancer Center
MDAPS	MD Anderson Prognostic Score
MDS/MPN	mixed or hybrid or overlap myelodysplastic syndrome/ myeloproliferative neoplasm
MDS/ MPN-U	myelodysplastic syndrome/myeloproliferative neoplasm unclassifiable
MEK	Mitogen-activated protein Kinase kinase
MMUD	mismatched unrelated donor
MPL	MPL protooncogene, thrombopoietin receptor
MPN	myeloproliferative neoplasm
MRD	matched related donor
MUD	matched unrelated donor
NF1	Neurofibromatosis type 1 gene
NRAS	NRAS protooncogene GTPase
NRM	non-relapse mortality
OS	overall survival
PB	peripheral blood
PCR	Polymerase Chain Reaction
PFS	progression-free survival
PTPN11	Protein Tyrosin Phosphatase Non-receptor 11
RAEB	refractory anemia with an excess of blasts
RAF	Rapidly Accelerated Fibrosarcoma
RAS	Rat Sarcoma gene
RFS	Relapse-free survival
RIC	Reduced Intensity Conditioning
RR	relapse rate
RUNX1	Runt-related transcription factor 1
SCT	Stem cell transplantation
SETBP1	SET binding protein 1
SOS1	SOS Ras/Rac guanine nucleotide exchange factor 1
SRSF2	Serine andarginine Rich Splicing Factor 2
TBI	total body irradiation
TET2	Ten-Eleven Translocation type 2 gene
TLI	total lymphoid irradiation
TRM	treatment-related mortality
UCB	umbilical cord blood
USA	United States of America
WBC	white blood cell
WHO	World Health Organization
WT1	Wilms Tumor 1 gene

Frontiers in Oncology

Advances knowledge of carcinogenesis and tumor progression for better treatment and management

The third most-cited oncology journal, which highlights research in carcinogenesis and tumor progression, bridging the gap between basic research and applications to imrpove diagnosis, therapeutics and management strategies.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact