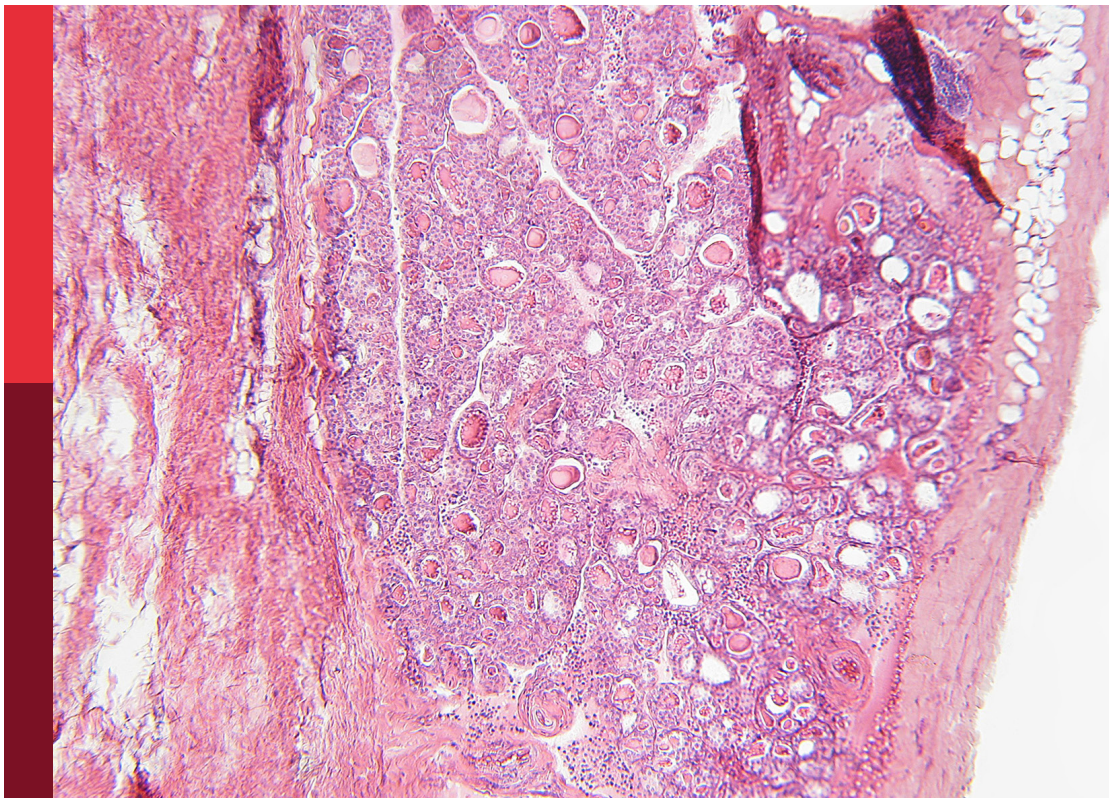
frontiers | Research Topics

# Advances in targeted therapy and biomarker research for endocrine-related cancers

**Edited by**
Zili Zhang, Xiaoqiang Qi and Min Tu

**Coordinated by**
Yang Wu

**Published in**
Frontiers in Endocrinology
Frontiers in Oncology



ISSN 1664-8714
ISBN 978-2-8325-5834-8
DOI 10.3389/978-2-8325-5834-8

## 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

# Advances in targeted therapy and biomarker research for endocrine-related cancers

**Topic editors**
Zili Zhang — Nanjing University of Chinese Medicine, China
Xiaoqiang Qi — University of Missouri, United States
Min Tu — Nanjing Medical University, China

**Topic coordinator**
Yang Wu — LMU Munich University Hospital, Germany

**Citation**
Zhang, Z., Qi, X., Tu, M., Wu, Y., eds. (2025). *Advances in targeted therapy and biomarker research for endocrine-related cancers*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-5834-8

# Table of contents

frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 11 December 2024
DOI 10.3389/fendo.2024.1533623



OPEN ACCESS

EDITED AND REVIEWED BY
Claire Perks,
University of Bristol, United Kingdom

*CORRESPONDENCE
Zili Zhang
zilizhang@njucm.edu.cn

RECEIVED 24 November 2024
ACCEPTED 29 November 2024
PUBLISHED 11 December 2024

CITATION
Wu Y and Zhang Z (2024) Editorial: Advances in targeted therapy and biomarker research for endocrine-related cancers.
*Front. Endocrinol.* 15:1533623.
doi: 10.3389/fendo.2024.1533623



# Editorial: Advances in targeted therapy and biomarker research for endocrine-related cancers

Yang Wu[1,2] and Zili Zhang[1]*

[1]School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing, China, [2]Pancreas Center, the First Affiliated Hospital of Nanjing Medical University, Nanjing, China

KEYWORDS

**targeted therapy, biomarker, endocrine-related cancers, molecular mechanisms, clinical applications**

Editorial on the Research Topic
Advances in targeted therapy and biomarker research for endocrine-related cancers

The field of endocrine-related cancers is undergoing rapid transformation, fueled by significant strides in deciphering the molecular and genetic foundations of these diseases (1). These cancers, which encompass a range of malignancies affecting the endocrine system, present unique challenges due to their intricate interactions with hormonal signaling pathways (2). In recent years, the surge in the development of targeted therapies has sparked optimism for more precise and personalized treatment options (3). Simultaneously, the identification and validation of biomarkers have become crucial for improving diagnosis, prognosis, and treatment decision-making, marking a significant shift toward precision medicine. Moreover, cutting-edge research techniques, including organoid cultures and organ-on-a-chip systems, have emerged, providing more accurate and reliable models for studying tumor biology and drug responses (4). These innovations highlight the growing importance of translational medicine, which bridges the gap between laboratory findings and clinical applications, opening new pathways for understanding and addressing endocrine-related cancers. This dynamic landscape calls for a comprehensive examination of both current trends and future directions in the research and treatment of these complex diseases.

The aim of this Research Topic was to accelerate pioneering research and deepen our understanding of endocrine-related cancers. By spotlighting groundbreaking studies on targeted therapies, biomarker discovery, and advanced modeling approaches like organoids and organ-on-a-chip platforms, we seek to foster a connection between experimental research and clinical practice. Our goal was to establish a collaborative forum that not only disseminates cutting-edge scientific insights but also stimulates the development of innovative therapeutic strategies, ultimately enhancing patient outcomes in the field of endocrine oncology. A series of articles have been published under this Research Topic, as shown below.

Head and neck paragangliomas (HNPGNs) are infrequent tumors that originate from the parasympathetic paraganglia located at the skull base and neck (5). Standard treatment options, including surgery and radiotherapy, carry considerable risks due to the tumors' close proximity to critical blood vessels and cranial nerves (6). Diagnosing HNPGNs is particularly challenging, as distinguishing between benign and malignant forms cannot be solely achieved through histology or imaging (7). Gaining a deeper understanding of the tumor microenvironment in HNPGNs could unveil key markers associated with tumor growth and malignancy, potentially enabling earlier detection, more effective targeted therapies, and strategies to prevent recurrences and metastasis. Therefore, Baruah et al. examined the profiles of fibroblasts and macrophages within HNPGNs. They identified the expression of fibroblast markers CD90 and PDPN in HNPGN tissue *in-situ* as well as presence of CD163 expressing macrophages. By isolating fibroblasts from HNPGN tissue, they confirmed CD90 expression *in vitro* and further detected monocarboxylate transporters (MCT1) in HNPGN-derived fibroblasts through flow cytometry. They also observed MCT1 and MCT4 expression in both tumor cells and macrophages within the HNPGN tissue. Further investigation of the phenotypic and functional characteristics of fibroblasts and macrophages in HNPGNs is crucial for better understanding the tumor microenvironment, which could lead to novel approaches for risk stratification and the development of targeted therapies.

Breast cancer is a leading global malignancy in women, with increasing incidence rates (8). It significantly impacts patients' quality of life and health. Despite treatment advancements, challenges persist, including delayed diagnosis, unpredictable outcomes, and drug resistance (9). Consequently, identifying new biomarkers and therapeutic targets is a key focus of current breast cancer research. Therefore, Song et al. conducted a comprehensive proteome-wide Mendelian randomization (MR) study to uncover potential biomarkers and therapeutic targets for breast cancer. Their study identified several proteins linked to increased breast cancer risk, including decreased levels of CASP8, DDX58, CPNE1, ULK3, PARK7, and BTN2A1, as well as elevated levels of TNFRSF9, TNXB, DNPH1, and TLR1. Among these, CASP8 and DDX58 were supported by tier-one evidence, while CPNE1, ULK3, PARK7, and TNFRSF9 received tier-two evidence support. The remaining proteins, TNXB, BTN2A1, DNPH1, and TLR1, were supported by tier-three evidence. Notably, several of these proteins, such as CASP8, DDX58, CPNE1, ULK3, PARK7, and TNFRSF9, are already recognized as potential drug targets. Moving forward, integrating multi-omics data, including expression quantitative trait loci (eQTL) and methylation quantitative trait loci (mQTL), alongside MR and two-sample MR approaches, could offer deeper insights into the molecular mechanisms of breast cancer and aid in the identification of personalized therapeutic targets.

Thyroid cancer originates in the thyroid gland, a butterfly-shaped organ located at the base of the neck, and its incidence has been rising in recent years (10). Key risk factors include radiation exposure, genetic mutations, and family history, with women being more frequently affected (11). Treatment options are largely determined by the cancer's type and stage, with surgery to remove the thyroid being a common approach. Remaining cancer cells are often treated with radioactive iodine therapy (12). Given the complexity of the disease and the need for personalized care, targeted therapies and biomarker research have become crucial areas of focus in thyroid cancer management. Guo et al. conducted a thorough review of conventional treatment strategies, the status of biomarker research, and the latest advancements in targeted therapy for thyroid cancer. They identified several critical molecular markers, including BRAF mutations, RAS mutations, RET/PTC rearrangements, PAX8/PPARγ rearrangements, and TERT promoter mutations, which are strongly associated with the disease. Understanding these biomarkers enables more precise diagnosis, prognostication, and tailored treatment plans for patients. Targeted treatments for thyroid cancer aim to disrupt the cancer cells' growth mechanisms by targeting specific molecules involved in tumor development. Among the key approaches identified are tyrosine kinase inhibitors, thyroid hormone receptor antagonists, radioactive iodine therapy, immunotherapy, and gene-targeted therapies. Further investigation is focused on discovering new therapeutic targets, improving treatment tolerance, and developing combination therapies. The evolving scientific landscape provides hope for more effective, personalized treatment options for thyroid cancer.

Liver hepatocellular carcinoma (LIHC) is the most prevalent primary liver cancer and the third leading cause of cancer-related deaths worldwide (13). It affects individuals with chronic liver diseases, such as those caused by viral hepatitis, alcohol-related liver damage, or non-alcoholic fatty liver disease (14). Identifying clinical and biochemical factors that can pinpoint high-risk groups is crucial for timely intervention through imaging or screening. Improving prevention efforts and developing novel therapies are essential for better outcomes. Yu et al. evaluated the causal impact of exposure factors, including Alzheimer's disease, platelet count, ambidextrousness, cigarettes smoked per day, alcohol consumption, and endocarditis, on the risk of LIHC using a two-sample MR study. Their findings provided compelling evidence supporting a causal relationship between reduced platelet levels and heightened vulnerability to LIHC in the European population. Therefore, it is recommended to prioritize the management of individuals with lower platelet counts to minimize their risk of developing LIHC. As a result, this study contributes to an expanding body of literature indicating that targeting platelet-related agents holds promise as a potential therapeutic approach for early detection and treatment of LIHC.

In summary, the articles collected under this Research Topic provide a comprehensive examination of targeted therapies and biomarkers for endocrine-related cancers. By focusing on the molecular and genetic underpinnings of these tumors, the studies presented offer critical insights into improving diagnosis, prognosis, and treatment personalization. The identification and validation of specific biomarkers have the potential to enhance early detection and guide more precise, individualized treatment approaches. The development of targeted therapies, aimed at disrupting key signaling pathways involved in tumor growth, represents a promising avenue for improving patient outcomes. These findings

contribute to shaping the future direction of clinical management for endocrine-related cancers. Lastly, we thank all our contributors who enriched this Research Topic by submitting manuscripts highlighting their highly valuable and interesting research studies.

## Author contributions

YW: Writing – original draft, Writing – review & editing. ZZ: Funding acquisition, Writing – original draft, Writing – review & editing.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The work was supported by the National Natural Science Foundation of China (No.82474164), the Natural Science Foundation of Jiangsu Province (No. BK20220467), Jiangsu Provincial Double-Innovation Doctor Program (No. JSSCBS20220452, JSSCBS20220472), Young Elite Scientists Sponsorship Program by CACM (No. 2022-QNRC2-B15), Outstanding Young Doctoral Training Program (No. 2023QB0124). The work was sponsored by Qing Lan Project.

## Acknowledgments

We appreciate all authors, reviewers, and journal editors who have contributed to this Research Topic.

## Conflict of interest

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

The author declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

## Publisher's note



## References

1. Revilla G, Cedó L, Tondo M, Moral A, Pérez JI, Corcoy R, et al. LDL, HDL and endocrine-related cancer: From pathogenic mechanisms to therapies. *Semin Cancer Biol*. (2021) 73:134–57. doi: 10.1016/j.semcancer.2020.11.012

2. Zhong W, Wang X, Wang Y, Sun G, Zhang J, Li Z. Obesity and endocrine-related cancer: The important role of IGF-1. *Front Endocrinol*. (2023) 14:1093257. doi: 10.3389/fendo.2023.1093257

3. Powers JF, Cochran B, Baleja JD, Sikes HD, Zhang X, Lomakin I, et al. A unique model for SDH-deficient GIST: an endocrine-related cancer. *Endocr Relat Cancer*. (2018) 25:943–54. doi: 10.1530/ERC-18-0115

4. Rodríguez-Rodero S, Delgado-Álvarez E, Fernández AF, Fernández-Morera JL, Menéndez-Torre E, Fraga MF. Epigenetic alterations in endocrine-related cancer. *Endocr Relat Cancer*. (2014) 21:R319–330. doi: 10.1530/ERC-13-0070

5. Sanna M, Pacak K, Taïeb D, Mariani-Costantini R. A congress on head and neck paragangliomas: advancing clinical care. *Nat Rev Endocrinol*. (2024) 20:383–4. doi: 10.1038/s41574-024-00987-9

6. Richter S, Constantinescu G, Fancello G, Paties CT, Mariani-Costantini R, Sanna M. Head and neck paragangliomas: Recent advances in translational and clinical research and guidelines for patient care. *Best Pract Res Clin Endocrinol Metab*. (2024) 11:101951. doi: 10.1016/j.beem.2024.101951

7. Fedorova M, Snezhkina A, Kalinin D, Pudova E, Lantsova M, Krasnov G, et al. Intratumoral microbiome in head and neck paragangliomas. *Int J Mol Sci*. (2024) 25:9180. doi: 10.3390/ijms25179180

8. Chen Q, Li Y, Hu J, Xu Z, Wang S, Cai N, et al. Local exosome inhibition potentiates mild photothermal immunotherapy against breast cancer. *Adv Sci*. (2024) 22:e2406328. doi: 10.1002/advs.202406328

9. Gong J, Cheng D, Liu C, Wu S, Sun N, Zhao L, et al. Hybrid cell membrane-coated nanoparticles for synergizing sonodynamic therapy and immunotherapy against triple-negative breast cancer. *Adv Healthc Mater*. (2024) 21:e2404184. doi: 10.1002/adhm.202404184

10. Zheng G, Chen S, Ma W, Wang Q, Sun L, Zhang C, et al. Spatial and single-cell transcriptomics unraveled spatial evolution of papillary thyroid cancer. *Adv Sci*. (2024) 14:e2404491. doi: 10.1002/advs.202404491

11. Jiang M, Yu Y, Yang A. Concerns regarding thermal ablation for papillary thyroid cancer. *JAMA Surg*. (2024) 159:1231–2. doi: 10.1001/jamasurg.2024.2744

12. Rezaei SJ, Chen ML, Kim J, John EM, Sunwoo JB, Linos E. Development of melanoma and other nonkeratinocyte skin cancers after thyroid cancer radiation. *JAMA Netw Open*. (2024) 7:e2434841. doi: 10.1001/jamanetworkopen.2024.34841

13. Zhai S, Li Y, Yang Y, Lang W, Liu X, Liu K, et al. Scinderin is a potential prognostic biomarker and correlated with immunological regulation: from pan-cancer analysis to liver hepatocellular carcinoma. *Front Immunol*. (2024) 15:1361657. doi: 10.3389/fimmu.2024.1361657

14. Sun X, Guo P, Wang N, Shi Y, Li Y. A refined therapeutic plan based on the machine-learning prognostic model of liver hepatocellular carcinoma. *Comput Biol Med*. (2024) 169:107907. doi: 10.1016/j.compbiomed.2023.107907

frontiers | Frontiers in Endocrinology

TYPE Systematic Review
PUBLISHED 28 February 2024
DOI 10.3389/fendo.2024.1254213



OPEN ACCESS

EDITED BY
Zili Zhang,
Nanjing University of Chinese Medicine, China

REVIEWED BY
Onur Baş,
Hacettepe University, Türkiye
Zheng Wang,
Shanghai Jiao Tong University, China

*CORRESPONDENCE
Wenjie Lv
lvwenjie_2021@163.com

RECEIVED 06 July 2023
ACCEPTED 12 January 2024
PUBLISHED 28 February 2024

CITATION
Wu P and Lv W (2024) Concurrent neoadjuvant endocrine therapy with chemotherapy in HR+HER2- breast cancer: a systematic review and meta-analysis. *Front. Endocrinol.* 15:1254213. doi: 10.3389/fendo.2024.1254213



# Concurrent neoadjuvant endocrine therapy with chemotherapy in HR+HER2- breast cancer: a systematic review and meta-analysis

Ping Wu and Wenjie Lv*

Department of Breast Surgery, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China

The role of simultaneous neoadjuvant endocrine therapy in chemotherapy in HR+HER2- breast cancer continues to be controversial. This systematic review and meta-analysis was conducted to further evaluate the effectiveness and safety of this strategy for HR+HER2- breast cancer patients. Trials in which HR+HER2- breast cancer patients were randomly assigned to either single or simultaneous endocrine-assisted neoadjuvant chemotherapy were eligible for inclusion. The prime endpoint was the pathological complete response (pCR) rate. The clinical response (complete clinical response: CR, partial response: PR) and safety were secondary endpoints. A random effect model was used for statistical analysis. A total of 690 patients from five trials were included. PCR rate was 10.43% in the concomitant endocrine group and 7.83% in control group (OR=1.37, 95%CI 0.72-2.60, P=0.34). The CR rate was 15.50% for the concomitant endocrine group and 10.26% for the control group. (OR=1.61, 95%CI 0.99-2.61, P=0.05). ORR (CR+PR) was significantly higher in the simultaneous endocrine group compared to the control group (79.53% (272/342) vs. 70.09% (239/341) , OR=1.70, 95%CI 1.19-2.43, P=0.004) and the meta-analysis approach showed no heterogeneity ($I^2$ = 0%, P=0.54) . Tamoxifen concurrent with chemotherapy could increase the frequency of adverse events, whereas aromatase inhibitors (AIs) would not. Our findings provide evidence for the efficacy and safety of concurrent neoadjuvant endocrine therapy (AIs) with chemotherapy as an available option to achieve a higher clinical response rate for HR+HER2- breast cancer patients compared with chemotherapy alone with low toxicity.

**Systematic review registration:** https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42022340725.

KEYWORDS

**concurrent, neoadjuvant endocrine therapy, chemotherapy, HR+HER2-breast cancer, meta-analysis**

# 1 Introduction

Neoadjuvant therapy has become the standard strategy for patients with locally advanced breast cancer. Pathologic complete response (pCR) to preoperative systemic therapy is associated with an extremely favorable disease-free and overall survival (1, 2).International guidelines recommend that a neoadjuvant approach be preferred in subtypes highly sensitive to chemotherapy, such as triple-negative and HER2+ (3–6). HR+HER2- carcinomas are generally less responsive to primary chemotherapy and may benefit less in neoadjuvant setting. Neoadjuvant endocrine therapy for this subtype is also not recommended by guidelines due to limited therapeutic efficacy. Small sample clinical trials have suggested equal rates of clinical response for endocrine therapy as for chemotherapy, though neither approach routinely achieves a rate of pCR>10% (7, 8).

Therefore, neoadjuvant concurrent endocrine therapy with chemotherapy for this particular type of tumor is worth further investigating, while whether concurrent endocrine therapy with chemotherapy in neoadjuvant setting can be of real clinical benefit has never been elaborated. Endocrine therapy needs to be sequenced after chemotherapy based on the previous understanding of adjuvant therapy. However, the prime purpose of neoadjuvant therapy is to shrink the tumor as early as possible with powerful regimens, and delaying endocrine therapy may deprive the patient of the best opportunity for treatment. The previous study suggests in patients with potentially hormone-sensitive metastatic breast cancer, chemohormonal therapy prolongs the time to treatment failure (TTF) for ER-positive patients (9). There are few studies of concurrent endocrine therapy with chemotherapy in neoadjuvant setting worldwide, as well as a lack of systematic reviews to objectively assess the efficacy of their concurrent treatment. This study seeks to provide more conclusive clinical evidence on this controversial topic by conducting a systematic review and meta-analysis on the data from randomized trials that investigated the role of concurrent endocrine therapy as a feasible strategy in neoadjuvant setting for patients with HR+Her2- breast cancer.

# 2 Methods

This systematic review and meta-analysis was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines (10, 11). A protocol was developed prior to the survey launch and presented to PROSPERO. (registration number CRD42022340725).

## 2.1 Search strategy

The PubMed, Embase, and Cochrane Library electronic medical publication databases were searched for relevant randomized controlled trials (RCTs) released before June 2022. Potential relevant RCTs were identified through various combinations of the following search terms: breast neoplasms, concurrent, neoadjuvant therapy and endocrine therapy.

## 2.2 Selection criteria

Randomized controlled trials that enrolled patients of HR+ HER2- breast cancer in neoadjuvant setting were included. In addition, studies were considered relevant if (a) the study concerned clinical research comparing concurrent chemo-endocrine therapy versus chemotherapy alone, (b) the pCR rates and clinical responses had to be reported, (c) the manuscript was published in English, (d) with full text available. Trials that only studied ovarian suppression were excluded. In-progress trials that have not yet been presented at conferences or published or available online at the time of the literature search have also been excluded.

Patients of HR+ HER2- breast cancer enrolled in the study were required to receive chemotherapy and endocrine therapy in neoadjuvant setting. Chemotherapy regimens include EC/AC-T (epirubicin/doxorubicin+ cyclophosphamide four cycles followed by docetaxel four cycles), TAC (epirubicin/doxorubicin+ cyclophosphamide+ docetaxel four cycles), TP (albumin paclitaxel + carboplatin/cisplatin),CMF (cyclophosphamide +methotrexate +5-fluorouracil) and FEC (5-fluorouracil+epirubicin +cyclophosphamide). Endocrine therapy includes tamoxifen/ aromatase inhibitor ± ovarian suppression.

## 2.3 Data extraction

This study aimed to evaluate both the efficacy and the safety of neoadjuvant chemotherapy with or without concurrent endocrine of patients with HR+ HER2- breast cancer. Primary end point was pCR rate. Secondary end points were clinical response and safety. For each eligible study we collected study design, number of patients enrolled overall and into the two study arms. Menopausal status, type of chemotherapy and endocrine therapy administered, the number of patients who achieved pCR and CR (complete response) or PR (partial response), toxicity and adverse events were also collected in both study arms.

Data from each of the included tests were thoroughly verified to ensure that they were consistent with their original publications. The discrepancies were discussed and resolved with the authors prior to aggregation into the final unified database used for the analysis.

## 2.4 Statistical analysis

All analyses were completed, including the total number of patients for which information was available for each specific endpoint. Odds ratios (ORs) with 95% confidence intervals [CI] comparing concurrent administration of neoadjuvant endocrine therapy with chemotherapy and chemotherapy alone were calculated from each article and the synthetic risk estimation was calculated using the DerSimonian and Laird Random Effect Model (12). OR < 1 indicated better outcome for chemotherapy arm, OR > 1 indicated favored prognosis for concurrent arm. Heterogeneity among studies was quantified by the Higgins $I^2$ index.

All statistical tests were two-sided, with P <.05 values considered statistically significant. Statistical analyses and the generation of forest plot were carried out by Reviewer Manager 5.3.

# 3 Results

Of the 94 entries returned during the initial database search, 89 were excluded because they failed to meet the inclusion criteria. In total, five separate randomized trials were considered eligible for this study (13–17). (Figure 1) Mohammadianpanah et al. (2011) and K. Sugiu et al. (2015) trials included 24 and 5 HER2+ patients, respectively. With no neoadjuvant targeted therapy, the findings were valid and the studies were included. Two studies (M. Mohammadianpanah (2011) and G. Minckwit (2001)) enrolled some ER- patients, balanced in both studies, hence also included.

In total, 690 patients were included, of which 345 were randomized to the study group (concurrent endocrine with chemotherapy) and 345 to the control group. Two of the five studies included ER(-) patients. Tamoxifen was used for endocrine therapy in 1 study, and aromatase inhibitors (AIs) were used in the rest. All premenopausal patients were given ovarian function suppression (goserelin/leucovorin) when AIs were administered. Table 1 summarizes the main characteristics of the five included trials.

## 3.1 PCR rates

All 690 patients were evaluated with pCR rates. The concurrent group has a slightly higher pCR rate than the control group (10.43%, 36/345 vs. 7.83%, 27/345), but the ORs did not reach significance (OR=1.37, 95%CI 0.72-2.60, P=0.34). (Figure 2) Of note, even in a study (M. Mohammadianpanah, 2011) including ER (-) patients, concurrent neoadjuvant endocrine therapy (AIs) still could achieve a higher pCR rate compared with chemotherapy alone (25.5% vs.10.2%, P=0.049). This indicated that even PR(+) patients might still benefit from endocrine therapy in neoadjuvant setting which was consistent with the current findings in adjuvant setting (18), although M. Mohammadianpanah believed ER status could be a potential predictor of a better clinical response.

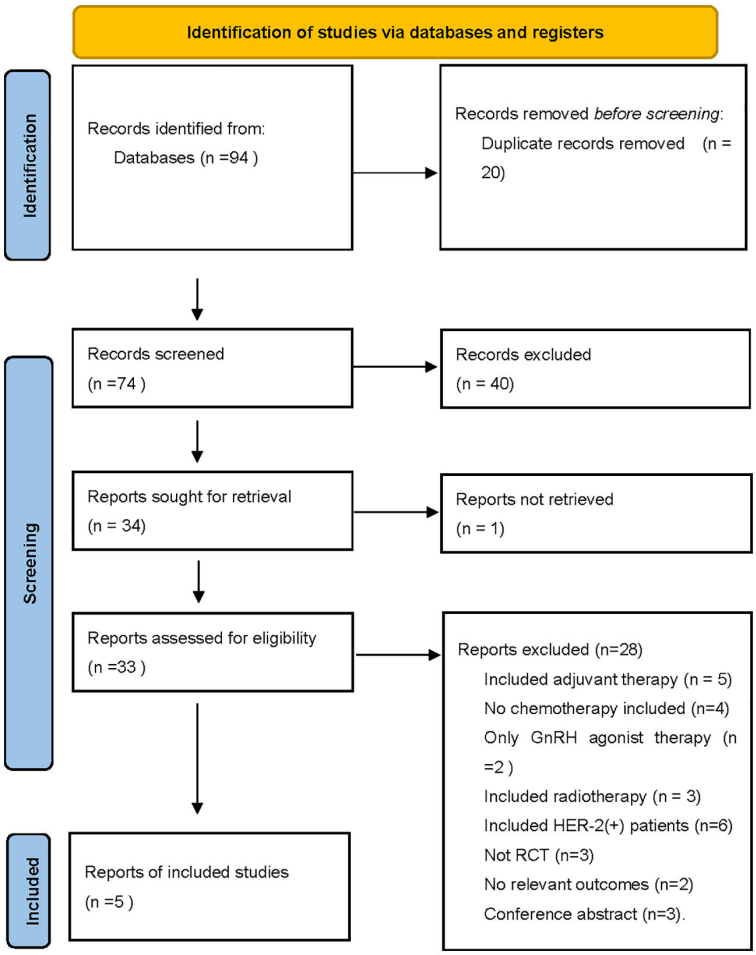



FIGURE 1
The PRISMA flow chart summarizing the process for the identification of the eligible studies.

TABLE 1 Characteristics of the five included randomized trials.

| Study | R.Matsunuma, 2020 (13) | K-D Yu, 2019 (14) | G.Minckwitz,2001 (15) | K.Sugiu, 2015 (16) | M. Mohammadianpanah, 2011 (17) |
|---|---|---|---|---|---|
| Country | Japan | China | German | Japan | Iran |
| Sample size | 70 | 249 | 247 | 28 | 96 |
| Control/Study | 35/35 | 124/125 | 125/122 | 12/16 | 49/47 |
| ER(-) | 0 | 0 | 80 | 0 | 33 |
| Menopausal status (pre/post) | 39/31 | 169/80 | 134/113 | 12/16 | 0/96 |
| Control arm | P-AC or EC | EC-T or FEC | AT | T-FEC | FAC |
| Study arm | +anastrozole ± leuprorelin | +letrozole ± leuproelin | +tamoxifen | +AI ± goserelin | +letrozole |
| Primary endpoint | pCR rate | ORR (CR+PR) | pCR rate | pCR rate | pCR rate |
| Secondary endpoints | Clinical response rate, toxicity, and health-related quality of life | Ki67,pCR rate, PFS,safety | Tumor regression | Tumor regression | CR rate |

P-AC, paclitaxel followed by doxorubicin; EC, epirubicin plus cyclophosphamide; FEC, fluorouracil, epirubicin, cyclophosphamide; AT, doxorubicin plus docetaxel; T-FEC, paclitaxel followed by fluorouracil, epirubicin, cyclophosphamide; FAC, fluorouracil, doxorubicin, cyclophosphamide.

## 3.2 Clinical response rates

Clinical response rates were evaluated in 683 of 690 patients. The complete response (CR) rate is 15.50% (53/342) in concurrent group and 10.26% (35/341) in control group. A trend, albeit non-significant, appears to favor the concurrent addition of endocrine therapy to chemotherapy for a higher CR rate (OR=1.61, 95%CI 0.99-2.61, P=0.05) and the heterogeneity was low ($I^2$ = 3%, P=0.39) . (Figure 3) When the objective clinical response rates (ORR=CR+PR) were compared between the two groups, concurrent endocrine therapy could achieve a significantly higher ORR than chemotherapy alone (79.53% (272/342) vs. 70.09% (239/341) , OR=1.70, 95%CI 1.19-2.43, P=0.004) and the meta-analysis approach showed no heterogeneity ($I^2$ = 0%, P=0.54. (Figure 4) Two studies assessed the relationship between baseline Ki67 levels and clinical response. K-D Yu (14) found patients with a high baseline Ki-67 (>20%) demonstrated a significantly better clinical response to the concurrent treatment (91.2% vs. 68.7%, P = .001). K.Sugiu (16) showed in the high-Ki67 group, both the concurrent-therapy (P=0.084) and chemotherapy-only groups (P=0.026) had relatively favorable decreases in tumor size.

Could higher clinical response rates result in higher breast conservation (BC) rates? Three studies evaluated BC rates of the patients. G.Minckwitz (15) assessed the overall BC rates and found the rate was identical in the two treatment groups (68.6% and 69.0%, with a 95% CI for the difference of -12.0% to+11.1%). The likelihood of retaining the breast in larger tumors was highly dependent on the clinical reaction to preoperative chemotherapy. Patients with tumors greater than 4 cm had a higher rate of breast conservations if they obtained a favorable remission. R.Matsunuma (13) and K.Sugiu (16) analyzed the BC rates of the patients who were considered ineligible for breast-conserving surgery at baseline. In the former study, 22 patients were able to undergo achieved BC surgery through preoperative therapy, 13 (59.1%) of whom received concurrent therapy and 9(40.9%) of whom received chemotherapy alone. In the latter study, of the 6 patients with BC surgery, 4 (66.7%) received concurrent therapy, and 2 (33.3%) received chemotherapy alone. Both studies fully demonstrated the benefit of concurrent endocrine therapy with chemotherapy over chemotherapy alone in further improving BC rates in patients ineligible for breast-conserving surgery prior to treatment.

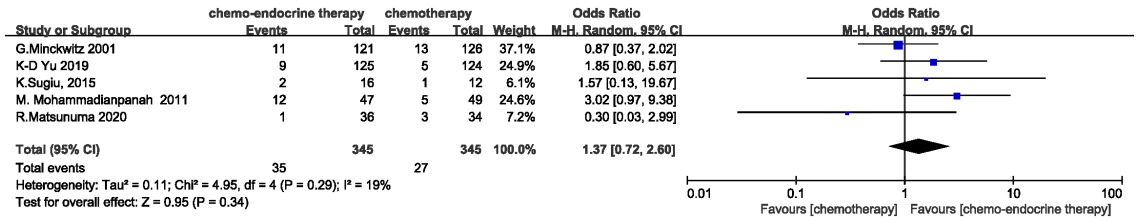



FIGURE 2
Odds ratio for pCR rate in the control arm versus concurrent administration of chemotherapy and endocrine therapy. The squares on the odds ratio plot are proportional to the weight of each study. CI, confidence interval.

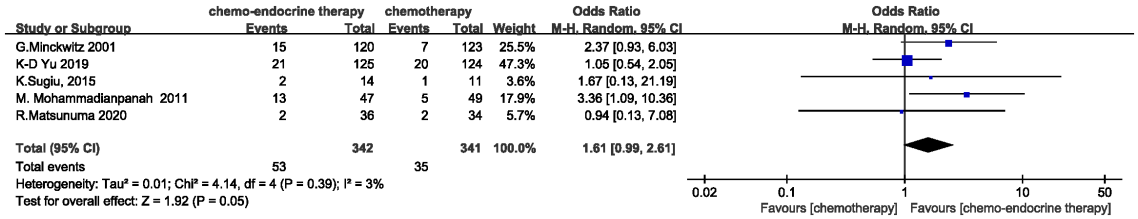



FIGURE 3
Odds ratio for CR rate in the control arm versus concurrent administration of chemotherapy and endocrine therapy.

## 3.3 Safety

A total of 625 patients in four studies (13–15, 17) were evaluated for safety. Hematologic toxicity such as leukopenia or neutropenia was seen in all studies. The decrease in leukocytes and neutrophils was more pronounced in concurrent tamoxifen group (15), while concurrent AIs would not increase hematologic toxicity. The most common grade ≥3 adverse events for non-hematologic toxicity were gastrointestinal effects, pruritus and peripheral neuropathy, rates of which did not significantly differ between the 2 treatment groups. The majority of endocrine-related adverse events, including hot flashes and musculoskeletal pain, were mild to moderate. One study (n=96) found the concurrent arm was associated with higher rate (23.4% vs. 6.1%, P = 0.016) of hot flushes compared with the control arm (17). In one study (15) (n=237) of concurrent tamoxifen, left ventricular ejection fraction fell after treatment. Three of these four events were associated with tamoxifen. Thromboembolic events were reported in 5 patients, of which 4 were on tamoxifen therapy. On the whole, concurrent endocrine therapy would not dramatically increase rates of serious adverse reactions (Grade 3 or 4) compared to chemotherapy alone.

# 4 Discussion

This meta-analysis of five trials investigated the role of additional concurrent endocrine therapy during chemotherapy for patients with HR+ HER2- breast cancer in neoadjuvant setting. Concurrent administration of endocrine and chemotherapy could significantly increase the clinical response rate with low toxicity.

The patients with HR+ HER2- breast cancer are less sensitive to neoadjuvant chemotherapy with a low pCR rate and lack of effective treatment. The efficacy of concurrent addition endocrine therapy to chemotherapy remains controversial. The timing of endocrine administration in neoadjuvant setting currently continues to refer to the adjuvant treatment. International guidelines still recommend sequential endocrine therapy (tamoxifen or aromatase inhibitors) after chemotherapy (3, 4). The latest edition of NCCN and ESMO guidelines both recommend sequential endocrine therapy based on the same phase 3, 3-arm RCT study (19) (tamoxifen alone, sequential tamoxifen with chemotherapy, concurrent tamoxifen with chemotherapy), in which 1477 patients were eligible for analysis after a maximum of 13 years of follow-up (median 8.94 years). The study confirmed therapy with chemotherapy plus tamoxifen combined (sequential or concurrent) was superior to tamoxifen alone for disease-free survival (DFS) (adjusted Cox regression hazard ratio [HR] 0.76, 95% CI 0.64–0.91, p=0.002) and marginally for overall survival (OS) (HR 0.83, 0.68–1.01, p=0.057). The adjusted HRs preferred sequential over concurrent but did not achieve significance for DFS (HR 0.84, 0.70–1.01, p=0.061) or OS (HR 0.90, 0.73–1.10, p=0.30). Similarly, the GEICAM 9401 study (20) compared the clinical benefit of sequential versus concurrent tamoxifen with chemotherapy. No significant difference in DFS at 5 years was found between the two groups with 70% in concurrent and 75% in the sequential group (adjusted HR 1.11, 95% CI 0.71–1.73, P = 0.64). Both studies failed to show an advantage of sequential over concurrent tamoxifen with chemotherapy. Aromatase inhibitor (AI) has emerged as an option for endocrine therapy in recent years, and AI is significantly superior to tamoxifen in both neoadjuvant and adjuvant settings (21–26), suggesting that there may be a potential benefit of concurrent AI administration in the neoadjuvant phase.

The pCR rate has been recognized as an indicator of efficacy after neoadjuvant therapy. The US Food and Drug Administration

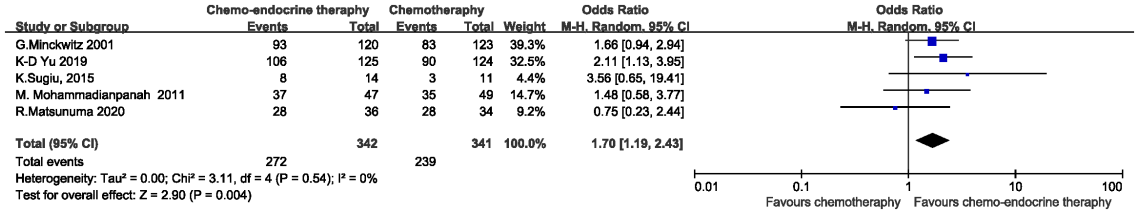



FIGURE 4
Odds ratio for ORR in the control arm versus concurrent administration of chemotherapy and endocrine therapy.

and European Medicines Agency support the use of pCR in early-stage neoadjuvant breast cancer randomized trials as a surrogate for long-term patient clinical outcomes, in the accelerated approval process of new drugs (26, 27). Almost all of the studies included in this meta-analysis investigated pCR rates as the primary endpoint, and the results indicated that concurrent therapy did not significantly improve pCR rates compared with chemotherapy alone. However, the value of pCR rates in predicting prognosis is controversial. The pCR rate to preoperative systemic therapy was previously believed to be associated with an extremely favorable disease-free and overall, but the correlation between pathological response and long-term outcome was highest for triple negative breast cancer (TNBC), slightly less for HER2-positive disease, and least for ER-positive disease (1, 2). Recent meta-analysis (28) confirmed that the weak relationship between pCR and long-term clinical outcomes was evident across all subgroups studied, and pCR should not be recommended as a surrogate for prognosis. Therefore, pCR may not be an appropriate primary endpoint to assess efficacy in neoadjuvant trials of HR+ HER2- breast cancer.

In this study, we assessed the clinical response as the secondary endpoint, and found that the concurrent therapy was more likely to achieve CR than chemotherapy alone, although a statistical difference was not yet reached; however, the concurrent therapy could achieve significantly high ORR than chemotherapy alone. This suggests that concurrent therapy can lead to higher clinical response rates than chemotherapy alone, furthermore, may result in improving BC rates for patients ineligible for breast-conserving surgery at baseline. Unfortunately, only two of the five studies with small samples explored the BC rates for patients ineligible for breast-conserving surgery at baseline, and further studies with large samples may still be needed. A high Ki67 level may serve as a predictor of good clinical response. Meanwhile, one study (14) showed that patients with a higher Ki67 level at baseline were more likely to benefit from disease-free progression survival (PFS rate) with concurrent therapy than with chemotherapy alone (2-year PFS rate 91.5% vs 76.5%, P=0.058), whereas patients with a lower Ki67 level did not (P=0.317). However, whether higher clinical response rates can lead to longer survival benefits was not mentioned in all studies and further investigation is needed.

The previous belief that tamoxifen could increase the incidence of thrombotic events and cardiovascular disease (18, 29) was similarly confirmed in the only study in which tamoxifen was used (15). Concurrent tamoxifen appears to be more prone to occur these adverse events than chemotherapy alone (3/4 vs. 1/4; 4/5 vs. 1/5), and hematologic toxicity was more pronounced in the concurrent tamoxifen group. While concurrent AI as endocrine therapy did not show serious endocrine-related adverse effects (≥ grade 3), and its symptoms such as hot flashes and bone pain were mild or moderate and well-tolerated for patients. Therefore, concurrent tamoxifen seems to increase the incidence of adverse events, while concurrent AI therapy is safe and low-toxicity.

Some limitations of the current study should be acknowledged. Sample sizes were uneven, with some studies having small sample sizes which appear to be inadequate by today's standard to make definitive conclusions. K.Sugiu et al. (2015) trial had a low sample size (n=28) and an imbalanced group allocation (only 1 T3 and 2 T4 tumors were included in the chem-endo group and none in the chemo-group). Some individual study was dated and did not use ovarian function suppression in premenopausal patients, which may have affected the efficacy of endocrine therapy. Also, since this study was not based on individual patient-level data, it was impossible to obtain treatment data for patients with different menopausal statuses, to perform subgroup analysis, and to analyze whether menopausal status affected the efficacy of concurrent endocrine therapy.

In conclusion, our systematic review and meta-analysis provides evidence for the efficacy and safety of concurrent endocrine therapy for patients of HR+HER2- breast cancer in neoadjuvant setting. The pCR rate should not be the primary end point for this subtype of breast cancer. Given the findings of our study, concurrent endocrine therapy should be considered as an available option to improve clinical response, or even maybe increase breast conservation rate. Concurrent AI therapy is safe and well-tolerated, without a significant increase in adverse effects. Further studies are still needed to investigate whether long-term survival benefits can be achieved from concurrent endocrine therapy in neoadjuvant setting.

## 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.

## Author contributions

PW: Data curation, Writing – original draft. WL: Conceptualization, Formal analysis, Investigation, Supervision, Writing – review & editing.

## 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 reviewer ZW declared a shared parent affiliation with the authors to the handling editor at the time of review.

## Publisher's note

# References


1. von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. *J Clin Oncol*. (2012) 30:1796–804. doi: 10.1200/JCO.2011.38.8595

2. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and longterm clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet*. (2014) 384:164–72. doi: 10.1016/S0140-6736(13)62422-8

3. Gradishar WJ, Moran MS, Abraham J, Aft R, Agnese D, Allison KH, et al. Breast cancer, version 3.2022, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. (2022) 20:691–722. doi: 10.6004/jnccn.2022.0030

4. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, et al. ESMO Guidelines Committee. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. (2019) 30:1674. doi: 10.1093/annonc/mdz189

5. Burstein HJ, Curigliano G, Thürlimann B, Weber WP, Poortmans P, Regan MM, et al. Panelists of the St Gallen Consensus Conference. Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. *Ann Oncol*. (2021) 32:1216–35. doi: 10.1016/j.annonc.2021.06.023

6. Korde LA, Somerfield MR, Carey LA, Crews JR, Denduluri N, Hwang ES, et al. Neoadjuvant chemotherapy, endocrine therapy, and targeted therapy for breast cancer: ASCO guideline. *J Clin Oncol*. (2021) 39:1485–505. doi: 10.1200/JCO.20.03399

7. Semiglazov VF, Semiglazov VV, Dashyan GA, Ziltsova EK, Ivanov VG, Bozhok AA, et al. Phase 2 randomized trial of primary endocrine therapy versus chemotherapy in postmenopausal patients with estrogen receptor-positive breast cancer. *Cancer*. (2007) 110:244–54. doi: 10.1002/cncr.22789

8. Kim HJ, Noh WC, Lee ES, Jung YS, Kim LS, Han W, et al. Efficacy of neoadjuvant endocrine therapy compared with neoadjuvant chemotherapy in premenopausal patients with oestrogen receptor-positive and HER2-negative, lymph node-positive breast cancer. *Breast Cancer Res*. (2020) 22:54. doi: 10.1186/s13058-020-01288-5

9. Sledge GW Jr, Hu P, Falkson G, Tormey D, Abeloff M. Comparison of chemotherapy with chemohormonal therapy as first-line therapy for metastatic, hormone-sensitive breast cancer: An Eastern Cooperative Oncology Group study. *J Clin Oncol*. (2000) 18:262–6. doi: 10.1200/JCO.2000.18.2.262

10. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta analyses: the PRISMA statement. *BMJ*. (2009) 339:b2535. doi: 10.1136/bmj.b2535

11. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. (2021) 29:372:n71. doi: 10.1136/bmj.n71

12. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. (1986) 7:177–88. doi: 10.1016/0197-2456(86)90046-2

13. Matsunuma R, Watanabe T, Hozumi Y, Koizumi K, Ito Y, Maruyama S, et al. Preoperative concurrent endocrine therapy with chemotherapy in luminal B-like breast cancer. *Breast Cancer*. (2020) 27:819–27. doi: 10.1007/s12282-020-01077-0

14. Yu KD, Wu SY, Liu GY, Wu J, Di GH, Hu Z, et al. Concurrent neoadjuvant chemotherapy and estrogen deprivation in patients with estrogen receptor-positive, human epidermal growth factor receptor 2-negative breast cancer (CBCSG-036): A randomized, controlled, multicenter trial. *Cancer*. (2019) 125:2185–93. doi: 10.1002/cncr.32057

15. von Minckwitz G, Costa SD, Raab G, Blohmer JU, Eidtmann H, Hilfrich J, et al. German Preoperative Adriamycin-Docetaxel and German Adjuvant Breast Cancer Study Groups. Dose-dense doxorubicin, docetaxel, and granulocyte colony-stimulating factor support with or without tamoxifen as preoperative therapy in patients with operable carcinoma of the breast: a randomized, controlled, open phase IIb study. *J Clin Oncol*. (2001) 19:3506–15. doi: 10.1200/JCO.2001.19.15.3506

16. Sugiu K, Iwamoto T, Kelly CM, Watanabe N, Motoki T, Ito M, et al. Neoadjuvant chemotherapy with or without concurrent hormone therapy in estrogen receptor-positive breast cancer: NACED-Randomized Multicenter Phase II Trial. *Acta Med Okayama*. (2015) 69:291–9. doi: 10.18926/AMO/53675

17. Mohammadianpanah M, Ashouri Y, Hoseini S, Amadloo N, Talei A, Tahmasebi S, et al. The efficacy and safety of neoadjuvant chemotherapy +/- letrozole in postmenopausal women with locally advanced breast cancer: a randomized phase III clinical trial. *Breast Cancer Res Treat*. (2012) 132:853–61. doi: 10.1007/s10549-011-1814-6

18. Eifel P, Axelson JA, Costa J, Crowley J, Curran WJ Jr, Deshler A, et al. National Institutes of Health Consensus Development Conference Statement: adjuvant therapy for breast cancer, November 1-3, 2000. *J Natl Cancer Inst*. (2001) 93:979–89. doi: 10.1093/jnci/93.13.979

19. Albain KS, Barlow WE, Ravdin PM, Farrar WB, Burton GV, Ketchel SJ, et al. Adjuvant chemotherapy and timing of tamoxifen in postmenopausal patients with endocrine-responsive, node-positive breast cancer: a phase 3, open-label, randomised controlled trial. *Lancet*. (2009) 374:2055–63. doi: 10.1016/S0140-6736(09)61523-3

20. Pico C, Martin M, Jara C, Barnadas A, Pelegri A, Balil A, et al. Epirubicin-cyclophosphamide adjuvant chemotherapy plus tamoxifen administered concurrently versus sequentially: randomized phase III trial in postmenopausal node-positive breast cancer patients. A GEICAM 9401 study. *Ann Oncol*. (2004) 15:79–87. doi: 10.1093/annonc/mdh016

21. Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M, Forbes JF, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet*. (2005) 365:60–2. doi: 10.1016/S0140-6736(04)17666-6

22. Smith IE, Dowsett M, Ebbs SR, Dixon JM, Skene A, Blohmer JU, et al. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. *J Clin Oncol*. (2005) 23:5108–16. doi: 10.1200/JCO.2005.04.005

23. Eiermann W, Paepke S, Appfelstaedt J, Llombart-Cussac A, Eremin J, Vinholes J, et al. Preoperative treatment of postmenopausal breast cancer patients with letrozole: a randomized double-blind multicenter study. *Ann Oncol*. (2001) 12:1527–32. doi: 10.1023/A:1013128213451

24. Dixon JM, Renshaw L, Bellamy C, Stuart M, Hoctin-Boes G, Miller WR, et al. The effects of neoadjuvant anastrozole (Arimidex) on tumor volume in postmenopausal women with breast cancer: a randomized, double-blind, single-center study. *Clin Cancer Res*. (2000) 6:2229–35.

25. Cataliotti L, Buzdar AU, Noguchi S, Bines J, Takatsuka Y, Petrakova K, et al. Comparison of anastrozole versus tamoxifen as preoperative therapy in postmenopausal women with hormone receptor-positive breast cancer: the pre-operative "Arimidex" compared to Tamoxifen (PROACT) trial. *Cancer*. (2006) 106:2095–103. doi: 10.1002/cncr.21872

26. US Department of Health and Human Services. US food and drug administration, center for drug evaluation and research (CDER): guidance for industry: pathological complete response in neoadjuvant treatment of high-risk early-stage breast cancer—Use as an endpoint to support accelerated approval. Available online at: www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm305501.pdf.

27. European Medicines Agency. EMA/CHMP/151853/2014: Draft guideline on the role of the pathological complete response as an endpoint in neoadjuvant breast cancer studies. Available online at: www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/04/WC500165781.pdf.

28. Conforti F, Pala L, Sala I, Oriecuia C, De Pas T, Specchia C, et al. Evaluation of pathological complete response as surrogate endpoint in neoadjuvant randomised clinical trials of early stage breast cancer: systematic review and meta-analysis. *BMJ*. (2021) 375:e066381. doi: 10.1136/bmj-2021-066381

29. Winer EP, Hudis C, Burstein HJ, Chlebowski RT, Ingle JN, Edge SB, et al. American Society of Clinical Oncology technology assessment on the use of aromatase inhibitors as adjuvant therapy for women with hormone receptor-positive breast cancer: status report 2002. *J Clin Oncol*. (2002) 20:3317–27. doi: 10.1200/JCO.2002.06.020

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 04 March 2024
DOI 10.3389/fendo.2024.1372553



OPEN ACCESS

EDITED BY
Min Tu,
Nanjing Medical University, China

REVIEWED BY
Feixiang Liu,
First Affiliated Hospital of Henan University of Traditional Chinese Medicine, China
Ping Qiu,
Zhejiang Chinese Medical University, China

*CORRESPONDENCE
Mei Guo
370110@njucm.edu.cn
Chun Zhang
260316@njucm.edu.cn

RECEIVED 18 January 2024
ACCEPTED 19 February 2024
PUBLISHED 04 March 2024

CITATION
Guo M, Sun Y, Wei Y, Xu J and Zhang C (2024) Advances in targeted therapy and biomarker research in thyroid cancer. *Front. Endocrinol.* 15:1372553. doi: 10.3389/fendo.2024.1372553



# Advances in targeted therapy and biomarker research in thyroid cancer

Mei Guo[1*], Yuqi Sun[2], Yuyao Wei[2], Jianxin Xu[2] and Chun Zhang[3*]

[1]School of Nursing, Nanjing University of Chinese Medicine, Nanjing, China, [2]School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing, China, [3]Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, China

Driven by the intricacy of the illness and the need for individualized treatments, targeted therapy and biomarker research in thyroid cancer represent an important frontier in oncology. The variety of genetic changes associated with thyroid cancer demand more investigation to elucidate molecular details. This research is clinically significant since it can be used to develop customized treatment plans. A more focused approach is provided by targeted therapies, which target certain molecular targets such as mutant BRAF or RET proteins. This strategy minimizes collateral harm to healthy tissues and may also reduce adverse effects. Simultaneously, patient categorization based on molecular profiles is made possible by biomarker exploration, which allows for customized therapy regimens and maximizes therapeutic results. The benefits of targeted therapy and biomarker research go beyond their immediate clinical impact to encompass the whole cancer landscape. Comprehending the genetic underpinnings of thyroid cancer facilitates the creation of novel treatments that specifically target aberrant molecules. This advances the treatment of thyroid cancer and advances precision medicine, paving the way for the treatment of other cancers. Taken simply, more study on thyroid cancer is promising for better patient care. The concepts discovered during this investigation have the potential to completely transform the way that care is provided, bringing in a new era of personalized, precision medicine. This paradigm shift could improve the prognosis and quality of life for individuals with thyroid cancer and act as an inspiration for advances in other cancer types.

KEYWORDS

**thyroid cancer, targeted therapy, biomarker research, disease treatment, clinical application**

# 1 Introduction

Thyroid cancer originates from the thyroid gland, a butterfly-shaped organ located near the base of the neck (1). There has been a rise in its occurrence during the last several years (2). Thyroid cancer risk factors include radiation exposure, particular genetic abnormalities, and a family history of the disease (3). Women are more likely than men to develop thyroid cancer, and the risk increases with age (3). Some regions with high iodine intake might have a higher prevalence of subtypes of thyroid cancer (4). Possible symptoms include a lump or swelling in the neck, swallowing difficulty, chronic neck pain, and hoarseness (4). The diagnosis procedure involves a physical examination, imaging tests including ultrasonography, and a biopsy to confirm the existence of cancerous cells (4). The potential treatment options are determined by the type and stage of thyroid cancer. Surgically removing the thyroid gland is a common approach, and any remaining cancer cells are treated with radioactive iodine therapy (5). Thyroid cancer usually has an excellent prognosis, especially if it is detected early and treated promptly (6). Regular follow-up treatment is crucial to monitor for any signs of a recurrence (6).

Molecular biomarkers are essential for thyroid cancer diagnosis, prognosis, and treatment because they offer important information on the molecular features of the illness (7). BRAF mutation (8), RAS mutation (9), RET/PTC rearrangement (10), PAX8/PPARγ rearrangement (11), and TERT promoter mutation (12), are among the major molecular indicators linked to thyroid cancer. The mutation V600E in the BRAF gene is frequently found in papillary thyroid carcinoma (PTC). Identifying this mutation improves prognosis and may have an impact on therapy choices (8). Thyroid tumors that are poorly differentiated and follicular frequently include mutations in the RAS gene. Detecting RAS mutations aids in improving the precision of diagnosis and forecasting the behavior of tumors (9). PTC is commonly associated with RET proto-oncogene rearrangements, such as RET/PTC. These reorganizations function as crucial indicators for diagnosis (10). A particular genetic change that is present in follicular thyroid carcinoma (FTC) and helps distinguish this thyroid cancer subtype from other types of thyroid cancer is the PAX8-PPARγ rearrangement (11). A worse prognosis is linked to aggressive forms of thyroid cancer that have mutations in the TERT promoter region. Risk categorization is informed by the discovery of TERT mutations (12). Comprehending these molecular biomarkers facilitates more accurate diagnosis, prognostication, and customized treatment plans for individuals with thyroid cancer.

Thyroid cancer targeted treatment aims to more precisely interfere with the development and multiplication of cancer cells using specialized ways to target molecules involved in cancer growth (13). Inhibitors of tyrosine kinase (14), inhibitors of thyroid hormone receptor (15), radioactive iodine treatment (16), immunotherapy (17), and gene targeted therapy (18) are some of the main targeted treatments for thyroid cancer. Drugs such as Sorafenib and Lenvatinib target abnormal activation of tyrosine kinase in thyroid cancer. These drugs prevent the enzyme from functioning, which hinders the development and division of cancer cells (14). Drugs such as Dabrafenib and Trametinib disrupt certain signaling pathways in malignant cells to treat unresectable or recurrent thyroid cancer (15). Despite difficulties during surgery or recurrence, radioactive iodine therapy continues to be efficient in eliminating any residual cancer cells using radioactive iodine (16). To treat resistant thyroid cancer, immune checkpoint inhibitors such as pembrolizumab and nivolumab are being studied. These inhibitors work by stimulating the immune system against cancer cells (17). To precisely restrict the proliferation of cancer cells, novel medications that specifically target gene abnormalities have been produced (18). Customized to the specific needs of each patient and kind of pathology, targeted treatment requires medical oversight to prevent adverse effects and maintain maximum effectiveness and quality of life.

Biomarker research and targeted therapeutic research offer unprecedented opportunities for the clinical treatment of thyroid cancer, but also present formidable challenges. In this article, we comprehensively analyze the conventional treatment strategies for thyroid cancer, the status of biomarker research, and the latest developments in targeted therapy.

# 2 Overview of thyroid cancer

## 2.1 Pathological features of thyroid cancer

Due to its heterogeneous nature, thyroid carcinoma exhibits a wide range of clinical features (19). Thyroid cancer's early asymptomatic state is one of its distinctive features. Many people don't know they have the disease unless they happen to feel thyroid nodules while getting regular checkups or imaging tests (19). Even though the majority of thyroid nodules are benign, an in-depth examination is necessary to rule out cancer (19). These nodules can be solitary or multi-located, and they can differ in size and consistency (20). A crucial diagnostic technique for determining these nodules and differentiating between benign and malignant lesions is fine-needle aspiration (FNA) biopsies (20). Thyroid imaging is a crucial approach for thyroid cancer diagnostics. Comprehensive details regarding nodule features, such as size, composition, and vascularity, can be obtained using ultrasonography (20). Computed tomography (CT) and magnetic resonance imaging (MRI) provide information on the degree of tumor invasion and the involvement of nearby structures, which helps with surgical planning (21). In cases of differentiated thyroid cancer, radioiodine scintigraphy is used to measure the thyroid tissue's uptake of radioactive iodine, which helps guide postoperative care and recurrence surveillance (21). In situations where iodine uptake is restricted, PET scans can be employed to obtain further insights into the dissemination of the disease.

Thyroid hormone levels can fluctuate in certain individuals, which may lead to symptoms like exhaustion, mood swings, and changes in weight (22). But it is important to remember that thyroid cancer can coexist with normal thyroid function, so a diagnosis based solely on hormonal alterations is inadequate (22). Clinical signs such as neck pain, discomfort, or a lump sensation may indicate an individual needs medical attention. Thyroid nodule expansion or involvement of other structures frequently causes

these symptoms (23). Swallowing difficulties and hoarseness can arise from tumor compression or infiltration of the esophagus or recurrent laryngeal nerve. Thyroid cancer frequently presents with the enlargement of the neck lymph nodes (23). Palpable lymph nodes, especially those exhibiting questionable features, require further imaging examinations and biopsies to assess the degree of disease dissemination (24). There is histological variation in thyroid cancer, with PTC being the most common subtype. Under a microscope, PTC frequently exhibits well-differentiated appearance with distinctive nuclear characteristics (25). Another histological subtype that has unique features and is linked to an increased risk of vascular invasion is called FTC (25). Despite being uncommon, one aggressive type of thyroid cancer known for its quick growth and dismal prognosis is anaplastic thyroid carcinoma (ATC). Parafollicular C cells are the source of medullary thyroid cancer (MTC), which has been linked to family disorders.

## 2.2 Health hazards of thyroid cancer

It is imperative that patients, healthcare providers, and the public comprehend the various consequences associated with thyroid cancer. First and foremost, thyroid cancer has the potential to disrupt the delicate hormone balance, which might affect the body's metabolism and energy regulation (26). These hormones' dysregulation, which is frequently observed in thyroid cancer cases, can cause symptoms like weariness, worry, emotional instability, and variations in weight (26). Thyroid cancer can present with physical symptoms up to the neck, where the primary tumor is located (27). It is possible for patients to feel pressure, discomfort, or pain in their necks. As the tumor grows larger, it may compress nearby structures and cause symptoms like hoarseness and difficulty swallowing (27). These physical symptoms lead to a lower quality of life in addition to interfering with daily activities (27). Thyroid cancer frequently involves lymph nodes, particularly if there is cervical lymphadenopathy (28). Palpable swelling, pain, and an increased level of intricacy in the disease can all result from enlarged lymph nodes in the neck. Thyroid cancer cells have the potential to spread throughout the circulation and cause distant metastases in organs including the lungs, bones, or other vital organs (28). The health risks are increased when tumor metastasis occurs, making treatment more difficult and raising the possibility of unfavorable results. The health risks are especially significant for some subtypes of thyroid cancer, such as ATC.

Aggressive growth, quick development, and a bleak prognosis are characteristics of ATC. The seriousness of the health hazards associated with specific thyroid cancer subtypes is highlighted by the fact that patients with ATC frequently encounter major hurdles regarding treatment response and overall survival (29). There are potential health risks associated with the diagnostic and therapy procedures itself. There is an inherent risk of problems with diagnostic tests, such as hemorrhage, infection, and injury to adjacent structures (29). These hazards include imaging examinations, surgical treatments, and fine-needle aspiration (FNA) biopsies (30). Furthermore, radiation induced malfunction of the salivary glands and its long-term repercussions are possible side effects and problems of radioactive iodine ablation, a popular postoperative treatment for thyroid cancer (31). Moreover, it is impossible to overstate the psychological effects of thyroid cancer (31). Receiving a cancer diagnosis is a transformative experience that frequently results in psychological anguish, anxiety, and despair. Individuals may struggle with uncertainty anxiety, worries about how their treatments will turn out, and the psychological weight of knowing they may die (32). A complete approach to patient care for thyroid cancer must address the psychological elements of the disease (32). Thyroid cancer has consequences for society and the economy in addition to personal costs. The financial burden on individuals and healthcare systems is exacerbated by the expenses related to thyroid cancer diagnosis, treatment, and long-term management (33). Furthermore, there are wider societal ramifications from the possible loss of productivity and quality of life for those impacted (33). The health risks associated with thyroid cancer are multifaceted, involving physical, physiological, and psychological factors.

## 2.3 Conventional treatment strategies for thyroid cancer

Surgical techniques, radioactive iodine therapy, and thyroid hormone replacement are commonly employed as conventional therapeutic procedures for thyroid cancer (34). Comprehending these therapeutic approaches is essential to maximizing results and guaranteeing the welfare of patients with thyroid cancer (34). The mainstay of treatment for thyroid cancer is surgery, which aims to remove the tumor and any nearby lymph nodes that may need to be removed (35). Tumor size, metastasis presence, and histological subtype all influence whether surgery is performed (35). Thyroidectomy is the most frequent surgical surgery in which the thyroid gland is removed whole or in part (36). For differentiated thyroid tumors, radioactive iodine treatment is frequently used after surgery (36). Radioactive iodine is used in this therapy to specifically target and eliminate tiny cancer cells and any surviving thyroid tissue (36). This therapy approach is quite particular because thyroid cells, especially malignant ones, have a special capacity to absorb iodine (36). A crucial part of postoperative treatment is thyroid hormone replacement medication (37). Individuals need to take thyroid hormone supplements for the rest of their lives to maintain normal physiological processes since thyroidectomy causes the loss of thyroid function (37). The most often recommended drug is levothyroxine, which is a synthetic version of the thyroid hormone thyroxine T4 (37). External beam radiation therapy may be an option for individuals whose aggressive or advanced thyroid tumors do not respond to standard care (37). The goal of this treatment is to stop the growth of the malignant tissues by applying specific radiation. Usually, external beam radiation is saved for situations in which radioactive iodine therapy and surgery are not enough to manage the illness. Advances in thyroid cancer therapy options are mostly dependent on clinical studies. These clinical

studies assess the safety and effectiveness of new medications, combinations of treatments, and therapy modalities. Enrolling in clinical trials provides patients with access to novel treatments that may result in better outcomes (38). A comprehensive approach to therapy is ensured by the collaboration of endocrinologists, surgeons, radiation oncologists, and medical oncologists in customizing treatment programs depending on the diagnosis of each patient (38). Tracking therapy response and identifying any recurrence signals requires routine monitoring with imaging scans, blood tests, and clinical evaluations (38). In summary, radioactive iodine therapy, surgery, and thyroid hormone replacement are traditional treatments for thyroid cancer. These interventions, which are based on the unique features of the tumor, are designed to get rid of cancerous cells, control symptoms, and stop the tumor from coming back.

# 3 Advances in biomarker research of thyroid cancer

Thyroid cancer molecular biomarker research has made great progress, providing important insights into the disease's complexity (7). Proteomics and genomics advances have revealed particular genetic abnormalities and molecular changes linked to distinct subtypes of thyroid cancer (7). Markers such as BRAF and RAS mutations have become critical for diagnosis and prognosis (8, 9). Furthermore, studies have shown intriguing biomarkers that indicate therapy response, which might help with treatment selection. By evaluating circulating tumor DNA, liquid biopsies provide a non-invasive way to track the course of the illness and identify recurrences before they become severe (39). Clinical practice may greatly benefit from the integration of molecular profiling in order to improve patient outcomes, risk classification, and customized treatment plans. The field of thyroid cancer research is being shaped by ongoing studies into new biomarkers, which are helping to better understand the condition and opening the way for innovative targeted therapies (Table 1) (Figure 1).

## 3.1 Genetic mutations

The BRAF mutation is a critical genetic modification in the landscape of thyroid cancer, notably in PTC (8). The substitution of glutamic acid for valine at codon 600 (V600E) is the most common BRAF mutation linked to thyroid cancer (8). The BRAF V600E mutant is present in a considerable number of PTC patients, which emphasizes its utility as a molecular biomarker (8). This mutation causes the MAPK pathway to become constitutively activated, which promotes unchecked cell division and growth. The disease development and severity of PTC are influenced by the downstream effects of this abnormal signaling cascade (40). The existence of the BRAF V600E mutant in clinical settings has consequences for both diagnosis and prognosis (40). It helps distinguish between benign thyroid nodules and malignant tumors, making it a useful diagnostic marker. Moreover, its discovery in PTC is linked to particular clinicopathological characteristics such lymph node metastases, extrathyroidal extension, and heightened recurrence risk (41). The BRAF V600E mutation has become a target for

TABLE 1 Biomarker research of thyroid cancer.

| Biomarkers | Notes | Diseases | Drugs | Functions | References |
|---|---|---|---|---|---|
| BRAF | V600E mutation | PTC | Vemurafenib, Dabrafenib | Activation of MAPK pathway | (40–42) |
| RAS | RAS mutation | FTC | Sotorasib | Cell growth and differentiation | (43–47) |
| TSHR | TSHR mutation | PTC, FTC | Protirelin | Activation of the receptor | (22, 48–52) |
| RET | RET/PTC rearrangement | PTC | Vandetanib Cabozantinib | Cell survival and differentiation | (10, 53–55) |
| PAX8/PPARγ | PAX8/PPARγ rearrangement | FTC | Pioglitazone | Lipid metabolism | (11, 56–58) |
| TERT | TERT promoter mutations | ATC | Under investigation | Cell division | (59–61) |
| miR-146b | Downregulation | PTC | Under investigation | Tumor characteristics | (63) |
| miR-221/222 | Upregulation | PTC, ATC | Under investigation | Cell proliferation and survival | (64) |
| miR-21 | Upregulation | PTC | Under investigation | Cell proliferation and migration | (62) |
| miR-29b | Downregulation | PTC, FTC | Under investigation | Modulation of ECM components | (62) |
| miR-96 | Upregulation | PTC, FTC | Under investigation | Cell proliferation and migration | (62) |
| Thyroglobulin | Upregulation | PTC, FTC | Under investigation | thyroid hormone biosynthesis | (66–69) |
| Calcitonin | Upregulation | MTC | Under investigation | Calcium homeostasis | (70–74) |
| Galectin-3 | Upregulation | PTC | Under investigation | Cell adhesion and apoptosis | (75–80) |
| CK19 | Upregulation | PTC | Under investigation | Tumor development and progression | (81–87) |
| ctDNA | Upregulation | PTC, FTC | Under investigation | Detection of specific genetic alterations | (88–91) |
| PD-L1 | Upregulation | PTC, FTC | Atezolizumab Durvalumab | Immunotherapy | (92–96) |

10.3389/fendo.2024.1372553

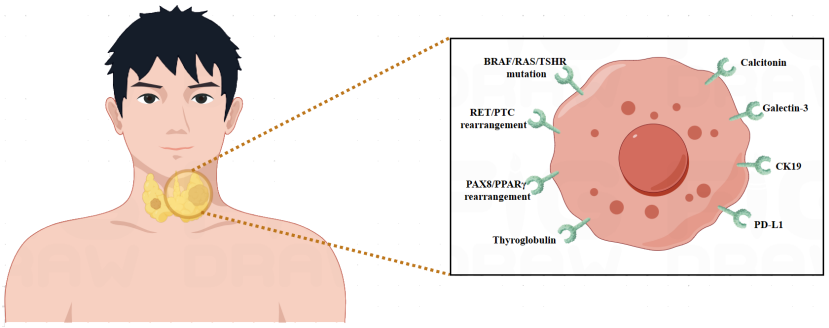



FIGURE 1
Overview of biomarkers for thyroid cancer.

innovative therapeutic approaches. Preclinical and clinical research have demonstrated the promise of inhibitors like vemurafenib and dabrafenib, which are specially made to target mutant BRAF proteins (41). These BRAF inhibitors stop the aberrant signaling cascade, which inhibits the growth of tumors and may open up new therapeutic options that are more focused and efficient (42). Comprehending the molecular details of the BRAF mutation has improved our understanding of the biology of thyroid cancer and opened the door to tailored treatment approaches. Treatment for thyroid cancer has changed dramatically with the introduction of targeted medications intended to block the effects of the BRAF V600E mutation.

RAS mutations are a key molecular change associated with thyroid cancer and specific forms of poorly differentiated and anaplastic thyroid carcinomas (9). RAS mutations are often linked to FTC in thyroid cancer, where they have a role in the development and course of the illness (43). Although these mutations are less common in well-differentiated PTC, they can still be detected in certain instances, particularly in those that have a follicular variation (44). The presence of RAS mutations frequently indicates a different molecular route in thyroid carcinogenesis than the more common BRAF-mutated PTC (44). RAS mutations have distinct clinical effects on thyroid carcinoma. RAS-mutant FTC frequently has a follicular growth pattern and may be linked to vascular invasion, which may increase the tumor's aggressiveness (45). By contrast, PTC with RAS mutations may exhibit unique histological characteristics, such as a follicular growth pattern, necessitating a correct diagnosis in order to implement the best course of treatment (46). The precise prognostic significance is still unclear despite several studies suggesting a relationship with more aggressive disease features, such as an increased risk of recurrence and a reduced overall survival rate (46). RAS mutations present therapeutic problems since they have demonstrated to be less responsive to targeted therapy than other variants, such as BRAF V600E. The deficiency of efficacious targeted inhibitors exclusively for cancers mutant in RAS highlights the necessity for substitute therapeutic approaches (47). In addition to improving diagnostic precision, knowledge of the molecular landscape of RAS mutations in thyroid cancer may influence therapy choices. Given the unique biological activity linked to RAS mutations, the discovery of these mutations may lead to increased surveillance and customized treatment strategies.

Thyroid stimulating hormone receptor (TSHR) mutations are genetic changes that affect the function of the TSHR gene, which is an important regulator of thyroid function (48). Thyroid function dysregulation can arise from TSHR mutations that either constitutively activate or inactivate the receptor (48). Depending on the type of mutation, these mutations can cause both hypothyroidism and hyperthyroidism, among other thyroid problems (49). Increased sensitivity to TSH as a result of constitutive activation of the TSHR mutation results in unregulated thyroid hormone (49). This situation is frequently linked to hyperthyroidism, including Graves' disease, an autoimmune condition in which autoantibodies activate TSHR (49). Conversely, mutations that render the TSHR inactive may result in a decreased thyroid hormone synthesis via decreasing response to TSH (50). This disorder is linked to hypothyroidism and may be a factor in thyroid dyshormonogenesis or congenital hypothyroidism (50). The particular mutation and its functional ramifications determine the clinical significance of TSHR mutations (51). When hypothyroidism is present, symptoms like lethargy, weight gain, and cold sensitivity may appear, whereas symptoms of hyperthyroidism may include anxiety, accelerated heart rate, and weight loss (51). Genetic testing can detect TSHR mutations, which is useful information for a precise diagnosis and for informing therapy choices (22). Management approaches for patients of hyperthyroidism may involve radioactive iodine treatment, antithyroid drugs, or surgery (22). Thyroid hormone replacement medication is commonly used to treat hypothyroidism brought on by TSHR mutations. Not only is it essential for clinical care to comprehend the genetic basis of TSHR mutations, but it also advances thyroid biology research (52). Our understanding of thyroid problems is improved by elucidating the molecular pathways behind these alterations, which opens the door to customized treatment methods and targeted medicines (52). The precise form of the mutation determines the clinical presentation and therapeutic approaches, highlighting the need of genetic testing in the all-encompassing care of thyroid problems.

## 3.2 Gene rearrangements

The RET/PTC rearrangement is a genetic change that contributes to the development of papillary thyroid carcinoma, the most frequent kind of thyroid cancer (10). This rearrangement includes the fusing of the RET proto-oncogene with the PTC gene, which results in a chimeric gene that promotes uncontrolled cell growth and proliferation (10). The fusion event in the setting of RET/PTC rearrangements results in a constitutively active RET kinase, which in turn stimulates downstream signaling pathways, most notably the MAPK pathway (10). The most prevalent fusion forms, with unique clinicopathological characteristics, are RET/PTC1 and RET/PTC3 (10). These reorganizations are linked to a particular histological pattern that has a noticeable papillary form (10). Finding RET/PTC rearrangements is important from a diagnostic standpoint. The existence of these rearrangements contributes to a more precise tumor categorization by helping to differentiate PTC from other thyroid lesions (53). Furthermore, risk assessment and treatment planning may be impacted by the discovery of RET/PTC rearrangements (53). Certain clinicopathological characteristics, such as a greater frequency in younger individuals and a higher probability of multifocality, are frequently seen in RET/PTC-positive tumors (54). The correlation with radiation exposure, especially during early life, emphasizes the environmental elements influencing the formation of these rearrangements (54). One therapeutic approach that may be used is to address the abnormal RET kinase activity. Tyrosine kinase inhibitors that target RET, such as vandetanib and cabozantinib, have showed promise in clinical studies, while particular inhibitors for RET/PTC-positive thyroid tumors are currently being investigated (54). By blocking the signaling pathways started by RET/PTC rearrangements, these targeted treatments hope to slow the growth of tumors and maybe improve the prognosis of patients who carry these particular genetic abnormalities (55). A specific molecular characteristic of PTC that adds to our knowledge of the disease's pathophysiology is the presence of RET/PTC rearrangements (55). Their detection has consequences for both diagnosis and prognosis, and continued research into targeted therapies might lead to more efficient treatments specifically designed for patients with thyroid malignancies that are positive for RET or PTC (55). As molecular insights emerge, deciphering the intricacies of these rearrangements holds promise for enhancing precision medicine in thyroid cancer treatment.

The PAX8/PPARγ rearrangement is a molecular abnormality found in thyroid carcinoma, especially FTC (11). The fusion of PAX8 and PPARγ genes creates a chimeric gene with carcinogenic potential (11). The follicular variety of FTC is characterized by the PAX8/PPARγ rearrangement, which offers molecular insights into the pathophysiology of this subtype (11). Although FTC is thought to be less aggressive than PTC, its ability to spread to other areas of the body and invade blood vessels presents management issues that require a fuller knowledge of the molecular basis of the disease (56). Accurate diagnosis of FTC is aided clinically by identifying the PAX8/PPARγ rearrangement (56). For the purpose of choosing the best course of treatment, it is essential to distinguish between FTC and follicular adenoma, a benign thyroid lesion that shares a similar histological appearance (57). Molecular testing is frequently used to validate the existence of this rearrangement, which helps to provide a more accurate classification of thyroid cancers (57). Histologically, FTCs that are positive for PAX8/PPARγ may display distinct characteristics including a solid or trabecular development. Furthermore, treatment planning and risk stratification may be affected by the discovery of this rearrangement (58). Treatment with radioactive iodine may be taken into consideration when there is vascular invasion or a higher chance of recurrence. Future targeted therapeutics targeting the PAX8/PPARγ fusion protein could result in more precise and efficacious interventions (58). A unique molecular characteristic linked to follicular thyroid cancer is the rearrangement of PAX8/PPARγ (58). Its detection guides the management of individuals with this particular genetic mutation and has diagnostic and potentially prognostic ramifications. There is hope for the development of targeted treatments that could improve treatment options and outcomes for those with PAX8/PPARγ-positive thyroid malignancies as research into these complex tumors continues.

## 3.3 TERT promoter mutations

Mutations in the telomerase reverse transcriptase (TERT) gene promoter area are linked to worse prognoses and more aggressive forms of thyroid cancer (12). TERT promoter mutations are genetic changes that take place in the telomerase reverse transcriptase promoter, which is the promoter region of the TERT gene (12). These mutations are linked to increased telomerase activity, which promotes cellular immortalization and aids in oncogenesis (12). TERT promoter mutations occur frequently in aggressive subtypes of thyroid cancer, including versions of differentiated thyroid carcinoma (12). These alterations are linked to more advanced stages of the disease, larger tumors, and an increased risk of lymph node metastases and extrathyroidal extension (59). Furthermore, TERT promoter mutations are linked to a worse chance of survival and recurrence of the disease, making them a predictor of poor prognosis (59). Finding TERT promoter mutations has important diagnostic and prognostic implications in the clinical setting. Their existence may suggest a more severe disease phenotype in thyroid cancer diagnosis, impacting postoperative care and treatment choices (60). When a tumor is difficult to distinguish based just on histopathological findings, TERT promoter mutation analysis is very pertinent (60). Therapeutic treatment planning may be affected by the presence of TERT promoter mutations. The discovery of TERT promoter mutations may lead to more careful monitoring and individualized treatment strategies, as tumors containing these mutations may show resistance to traditional medicines (61). Nevertheless, there are currently few readily accessible targeted treatments that directly suppress mutations in the TERT promoter. Researching the molecular effects of TERT promoter mutations may have therapeutic applications in addition to improving thyroid cancer diagnosis and prognostication (61). TERT promoter mutation analysis may eventually be included into standard clinical practice, which could improve the accuracy of managing thyroid cancer and open up new possibilities for individualized treatment plans.

## 3.4 microRNA expression profiles

Dysregulation of microRNA (miRNA) expression is observed in thyroid cancer. Particular miRNA patterns are linked to the development, spread, and response to therapy of tumors (62). The complex molecular signatures known as miRNA expression profiles offer important new perspectives on the cellular mechanisms governing gene expression (62). MiRNA expression profiles have become important resources in the field of cancer research, particularly thyroid cancer, since they provide insight into the etiology, prognosis, and possible therapeutic approaches of the illness (62). A typical characteristic of cancer is the dysregulation of miRNAs, whose abnormal expression aids in the development, advancement, and metastasis of malignancies (62). Different histological subtypes of thyroid cancer have been found to have diverse miRNA expression profiles, which helps with the molecular classification of malignancies (62). miR-146b is known for its ability to modulate inflammation, and it is frequently downregulated in PTC (63). The miR-221/222 cluster is often overexpressed in thyroid cancer and has a role in controlling important signaling pathways that support the growth and survival of cells (64). Thyroid cancer is associated with an upregulation of miR-155, which facilitates the migration and proliferation of cancer cells (65). Higher expression of miR-21 has been linked to a number of malignancies, including thyroid carcinoma (62). It encourages cell invasion and survival, which adds to the aggressive nature of thyroid cancers (62). Tumor suppressor miR-34a is frequently downregulated in thyroid carcinoma. Advanced stages of the disease are linked to its decreased expression (62). MiR-29b downregulation has been linked to aggressive thyroid carcinoma characteristics. Its functions include suppressing metastasis and modifying the components of the extracellular matrix (62). MiRNA expression profiles function as predictive and diagnostic indicators. Preoperative diagnosis accuracy can be improved by using specific miRNA signatures that can distinguish between benign and malignant thyroid lesions (62–64). MiRNA expression patterns have a wide range of potential therapeutic applications. To restore or suppress certain miRNAs for therapeutic purposes, researchers are investigating the creation of miRNA-based therapies, such as miRNA mimics and inhibitors (62–64). Technological developments like microarray studies and high-throughput sequencing have made it possible to profile miRNAs in a wide range of biological samples (62–64). These methods enable researchers to find important regulatory networks, discover patterns of global miRNA expression, and locate putative illness biomarkers (62–64). MiRNA expression profiles provide a complex layer of biological data that aids our knowledge of thyroid cancer and other disorders (62–65). The complex interaction between miRNAs and gene regulation sheds light on the molecular landscape of malignancies, opening up new diagnostic, prognostic, and therapeutic prospects. As research advances, incorporating miRNA expression profiling into clinical practice has the potential to improve the accuracy of thyroid cancer care and advance customized therapy methods.

## 3.5 Thyroglobulin

Thyroglobulin is a protein generated by both healthy and malignant thyroid cells (66). Serum thyroglobulin levels are measured as a biomarker of recurrence following thyroidectomy (66). Elevated levels might suggest persistent or recurring illness. Thyroglobulin is generated and maintained in thyroid follicular cells, where it is essential in the intricate process of thyroid hormone production (66). Thyroglobulin is a big protein with a molecular weight of about 660 kDa. It consists of a single polypeptide chain with numerous tyrosine residues that are iodinated during thyroid hormone production (66). Thyroglobulin is synthesized in the endoplasmic reticulum of thyroid follicular cells before being transferred to the thyroid follicular colloid, where thyroid hormone is synthesized (67). Thyroglobulin's principal role is to act as a framework for thyroid hormone production and storage, namely thyroxine (T4) and triiodothyronine (T3). Thyroid peroxidase enzymes iodize particular tyrosine residues on thyroglobulin, resulting in monoiodotyrosine (MIT) and diiodotyrosine. These iodinated tyrosine residues are coupled together to create T3 and T4 inside the thyroglobulin structure (67). When triggered by thyroid-stimulating hormone (TSH), thyroid follicular cells transport thyroglobulin from the colloid to vesicles. Enzymes in these vesicles breakdown thyroglobulin, releasing T3 and T4 into the circulation. Thyroglobulin is also secreted into the bloodstream, acting as a quantifiable indication of thyroid function. Serum thyroglobulin levels are an important clinical tool for assessing thyroid diseases (68). Thyroglobulin levels are typically modest in healthy people, but increased levels might suggest a variety of diseases. Following thyroidectomy for thyroid cancer, serum thyroglobulin levels are measured as a tumor marker (68). An increase in thyroglobulin levels might indicate remaining or recurring thyroid cancer cells. Thyroglobulin testing is also used to determine the efficacy of thyroid cancer therapies such radioactive iodine therapy (68). While thyroglobulin is an important clinical marker, measuring it might be hindered by anti-thyroglobulin antibodies (TgAbs). This can interfere with reliable thyroglobulin tests, necessitating cautious interpretation in clinical situations (68). Thyroglobulin is a key glycoprotein that plays an important role in thyroid hormone production and storage. Its blood levels give useful information for measuring thyroid function, treating thyroid diseases, and tracking thyroid cancer therapy results. The intricate interaction of thyroglobulin inside the thyroid gland emphasizes its importance in maintaining hormonal balance and general thyroid function.

## 3.6 Calcitonin

Calcitonin is an important biomarker in the setting of thyroid cancer, particularly in the diagnosis and monitoring of MTC (69). MTC is an uncommon form of thyroid cancer that arises from parafollicular C cells, accounting for just 1-2% of all thyroid malignancies (70). Calcitonin is a hormone generated by the

thyroid gland's C cells. Its principal physiological duty is to maintain calcium homeostasis by reducing osteoclast activity in bones, resulting in less calcium release into the circulation (70). Calcitonin levels are considerably higher in MTC patients (71). Unlike other types of thyroid cancer, where thyroglobulin is the predominant tumor marker, calcitonin is highly unique to MTC, making it an important diagnostic tool for this subtype (71). Serum calcitonin levels can help in early identification and diagnosis of MTC. Elevated calcitonin levels, particularly in the baseline state and after stimulation tests, indicate the existence of MTC (72). Calcitonin is used as a biomarker not just for initial diagnosis, but also to detect residual or recurrent illness following surgery (72). Calcitonin levels at baseline in MTC patients can be used to predict prognosis (72). Higher preoperative calcitonin levels are frequently linked with more advanced disease stages, which can impact treatment options and postoperative care (73). Calcitonin can be released via stimulation tests such as the calcium or pentagastrin stimulation test. The extent of the calcitonin rise in response to these tests reveals more about the MTC's functioning and aggressiveness (73). Following surgical thyroid gland removal in MTC patients, blood calcitonin levels must be monitored to determine therapy success and identify illness recurrence (73). Persistent or rising calcitonin levels during follow-up may suggest residual or recurrent MTC, necessitating further imaging examinations and treatment measures (74). Given its prominent involvement in MTC, calcitonin has been investigated as a possible treatment target. Some medications try to reduce calcitonin synthesis and secretion, providing a more tailored approach to treating MTC (74). Calcitonin is a key biomarker for medullary thyroid cancer. Its assessment is critical in the diagnosis, monitoring, and prognosis of MTC, emphasizing its clinical importance in the overall therapy of this rare but specific kind of thyroid cancer (74). Current investigation seeks to improve understanding of calcitonin's role and its potential as a therapeutic target in medullary thyroid cancer.

## 3.7 Galectin-3

Belonging to the galectin family of β-galactoside-binding proteins, galectin-3 is important when it comes to thyroid cancer, especially when it comes to the diagnosis and prognosis of thyroid nodules (75). This multifunctional protein participates in a variety of biological activities, including cell adhesion, proliferation, differentiation, and death (75). Galectin-3 has emerged as an important biomarker in thyroid cancer, with implications for risk classification and treatment decisions (75). Galectin-3 is significantly overexpressed in thyroid cancer, especially in PTC, one of the most frequent kinds of thyroid cancer (76). The level of expression is insufficient or absent in normal thyroid tissue (76). Galectin-3 immunohistochemistry has proven to be an effective method for discriminating between benign and malignant thyroid nodules (76). High galectin-3 expression indicates malignancy, which aids in the preoperative evaluation of thyroid nodules (77). Galectin-3 expression is linked to more aggressive characteristics in thyroid cancer (77). High galectin-3 levels have been associated to increased tumor growth, extrathyroidal extension, and lymph node metastasis in PTC (78). Incorporating galectin-3 testing into diagnostic algorithms helps stratify the risk of malignancy in thyroid nodules, aiding doctors in deciding the best course of action, such as surgery or careful observation (78). Galectin-3 expression is used as a prognostic indication in thyroid cancer. Its overexpression is linked to an increased risk of illness recurrence and may alter postoperative care options (78). Patients with PTC who have high levels of galectin-3 may benefit from more aggressive therapy methods or diligent postoperative surveillance to detect possible recurrences early (79). Galectin-3 regulates a variety of biological functions, including cell adhesion and death. In thyroid cancer, dysregulation leads to the disruption of normal cellular activities, which promotes tumor growth (79). The specific molecular processes by which galectin-3 promotes thyroid cancer formation and progression are now being investigated, offering insights into prospective treatment targets (80). Researchers expect to uncover targets for precision medicine techniques by unraveling the complicated molecular connections involving galectin-3 (80). Recent research is helping to improve our grasp of galectin-3's function in thyroid cancer biology and its potential implications for individualized treatment.

## 3.8 Cytokeratin 19

CK19 is an important biomarker for thyroid cancer detection, risk stratification, and prognosis (81). CK19 belongs to the cytokeratin family of intermediate filament proteins and is found in a variety of epithelial tissues, including the thyroid gland (81). In thyroid cancer, CK19 has received attention for its ability to discriminate between benign and malignant thyroid tumors (81). The thyroid gland's epithelial cells typically express CK-19. However, its upregulation is observed in thyroid cancer, particularly PTC (81). Immunohistochemical staining for CK19 has become an important technique in pathology, assisting in the distinguishing of thyroid nodules (82). The identification of CK19 expression is especially beneficial in separating benign thyroid lesions from malignancies, allowing for more accurate preoperative diagnosis and treatment decisions (82). Elevated CK19 expression is related with more aggressive characteristics in thyroid carcinoma (83). In PTC, CK19 positive has been associated with increased tumor size, lymph node metastasis, and extrathyroidal extension (83). The presence of CK19 is used as a prognostic indication to assist identify patients who are at a higher risk of illness recurrence (84). This information assists doctors in developing postoperative treatment regimens and identifying the need for further medications or increased surveillance (84). CK19 expression aids in risk classification in thyroid cancer. Its evaluation, frequently in combination with other indicators, assists in classifying thyroid nodules into risk categories, allowing for a more tailored approach to patient care (85). High CK19 expression may impact surgical extent and the necessity for postoperative radioactive iodine therapy, giving useful information for optimizing treatment regimens (85). The upregulation of CK19 in thyroid cancer reflects molecular changes that occur in malignant thyroid cells. Understanding the molecular pathways involving CK19

sheds light on the underlying processes of tumor formation and progression (86). The importance of CK19 in maintaining cellular shape and integrity implies that it may be involved in thyroid cancer cells' invasive characteristics (86). While CK19 is not a direct therapeutic target, its significance as a diagnostic and prognostic marker aids in the management of thyroid cancer (87). Identifying patients with high CK19 expression enables a more personalized and focused approach to therapy, stressing precision medicine tactics (87). CK19 appears as an important biomarker in the context of thyroid carcinoma (87). Its expression patterns give crucial diagnostic information, help in risk stratification, and shed light on the prognosis of people with thyroid cancer (87). The continuous investigation of CK19's molecular contributions advances our understanding of thyroid cancer biology and may have future implications for treatment strategy refinement.

## 3.9 Circulating tumor DNA

ctDNA has emerged as a potential molecular biomarker in thyroid cancer, providing non-invasive insights into the disease's progression (88). The term ctDNA refers to fragmented DNA shed into the circulation by tumor cells, allowing for a liquid biopsy method that has the potential to transform thyroid cancer detection, monitoring, and therapy. ctDNA is a non-invasive way to identify and monitor thyroid cancer (88). CtDNA analysis includes extracting cell-free DNA from a blood sample, which eliminates the need for invasive procedures such as conventional biopsies (88). This is particularly useful for tracking disease development and therapy response over time (88). ctDNA enables the diagnosis of little residual illness or early-stage thyroid cancer. ctDNA enables the diagnosis of little residual illness or early-stage thyroid cancer (89). Its sensitivity allows doctors to identify genetic changes linked with thyroid cancer, giving them a tool for early detection and intervention (89). ctDNA analysis involves the identification of particular genetic abnormalities, such as mutations or rearrangements. ctDNA analysis can detect prevalent genetic abnormalities in thyroid cancer, such as BRAF and RAS mutations and RET/PTC rearrangements (89). These molecular fingerprints help in tumor profiling and influence therapy recommendations. ctDNA analysis is useful for assessing therapy response and illness recurrence (90). Variations in ctDNA levels or the appearance of particular mutations during or after therapy might indicate treatment success or the need to modify therapeutic techniques (90). The dynamic nature of ctDNA enables real-time evaluation of the tumor landscape. This is especially important in thyroid cancer, where tumors can be heterogeneous, and identifying emerging genetic changes allows for more precise and focused therapy (90). ctDNA analysis gives prognostic information, which can assist predict illness recurrence or progression. Specific ctDNA patterns may suggest a greater probability of aggressive tumor activity, which might influence postoperative therapy and monitoring tactics (90). The molecular information gained from ctDNA allows for the formulation of individualized treatment methods. Targeted medicines can be tailored to particular genetic abnormalities found in ctDNA, improving the accuracy of thyroid cancer treatment (91). ctDNA is increasingly being used in clinical trials and research projects to investigate new treatments for thyroid cancer. Its involvement in finding actionable genomic targets helps to create novel treatment options and advances precision medicine (91). ctDNA is a revolutionary tool in the molecular landscape of thyroid cancer. Its non-invasive nature, ability to detect genetic alterations, and dynamic monitoring capabilities all help to improve thyroid cancer diagnosis, treatment decisions, and overall patient management (91). As technology and research advance, ctDNA is anticipated to play an increasingly important role in developing individualized and targeted therapy for thyroid cancer.

## 3.10 Programmed death-ligand 1

PD-L1 has received a lot of interest as a molecular biomarker for thyroid cancer, especially in the setting of immunotherapy (92). PD-L1 is a cell surface protein that regulates the immune response by interacting with the PD-1 receptor on immune cells (92). In thyroid cancer, PD-L1 expression influences prognosis and treatment options, particularly in the age of immune checkpoint inhibitors (92). PD-L1 is an important target in immunotherapy, especially immune checkpoint blocking. Tumors that express PD-L1 can use this route to avoid the immune system, resulting in immunological tolerance and allowing cancer cells to spread unabated (93). PD-L1 expression in thyroid carcinoma is related with a more aggressive disease progression and a worse prognosis (93). High levels of PD-L1 are frequently associated with increased tumor invasiveness, metastasis, and resistance to conventional therapies (93). The presence of PD-L1 in thyroid carcinoma is a critical factor influencing the responsiveness to immune checkpoint inhibitors (93). Tumors with high PD-L1 expression are more likely to react successfully to immunotherapy, emphasizing the value of PD-L1 testing in guiding treatment decisions (94). PD-L1 is a companion diagnostic marker for immune checkpoint inhibitor treatments. Determining PD-L1 expression levels in tumor tissues aids doctors in determining the most effective treatment approaches (94). PD-L1 expression can be dynamic, driven by a variety of variables such as the tumor microenvironment and therapeutic treatments (94). Monitoring PD-L1 levels over time provides a more nuanced knowledge of the tumor's response to therapy and the possible formation of resistance mechanisms (95). PD-L1 status influences the development of combination therapy in thyroid cancer. Understanding the interactions between PD-L1 expression and other molecular factors can help drive the development of personalized treatment methods that combine immunotherapy with other targeted medicines (95). Present study investigates the function of PD-L1 in thyroid cancer biology. Investigating the variables controlling PD-L1 expression and its interactions with the immune system sheds light on the intricate processes that drive thyroid cancer growth and immune evasion (95). PD-L1 is a critical molecular biomarker in thyroid cancer, impacting treatment decisions and prognosis, notably in the field of immunotherapy (95). The evaluation of PD-L1 expression is critical to the developing landscape of precision medicine, assisting doctors in personalizing therapy regimens for patients with thyroid cancer (96). Future research aims to improve our understanding of PD-

L1's significance and broaden the scope of targeted and immunotherapeutic therapies in thyroid cancer.

# 4 Advances in targeted therapy of thyroid cancer

The current emphasis of thyroid cancer targeted therapy research is to find and exploit specific biochemical pathways in order to improve treatment results (97). Tyrosine kinase inhibitors (TKIs) are a popular research topic because they target critical signaling pathways involved in thyroid cancer development and progression (97). TKIs, such as lenvatinib and sorafenib, have demonstrated success in advanced thyroid malignancies, particularly those that are resistant to traditional therapies (97). Additionally, efforts are being made to study and target genetic abnormalities such as BRAF and RET changes, which are frequent in thyroid cancer (98). BRAF inhibitors, such as vemurafenib and dabrafenib, have shown potential in treating BRAF-mutant thyroid tumors (98). Immunotherapy, particularly immune checkpoint inhibitors such as pembrolizumab, is being investigated, with a focus on improving the immune response against thyroid cancer cells (98). Combining immunotherapy with additional targeted medicines is a promising method for increasing therapeutic efficacy. Precision medicine techniques based on molecular profiling are gaining traction (98). Comprehensive genetic analysis aids in the identification of particular mutations in individual tumors, allowing for the development of personalized therapy based on the unique molecular landscape of each patient's thyroid cancer (99). Despite these advances, there are still obstacles, including as dealing with treatment resistance and unwanted effects (99). Ongoing research seeks to identify new therapeutic targets, increase therapy tolerance, and develop combination methods. The changing scientific environment offers out hope for more effective and tailored targeted medicines in the treatment of thyroid cancer (Table 2).

## 4.1 Tyrosine kinase inhibitors

TKIs are a kind of cancer therapy that inhibits the activity of tyrosine kinases, enzymes involved in a variety of biological functions, including cell growth and division (100). In the setting of thyroid cancer, TKIs have demonstrated effectiveness in blocking particular pathways implicated in tumor development. TKIs function by suppressing the activity of tyrosine kinases, enzymes that phosphorylate tyrosine residues in proteins, a step necessary for intracellular signal transmission (100). TKIs in thyroid cancer typically target receptors such vascular endothelial growth factor receptors (VEGFR), epidermal growth factor receptors (EGFR), and other kinases involved in angiogenesis and tumor development (100). Lenvatinib targets the VEGFR, FGFR, PDGFR, and RET. It is authorized for the treatment of advanced differentiated thyroid carcinoma. Another TKI for advanced thyroid cancer patients is sorafenib, which inhibits PDGFR, RAF, and VEGFR (101). TKIs are frequently used when traditional therapies such as surgery or radioactive iodine are inadequate, or when advanced thyroid cancer cannot be physically removed (101). They are especially beneficial for aggressive thyroid tumors that have progressed outside the thyroid gland. One important feature of TKI activity is the prevention of angiogenesis, the creation of new blood vessels on which tumors rely for nutrition and oxygen (101). TKIs that target VEGFR impede the development of blood vessels, depriving the tumor of vital supplies and slowing its growth (102). TKIs may cause tiredness, hypertension, diarrhea, and skin problems. Normal monitoring is important to treat those adverse symptoms adequately (102). Eventually, certain cancers may acquire resistance to TKIs. Ongoing study seeks to identify and overcome resistance mechanisms. Combination treatments, such as TKIs combined with other targeted drugs or immunotherapy, are being investigated in order to improve therapeutic effectiveness and overcome resistance (103). The molecular features of the tumor are frequently used to choose a certain TKI. Tumor molecular profiling identifies particular genetic changes that might assist guide therapy decisions (103). Continuous research is being conducted to find novel targets and increase the efficacy of TKIs. Clinical trials are investigating novel combinations and determining their efficiency in various thyroid cancer subtypes (103). In conclusion, TKIs are an important class of targeted medicines in thyroid cancer therapy. TKIs have been shown to be beneficial in limiting tumor development and improving outcomes by specifically interfering with critical signaling pathways, especially in circumstances when standard therapies may be ineffective. Current research and clinical studies attempt to improve their efficacy, resolve resistance concerns, and expand their therapeutic potential.

## 4.2 BRAF inhibitors

BRAF inhibitors are a type of targeted therapy that inhibits the action of a mutant BRAF gene, which regulates cell growth and

TABLE 2 Targeted therapy of thyroid cancer.

| Targeted therapy | Notes | Diseases | Drugs | References |
|---|---|---|---|---|
| Tyrosine kinase inhibitors | Inhibiting VEGFR, FGFR, PDGFR, and RET | PTC, FTC | Lenvatinib, Sorafenib | (100–103) |
| BRAF inhibitors | Inhibiting BRAF | PTC | Vemurafenib, Dabrafenib | (46, 104–106) |
| Immunotherapy | Inhibiting PD-L1 | PTC, FTC | Atezolizumab, Durvalumab | (17, 107–109) |
| RET inhibitors | Inhibiting RET | PTC | Selpercatinib, Pralsetinib | (110–114) |
| MEK inhibitors | Inhibiting MEK | PTC, FTC | Trametinib, Cobimetinib | (115–117) |

division (104). These inhibitors are especially important for malignancies with specific BRAF mutations, such as thyroid carcinoma (104). BRAF inhibitors work by targeting the mutant BRAF gene, most often the V600E mutation (104). This mutation results in a constitutively active BRAF protein, which contributes to uncontrolled cell proliferation (104). By blocking the action of mutant BRAF, these inhibitors hope to disrupt the signaling cascade known as the MAPK/ERK pathway, which is abnormally active in cells with the V600E mutation (105). Vemurafenib and Dabrafenib are both well-known BRAF inhibitors (105). Vemurafenib was first designed for melanoma, and it has also been used to treat thyroid carcinoma with BRAF mutations (105). Dabrafenib is another BRAF inhibitor used to treat BRAF-mutant thyroid carcinoma (105). BRAF inhibitors are mostly utilized in thyroid cancer patients with the BRAF V600E mutation (46). They are commonly used when standard therapies, such as surgery and radioactive iodine, are ineffective or in situations of advanced thyroid cancer (46). BRAF inhibitors have demonstrated considerable effectiveness in lowering tumor size and slowing disease progression in BRAF-mutant thyroid malignancies (46). Response rates differ amongst individuals, and the length of response may be impacted by factors such as the existence of other genetic abnormalities (106). Combining BRAF inhibitors with other targeted medicines or immunotherapies is an ongoing research topic. Combinations are intended to improve treatment results, overcome resistance, and address the heterogeneity of thyroid cancer (106). Common side effects of BRAF inhibitors include skin problems, fatigue, fever, and joint pain. Monitoring for side effects and adjusting treatment as necessary are critical components of patient care. Some cancers may become resistant to BRAF inhibitors over time (106). Ongoing research aims to better understand the processes of resistance and find solutions to overcome this issue. Clinical studies for new BRAF inhibitors and combination therapy are now underway, adding to the developing landscape of precision medicine in thyroid cancer (106). Molecular profiling of tumors is critical for identifying individuals with BRAF mutations who might benefit from BRAF inhibitors. Patient selection based on genetic features improves therapy outcomes (106). To summarize, BRAF inhibitors are an effective targeted treatment for particular subtypes of thyroid carcinoma with BRAF mutations. These inhibitors, which directly block the aberrantly active BRAF protein, have showed promise in limiting tumor development. Ongoing research seeks to improve their usage, address resistance mechanisms, and investigate combination tactics to increase their efficacy in treating thyroid cancer.

## 4.3 Immunotherapy

Immunotherapy is a novel method to cancer treatment that uses the immune system of the patient to identify and remove cancer cells (17). Immunotherapy has showed promise in the treatment of thyroid cancer, especially in advanced instances where standard therapies may be ineffective (17). Immune checkpoint inhibitors are important components of immunotherapy for thyroid cancer. These medications target particular proteins on immunological or cancer cells, altering inhibitory signals that keep the immune system from successfully targeting cancer cells (17). Pembrolizumab and nivolumab are PD-1 inhibitors, whereas atezolizumab and durvalumab are PD-L1 inhibitors. They have been examined in several malignancies, including thyroid carcinoma (107). Tumors can use immunological checkpoints, such as PD-1/PD-L1, to avoid immune detection. Immunotherapy suppresses these checkpoints, allowing T cells to better detect and destroy cancer cells (107). Immunotherapy is often used in situations of advanced thyroid cancer that has not responded well to conventional treatments such as surgery, radioactive iodine, or targeted therapies (107). PD-1/PD-L1 inhibitors have shown benefit in certain types of thyroid cancer patients, notably those with poorly differentiated or anaplastic thyroid carcinoma (107). Immunotherapy has shown long-term responses in certain patients, resulting in tumor reduction and increased overall survival (108). Immunotherapy responses might vary, and ongoing research is aimed at identifying predictive indicators to help select individuals who would benefit (108). Combinations of immunotherapy with other targeted treatments, chemotherapy, or radiation are being investigated in order to improve treatment results and overcome resistance mechanisms. While immunotherapy is typically well tolerated, it can cause immune-related side effects such as tiredness, skin rash, and organ inflammation (108). Prompt detection and control of these adverse effects is critical to patient safety. Biomarker testing, such as PD-L1 expression, is frequently used to identify individuals with a higher likelihood of responding to immunotherapy (109). However, reactions can still occur in people who have minimal or no PD-L1 expression (109). To summarize, immunotherapy represents a game-changing technique in the treatment of thyroid cancer. It provides new hope to patients with advanced or resistant illness by releasing the immune system's strength. Ongoing research aims to improve its usage, broaden its application to diverse subtypes, and increase immunotherapy's overall success in the treatment of thyroid cancer.

## 4.4 RET inhibitors

RET inhibitors are a type of targeted therapy that interferes with the action of the RET protein, notably in malignancies with RET mutations or fusions (110). These inhibitors have demonstrated effectiveness in inhibiting the aberrant signaling associated with RET-driven malignancies (110). RET is a receptor tyrosine kinase that regulates cell development and differentiation (110). In tumors with RET changes, such as rearrangements or mutations, the RET signaling system is abnormally active (110). RET inhibitors work by targeting the RET protein or disrupting downstream signaling pathways to prevent cancer cells from growing and dividing uncontrollably (111). Selpercatinib is authorized for a variety of malignancies with RET mutations, including several kinds of thyroid carcinoma (111). Pralsetinib is another RET inhibitor utilized to treat tumors with RET mutations (111). RET inhibitors are generally utilized in malignancies where RET mutations are detected by molecular testing (111). These changes can occur in both papillary and medullary thyroid tumors (112). RET inhibitors have shown effectiveness in reducing tumor development and

inducing significant responses in individuals with RET-altered malignancies (112). Patients' responses may vary, and continuing study strives to better understand the elements that influence therapy results (112). Clinical trials are looking into the potential benefits of combining RET inhibitors with other targeted medicines or immunotherapy to improve treatment efficacy and address resistance mechanisms (112). Fatigue, hypertension, gastrointestinal difficulties, and changes in liver enzyme levels are some of the most common RET inhibitor adverse effects. Regular monitoring and control of side effects are critical components of patient care (113). Resistance to RET inhibitors can develop over time, and ongoing research seeks to uncover and overcome these pathways (113). Further research is being conducted to determine the appropriate therapy sequence and the feasibility of combining RET inhibitors with other treatment methods (113). Molecular profiling of cancers is critical for identifying individuals with RET mutations who might benefit from RET inhibitors (113). Targeted medicines are most successful when they are customized to the unique genetic features of each tumor (114). In conclusion, RET inhibitors offer a viable treatment alternative for malignancies caused by RET mutations, including some kinds of thyroid cancer. These inhibitors have been shown to be effective in reducing tumor development and improving outcomes by addressing the underlying molecular abnormalities. Ongoing research is required to improve their usage, address resistance concerns, and investigate combination tactics to increase their efficacy in treating malignancies with RET mutations.

### 4.5 MEK inhibitors

The MAPK pathway is a signaling route that regulates cell growth, differentiation, and survival (115). MEK inhibitors are a kind of targeted therapy that interferes with the action of MEK (MAPK/ERK kinase), a critical enzyme in this pathway (115). These inhibitors are especially useful in malignancies when the MAPK pathway is dysregulated (115). MEK inhibitors work by selectively targeting MEK, which phosphorylates and activates ERK in the MAPK pathway (116). By inhibiting MEK, these inhibitors impair the downstream signaling cascade, affecting cell growth and proliferation (116). Trametinib is licensed for the treatment of certain malignancies with BRAF mutations and is frequently used in conjunction with BRAF inhibitors (116). Cobimetinib is another MEK inhibitor that is utilized in conjunction with BRAF inhibitors in some malignancies, including melanoma (116). MEK inhibitors are typically utilized in malignancies characterized by MAPK pathway dysregulation, which is frequently caused by mutations in BRAF or other pathway components (117). They are frequently used in conjunction with BRAF inhibitors in malignancies with BRAF mutations, such as melanoma and some kinds of thyroid cancer (117). MEK inhibitors, particularly when combined with BRAF inhibitors, have been found to effectively suppress tumor development and improve outcomes in some malignancies (117). Responses can differ between individuals, and ongoing research tries to uncover characteristics that influence therapy results (117). Combining MEK inhibitors with other targeted treatments, immunotherapy, or chemotherapy is an important research topic to improve therapeutic efficacy and overcome possible resistance mechanisms. MEK inhibitors may cause skin problems, gastrointestinal discomfort, exhaustion, and changes in blood cell counts. Regular monitoring and control of adverse effects is required (117). Resistance to MEK inhibitors can develop, necessitating continued research to identify and overcome these resistance pathways (117). Investigational studies investigate the use of MEK inhibitors in various combinations and sequencing to maximize treatment options (118). Molecular screening of malignancies is critical for identifying individuals with particular mutations or dysregulation in the MAPK pathway who may benefit from MEK inhibitors (118). Personalized therapy options are critical for maximizing therapeutic outcomes (118). In summary, MEK inhibitors are an important class of targeted treatments, especially in malignancies with dysregulated MAPK pathway signaling. These inhibitors have shown effective in suppressing tumor development by interfering with this essential mechanism. Future research aims to improve their usage, address resistance mechanisms, and investigate combination tactics to boost their efficacy in a variety of malignancies.

## 5 Conclusion and perspectives

The progress of targeted therapy and biomarker research in thyroid cancer represents a possible paradigm change in therapeutic methods, aimed at precision and individualization. Here are some recommendations for investigating targeted therapy and biomarker studies in thyroid cancer: (1) Improve molecular diagnosis and tailored therapy. Advocating for the wider use of molecular diagnostic tools in thyroid cancer patients, such as genetic mutation analysis, protein expression profiling, and biomarker identification. Promoting the use of thorough molecular profiling, which includes identification of common mutations such as BRAF, RET, and RAS, to accurately guide the selection of appropriate targeted therapy. Teaching healthcare personnel how to create individualized treatment regimens based on a patient's molecular traits and biomarker data. (2) Promote multi-center collaborative research projects. Supporting the formation of multi-center collaborative research initiatives that allow for the sharing of patient samples and clinical data, therefore speeding our understanding of biomarkers and targeted therapeutics in thyroid cancer. Encourage cross-institutional and worldwide collaboration to expand study sample sizes, resulting in more compelling and therapeutically relevant research outputs. (3) In-depth research on critical targets such as BRAF and RET. Intensifying research into important molecular targets such as BRAF and RET to better understand their involvement in thyroid cancer development. Driving research into new and more effective BRAF and RET inhibitors, as well as developing novel techniques to potentially reduce therapy resistance. (4) Use of bioinformatics and artificial intelligence in biomarker analysis. Increasing the use of bioinformatics and artificial intelligence technology in biomarker research to decipher complicated biological interaction networks and predict treatment outcomes. Using big data analytics to identify

possible new biomarkers, resulting in more complete information for thyroid cancer therapy. (5) Investigate the possibilities of immunotherapy. Increasing research examines the possibilities of immunotherapy in thyroid cancer, includes the study of immune-related indicators including PD-L1 expression and tumor-infiltrating lymphocytes (TILs). Driving clinical studies to study the combination of immunotherapy and targeted treatments in order to improve therapeutic effectiveness. (6) Concentrate on the issue of treatment resistance and recurrence. To develop more successful treatment techniques, researchers are looking at treatment resistance mechanisms, including as recurrence patterns following targeted therapy and immunotherapy. Promoting the start of long-term follow-up studies to better understand post-treatment survival outcomes and the quality of life of thyroid cancer patients. (7) Patient education and engagement. Emphasizing the necessity of patient education about the advantages and potential negative effects of targeted therapy, as well as the value of biomarker testing. Encouraging patient participation in clinical trials, creating a collaborative approach to research, and enhancing treatment alternatives. In conclusion, these guidelines highlight the need of a holistic and collaborative approach to thyroid cancer research, utilizing advances in molecular diagnostics, targeted therapeutics, and biomarker analysis. Such activities are crucial for increasing precision medicine in thyroid cancer treatment and improving patient outcomes.

## Author contributions

MG: Conceptualization, Funding acquisition, Software, Writing – original draft, Writing – review & editing. YS: Conceptualization, Writing – original draft. YW: Conceptualization, Writing – original draft. JX: Conceptualization, Writing – original draft. CZ: Funding acquisition, Software, Validation, Writing – review & editing.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The work was supported by the National Natural Science Foundation of China (No. 82305046, 82304902), the Natural Science Foundation of Jiangsu Province (No. BK20220467), the Major Project of the Natural Science Research of Jiangsu Higher Education Institutions (No.22KJB310013), Jiangsu Provincial Double-Innovation Doctor Program (No. JSSCBS20220452, JSSCBS20220472), Young Elite Scientists Sponsorship Program by CACM (2022-QNRC2-B15), Outstanding Young Doctoral Training Program (2023QB0124).

## 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. Ma Q, Li Y, An L, Guo L, Liu X. Assessment of causal association between differentiated thyroid cancer and disordered serum lipid profile: a Mendelian randomization study. *Front Endocrinol (Lausanne)*. (2023) 14:1291445. doi: 10.3389/fendo.2023.1291445

2. English C. Improving care for head-and-neck and thyroid cancer patients. *Br J Nurs*. (2024) 33:48. doi: 10.12968/bjon.2024.33.1.48

3. Cao J, He X, Li X, Sun Y, Zhang W, Li Y, et al. The potential association of peripheral inflammatory biomarkers in patients with papillary thyroid cancer before radioiodine therapy to clinical outcomes. *Front Endocrinol (Lausanne)*. (2023) 14:1253394. doi: 10.3389/fendo.2023.1253394

4. Boucai L, Zafereo M, Cabanillas ME. Thyroid cancer: A review. *JAMA*. (2024) 331:425–35. doi: 10.1001/jama.2023.26348

5. Maurea S, Stanzione A, Klain M. Thyroid cancer radiomics: navigating challenges in a developing landscape. *Cancers (Basel)*. (2023) 15:5884. doi: 10.3390/cancers15245884

6. Liang N, Zhang H, Sui C, Du R, Li C, Li J, et al. Surgical resection of recurrent differentiated thyroid cancer: patterns, detection, staging, and treatment of 683 patients. *Front Endocrinol (Lausanne)*. (2023) 14:1301620. doi: 10.3389/fendo.2023.1301620

7. Xie K. A biomarker and molecular mechanism investigation for thyroid cancer. *Cent Eur J Immunol*. (2023) 48:203–18. doi: 10.5114/ceji.2023.132163

8. Abdulhaleem M, Bandargal S, Pusztaszeri MP, Rajab M, Greenspoon H, Krasner JR, et al. The impact of BRAF V600E mutation allele frequency on the histopathological characteristics of thyroid cancer. *Cancers (Basel)*. (2023) 16:113. doi: 10.3390/cancers16010113

9. Marín F, Del Nuevo E, Belinchón A, Acevedo A. Bilateral follicular variant of papillary thyroid cancer with different RAS mutations detected with next-generation sequencing: Report of an unusual case and literature review. *Diagn Cytopathol*. (2022) 50:E275–9. doi: 10.1002/dc.25004

10. Schweppe RE, Pozdeyev N, Pike LA, Korch C, Zhou Q, Sams SB, et al. Establishment and characterization of four novel thyroid cancer cell lines and PDX models expressing the RET/PTC1 rearrangement, BRAFV600E, or RASQ61R as drivers. *Mol Cancer Res*. (2019) 17:1036–48. doi: 10.1158/1541-7786.MCR-18-1026

11. Leeman-Neill RJ, Brenner AV, Little MP, Bogdanova TI, Hatch M, Zurnadzy LY, et al. RET/PTC and PAX8/PPARγ chromosomal rearrangements in post-Chernobyl thyroid cancer and their association with iodine-131 radiation dose and other characteristics. *Cancer*. (2013) 119:1792–9. doi: 10.1002/cncr.27893

12. Liu R, Zhu G, Tan J, Shen X, Xing M. Genetic trio of BRAF and TERT mutations and rs2853669TT in papillary thyroid cancer aggressiveness. *J Natl Cancer Inst*. (2023) 19:djad265. doi: 10.1093/jnci/djad265

13. Capdevila J, Awada A, Führer-Sakel D, Leboulleux S, Pauwels P. Molecular diagnosis and targeted treatment of advanced follicular cell-derived thyroid cancer in the precision medicine era. *Cancer Treat Rev*. (2022) 106:102380. doi: 10.1016/j.ctrv.2022.102380

14. Gunda V, Ghosh C, Hu J, Zhang L, Zhang YQ, Shen M, et al. Combination BRAFV600E inhibition with the multitargeting tyrosine kinase inhibitor axitinib shows additive anticancer activity in BRAFV600E-mutant anaplastic thyroid cancer. *Thyroid*. (2023) 33:1201–14. doi: 10.1089/thy.2023.0201

15. Davidson CD, Bolf EL, Gillis NE, Cozzens LM, Tomczak JA, Carr FE. Thyroid hormone receptor beta inhibits PI3K-akt-mTOR signaling axis in anaplastic thyroid

cancer via genomic mechanisms. *J Endocr Soc*. (2021) 5:bvab102. doi: 10.1210/jendso/bvab102

16. Cai Y, Yang Y, Pang X, Li S. The effect of radioactive iodine treatment for differentiated thyroid cancer on male gonadal function: a meta-analysis. *Endocr Connect*. (2023) 12:e230299. doi: 10.1530/EC-23-0299

17. Tao Y, Li P, Feng C, Cao Y. New insights into immune cells and immunotherapy for thyroid cancer. *Immunol Invest*. (2023) 52:1039–64. doi: 10.1080/08820139.2023.2268656

18. Li J, Li Z, Zhao P. Diagnosis and prognosis of thyroid cancer by immune-related genes. *Am J Clin Oncol*. (2024) 47:1–10. doi: 10.1097/COC.0000000000001048

19. Chen DW, Lang BHH, McLeod DSA, Newbold K, Haymart MR. Thyroid cancer. *Lancet*. (2023) 401:1531–44. doi: 10.1016/S0140-6736(23)00020-X

20. Kang YJ, Stybayeva G, Hwang SH. Surgical completeness and safety of minimally invasive thyroidectomy in patients with thyroid cancer: A network meta-analysis. *Surgery*. (2023) 173:1381–90. doi: 10.1016/j.surg.2023.02.021

21. Li LR, Song JL, Liu HQ, Chen C. Metabolic syndrome and thyroid Cancer: risk, prognosis, and mechanism. *Discovery Oncol*. (2023) 14:23. doi: 10.1007/s12672-022-00599-7

22. Jin J, Li J, Liu Y, Shi Q, Zhang B, Ji Y, et al. Thyroid hormone changes correlate to combined breast cancer with primary thyroid cancer. *Breast Cancer (Dove Med Press)*. (2024) 16:15–22. doi: 10.2147/BCTT.S442707

23. Soares MN, Borges-Canha M, Neves C, Neves JS, Carvalho D. The role of Graves' disease in the development of thyroid nodules and thyroid cancer. *Eur Thyroid J*. (2023) 12:e230055. doi: 10.1530/ETJ-23-0055

24. Feng X, Wang F, Yang W, Zheng Y, Liu C, Huang L, et al. Association between genetic risk, adherence to healthy lifestyle behavior, and thyroid cancer risk. *JAMA Netw Open*. (2022) 5:e2246311. doi: 10.1001/jamanetworkopen.2022.46311

25. Nagayama Y, Hamada K. Reprogramming of cellular metabolism and its therapeutic applications in thyroid cancer. *Metabolites*. (2022) 12:1214. doi: 10.3390/metabo12121214

26. Roseland ME, Dewaraja YK, Wong KK. Advanced imaging and theranostics in thyroid cancer. *Curr Opin Endocrinol Diabetes Obes*. (2022) 29:456–65. doi: 10.1097/MED.0000000000000740

27. Khosropour S, Mastoori Z, Hosseinzadegan R, Miri M, Shojaee M, Noori S. Novel and emerging concepts in the role of steroids in thyroid cancer promotion and progression. *Bratisl Lek Listy*. (2022) 123:672–7. doi: 10.4149/BLL_2022_107

28. Yu Q, Zhang X, Li L, Zhang C, Huang J, Huang W. Molecular basis and targeted therapies for radioiodine refractory thyroid cancer. *Asia Pac J Clin Oncol*. (2023) 19:279–89. doi: 10.1111/ajco.13836

29. Kaliszewski K, Ludwig M, Ludwig B, Mikuła A, Greniuk M, Rudnicki J. Update on the diagnosis and management of medullary thyroid cancer: what has changed in recent years? *Cancers (Basel)*. (2022) 14:3643. doi: 10.3390/cancers14153643

30. Pacilio M, Conte M, Frantellizzi V, De Feo MS, Pisani AR, Marongiu A, et al. Personalized dosimetry in the context of radioiodine therapy for differentiated thyroid cancer. *Diagn (Basel)*. (2022) 12:1763. doi: 10.3390/diagnostics12071763

31. Li Y, Zhang J, Zhou H, Du Z. Anticancer effects of natural phytochemicals in anaplastic thyroid cancer. *Oncol Rep*. (2022) 48:156. doi: 10.3892/or.2022.8368

32. Zhao X, Bie F, Luo C, Zhang JE. Distress, illness perception and coping style among thyroid cancer patients after thyroidectomy: A cross-sectional study. *Eur J Oncol Nurs*. (2024) 69:102517. doi: 10.1016/j.ejon.2024.102517

33. Bytnar JA, Enewold L, Shriver CD, Zhu K. Incidence of papillary thyroid cancer: Comparison of the military and the general population by race and tumor stage/size. *Cancer Epidemiol*. (2024) 89:102539. doi: 10.1016/j.canep.2024.102539

34. Vujovic D, Alsen M, Vasan V, Genden E, van Gerwen M. Anxiety and depression as potential predictors for shorter time to undergo initial surgical treatment for papillary thyroid cancer. *Cancers (Basel)*. (2024) 16:545. doi: 10.3390/cancers16030545

35. He J, Sun P, Lin J, Shen J, Lin H, Jiang H, et al. Application of carbon nanoparticles in endoscopic thyroid cancer surgery: a systematic review and meta-analysis. *Front Surg*. (2024) 10:1283573. doi: 10.3389/fsurg.2023.1283573

36. Cortas C, Charalambous H. Tyrosine kinase inhibitors for radioactive iodine refractory differentiated thyroid cancer. *Life (Basel)*. (2023) 14:22. doi: 10.3390/life14010022

37. Yang G, Pu J, Zhu S, Shi Y, Yang Y, Mao J, et al. Optimizing levothyroxine replacement: A precision dosage model for post-thyroidectomy patients. *Int J Gen Med*. (2024) 17:377–86. doi: 10.2147/IJGM.S438397

38. Peckham M, Spencer HJ, Syed S, Armstrong WB, Farwell DG, Gal TJ, et al. Breast and thyroid cancer: A multicenter study with Accrual to Clinical Trials Network. *J Surg Oncol*. (2022) 125:1211–7. doi: 10.1002/jso.26825

39. Ricci C, Salvemini A, Dalmiglio C, Castagna MG, Cantara S. From circulating tumor cells to mirna: new challenges in the diagnosis and prognosis of medullary thyroid cancer. *Cancers (Basel)*. (2023) 15:4009. doi: 10.3390/cancers15154009

40. Subash A, Sinha P, Singh A. BRAF mutation and age in differentiated thyroid cancer risk stratification: Two sides of the same coin. *Oral Oncol*. (2020) 106:104732. doi: 10.1016/j.oraloncology.2020.104732

41. Su X, Li P, Han B, Jia H, Liang Q, Wang H, et al. Vitamin C sensitizes BRAFV600E thyroid cancer to PLX4032 via inhibiting the feedback activation of MAPK/ERK signal by PLX4032. *J Exp Clin Cancer Res*. (2021) 40:34. doi: 10.1186/s13046-021-01831-y

42. Chen P, Pan L, Huang W, Feng H, Ouyang W, Wu J, et al. BRAF V600E and lymph node metastases in papillary thyroid cancer. *Endocr Connect*. (2020) 9:999–1008. doi: 10.1530/EC-20-0420

43. Zaballos MA, Acuña-Ruiz A, Morante M, Crespo P, Santisteban P. Regulators of the RAS-ERK pathway as therapeutic targets in thyroid cancer. *Endocr Relat Cancer*. (2019) 26:R319–44. doi: 10.1530/ERC-19-0098

44. Su X, Chen D, Zhu L, Jia H, Cai J, Li P, et al. SGSM2 inhibits thyroid cancer progression by activating RAP1 and enhancing competitive RAS inhibition. *Cell Death Dis*. (2022) 13:218. doi: 10.1038/s41419-022-04598-y

45. Marotta V, Bifulco M, Vitale M. Significance of RAS mutations in thyroid benign nodules and non-medullary thyroid cancer. *Cancers (Basel)*. (2021) 13:3785. doi: 10.3390/cancers13153785

46. Bonaldi E, Gargiuli C, De Cecco L, Micali A, Rizzetti MG, Greco A, et al. BRAF inhibitors induce feedback activation of RAS pathway in thyroid cancer cells. *Int J Mol Sci*. (2021) 22:5744. doi: 10.3390/ijms22115744

47. Soares P, Póvoa AA, Melo M, Vinagre J, Máximo V, Eloy C, et al. Molecular pathology of non-familial follicular epithelial-derived thyroid cancer in adults: from RAS/BRAF-like tumor designations to molecular risk stratification. *Endocr Pathol*. (2021) 32:44–62. doi: 10.1007/s12022-021-09666-1

48. Gimblet GR, Whitt J, Houson HA, Lin D, Guenter R, Rao TC, et al. Thyroid-stimulating hormone receptor (TSHR) as a target for imaging differentiated thyroid cancer. *Surgery*. (2024) 175:199–206. doi: 10.1016/j.surg.2023.05.045

49. Song YS, Kim MJ, Sun HJ, Kim HH, Shin HS, Kim YA, et al. Aberrant thyroid-stimulating hormone receptor signaling increases VEGF-A and CXCL8 secretion of thyroid cancer cells, contributing to angiogenesis and tumor growth. *Clin Cancer Res*. (2019) 25:414–25. doi: 10.1158/1078-0432.CCR-18-0663

50. Zheng H, Xu J, Hao S, Liu X, Ning J, Song X, et al. Expression of BANCR promotes papillary thyroid cancer by targeting thyroid stimulating hormone receptor. *Oncol Lett*. (2018) 16:2009–15. doi: 10.3892/ol.2018.8810

51. Chia SY, Milas M, Reddy SK, Siperstein A, Skugor M, Brainard J, et al. Thyroid-stimulating hormone receptor messenger ribonucleic acid measurement in blood as a marker for circulating thyroid cancer cells and its role in the preoperative diagnosis of thyroid cancer. *J Clin Endocrinol Metab*. (2007) 92:468–75. doi: 10.1210/jc.2006-2088

52. Liang JJ, Feng WJ, Li R, Xu RT, Liang YL. Analysis of the value and safety of thyroid-stimulating hormone in the clinical efficacy of patients with thyroid cancer. *World J Clin Cases*. (2023) 11:1058–67. doi: 10.12998/wjcc.v11.i5.1058

53. Su X, He C, Ma J, Tang T, Zhang X, Ye Z, et al. RET/PTC rearrangements are associated with elevated postoperative TSH levels and multifocal lesions in papillary thyroid cancer without concomitant thyroid benign disease. *PloS One*. (2016) 11:e0165596. doi: 10.1371/journal.pone.0165596

54. Halkova T, Dvorakova S, Vaclavikova E, Sykorova V, Vcelak J, Sykorova P, et al. A novel RET/PTC variant detected in a pediatric patient with papillary thyroid cancer without ionization history. *Hum Pathol*. (2015) 46:1962–9. doi: 10.1016/j.humpath.2015.08.013

55. Castro L, Alves S, Chaves SR, Costa JL, Soares P. RAF-1 promotes survival of thyroid cancer cells harboring RET/PTC1 rearrangement independently of ERK activation. *Mol Cell Endocrinol*. (2015) 415:64–75. doi: 10.1016/j.mce.2015.08.006

56. Eberhardt NL, Grebe SK, McIver B, Reddi HV. The role of the PAX8/PPARgamma fusion oncogene in the pathogenesis of follicular thyroid cancer. *Mol Cell Endocrinol*. (2010) 321:50–6. doi: 10.1016/j.mce.2009.10.013

57. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. *Nat Rev Endocrinol*. (2011) 7:569–80. doi: 10.1038/nrendo.2011.142

58. Aydin K, Aydin C, Dagdelen S, Tezel GG, Erbas T. Genetic alterations in differentiated thyroid cancer patients with acromegaly. *Exp Clin Endocrinol Diabet*. (2016) 124:198–202. doi: 10.1055/s-0035-1565061

59. Xue J, Li S, Shi P, Chen M, Yu S, Hong S, et al. The ETS inhibitor YK-4-279 suppresses thyroid cancer progression independent of TERT promoter mutations. *Front Oncol*. (2021) 11:649323. doi: 10.3389/fonc.2021.649323

60. Chen Z, Wang W, Xu J, Song Y, Zhu H, Ma T, et al. Tumor mutation burden-assisted risk stratification for papillary thyroid cancer. *Endocrine*. (2022) 78:296–305. doi: 10.1007/s12020-022-03154-0

61. Cao J, Zhu X, Sun Y, Li X, Yun C, Zhang W. The genetic duet of BRAF V600E and TERT promoter mutations predicts the poor curative effect of radioiodine therapy in papillary thyroid cancer. *Eur J Nucl Med Mol Imaging*. (2022) 49:3470–81. doi: 10.1007/s00259-022-05820-x

62. Rogucki M, Sidorkiewicz I, Niemira M, Dzięcioł JB, Buczyńska A, Adamska A, et al. Expression profile and diagnostic significance of microRNAs in papillary thyroid cancer. *Cancers (Basel)*. (2022) 14:2679. doi: 10.3390/cancers14112679

63. Santa-Inez DC, Fuziwara CS, Saito KC, Kimura ET. Targeting the Highly Expressed microRNA miR-146b with CRISPR/Cas9n Gene Editing System in Thyroid Cancer. *Int J Mol Sci*. (2021) 22:7992. doi: 10.3390/ijms22157992

64. Jia H, Sun W, Li X, Xu W. Melatonin promotes apoptosis of thyroid cancer cells via regulating the signaling of microRNA-21 (miR-21) and microRNA-30e (miR-30e). *Bioengineered*. (2022) 13:9588–601. doi: 10.1080/21655979.2022.2054206

65. Zhang W, Ji W, Zhao X. MiR-155 promotes anaplastic thyroid cancer progression by directly targeting SOCS1. *BMC Cancer*. (2019) 19:1093. doi: 10.1186/s12885-019-6319-4

66. Agata B, Renata ŚS. Clinical use of thyroglobulin: not only thyroid cancer. *Endocrine*. (2024). doi: 10.1007/s12020-023-03658-3

67. Javan FN, Askari E, Shafiei S, Roshanravan V, Aghaei A, Ayati N, et al. The prognostic power of preablation stimulated thyroglobulin in children with differentiated thyroid cancer. *Endocr Pract*. (2023), S1530–891X(23)00782-6. doi: 10.1016/j.eprac.2023.12.005

68. Shuai JH, Leng ZF, Wang P, Ji YC. Correlation analysis of serum thyroglobulin, thyroid-stimulating hormone levels, and thyroid-cancer risk in thyroid nodule surgery. *World J Clin Cases*. (2023) 11:6407–14. doi: 10.12998/wjcc.v11.i27.6407

69. Fanget F, Demarchi MS, Maillard L, Lintis A, Decaussin M, Lifante JC. Medullary thyroid cancer outcomes in patients with undetectable versus normalized postoperative calcitonin levels. *Br J Surg*. (2021) 108:1064–71. doi: 10.1093/bjs/znab106

70. Kartal Baykan E, Erdoğan M. Basal and pentagastrin-stimulated calcitonin cut-off values in diagnosis of preoperative medullary thyroid cancer. *Turk J Med Sci*. (2021) 51:650–6. doi: 10.3906/sag-2003-182

71. Montgomery G, Collins L, Coghlin C, Ullah R. Calcitonin negative medullary thyroid cancer in ectopic thyroid tissue: a rare diagnosis in an unusual location. *BMJ Case Rep*. (2020) 13:e236865. doi: 10.1136/bcr-2020-236865

72. Machens A, Dralle H. Significance of marginally elevated calcitonin levels in micromedullary thyroid cancer. *Ann Surg Oncol*. (2009) 16:2960. doi: 10.1245/s10434-009-0642-y

73. Pirich C, Rendl G, Hauser-Kronberger C, Häusler I. Failure of pentagastrin-stimulated calcitonin testing in early manifestation of familial medullary thyroid cancer. *Wien Klin Wochenschr*. (2012) 124:723–4. doi: 10.1007/s00508-012-0241-y

74. Diazzi C, Madeo B, Taliani E, Zirilli L, Romano S, Granata AR, et al. The diagnostic value of calcitonin measurement in wash-out fluid from fine-needle aspiration of thyroid nodules in the diagnosis of medullary thyroid cancer. *Endocr Pract*. (2013) 19:769–79. doi: 10.4158/EP12420.OR

75. Lee JJ, Hsu YC, Li YS, Cheng SP. Galectin-3 inhibitors suppress anoikis resistance and invasive capacity in thyroid cancer cells. *Int J Endocrinol*. (2021) 2021:5583491. doi: 10.1155/2021/5583491

76. Li J, Vasilyeva E, Wiseman SM. Beyond immunohistochemistry and immunocytochemistry: a current perspective on galectin-3 and thyroid cancer. *Expert Rev Anticancer Ther*. (2019) 19:1017–27. doi: 10.1080/14737140.2019.1693270

77. Samija I, Mateša N, Lukač J, Kusić Z. Galectin-3 and CD44v6 as markers for preoperative diagnosis of thyroid cancer by RT-PCR. *Diagn Mol Pathol*. (2011) 20:233–41. doi: 10.1097/PDM.0b013e31821a59f1

78. Weber KB, Shroyer KR, Heinz DE, Nawaz S, Said MS, Haugen BR. The use of a combination of galectin-3 and thyroid peroxidase for the diagnosis and prognosis of thyroid cancer. *Am J Clin Pathol*. (2004) 122:524–31. doi: 10.1309/UUQT-E505-PTN5-QJ7M

79. Lin CI, Whang EE, Donner DB, Jiang X, Price BD, Carothers AM, et al. Galectin-3 targeted therapy with a small molecule inhibitor activates apoptosis and enhances both chemosensitivity and radiosensitivity in papillary thyroid cancer. *Mol Cancer Res*. (2009) 7:1655–62. doi: 10.1158/1541-7786.MCR-09-0274

80. Shankar J, Wiseman SM, Meng F, Kasaian K, Strugnell S, Mofid A, et al. Coordinated expression of galectin-3 and caveolin-1 in thyroid cancer. *J Pathol*. (2012) 228:56–66. doi: 10.1002/path.4041

81. Giovanella L, Treglia G, Verburg FA, Salvatori M, Ceriani L. Serum cytokeratin 19 fragments: a dedifferentiation marker in advanced thyroid cancer. *Eur J Endocrinol*. (2012) 167:793–7. doi: 10.1530/EJE-12-0660

82. Giovanella L, Imperiali M, Trimboli P. Role of serum cytokeratin 19 fragment (Cyfra 21.1) as a prognostic biomarker in patients with differentiated thyroid cancer. *Sci Rep*. (2017) 7:7359. doi: 10.1038/s41598-017-07915-0

83. Frasca F, Piticchio T, Le Moli R, Tumino D, Cannavò S, Ruggeri RM, et al. Early detection of suspicious lymph nodes in differentiated thyroid cancer. *Expert Rev Endocrinol Metab*. (2022) 17:447–54. doi: 10.1080/17446651.2022.2112176

84. Arcolia V, Journe F, Renaud F, Leteurtre E, Gabius HJ, Remmelink M, et al. Combination of galectin-3, CK19 and HBME-1 immunostaining improves the diagnosis of thyroid cancer. *Oncol Lett*. (2017) 14:4183–9. doi: 10.3892/ol.2017.6719

85. Wiseman SM, Melck A, Masoudi H, Ghaidi F, Goldstein L, Gown A, et al. Molecular phenotyping of thyroid tumors identifies a marker panel for differentiated thyroid cancer diagnosis. *Ann Surg Oncol*. (2008) 15:2811–26. doi: 10.1245/s10434-008-0034-8

86. Pazaitou-Panayiotou K, Mygdakos N, Boglou K, Kiziridou A, Chrisoulidou A, Destouni C. The immunocytochemistry is a valuable tool in the diagnosis of papillary thyroid cancer in FNA's using liquid-based cytology. *J Oncol*. (2010) 2010:963926. doi: 10.1155/2010/963926

87. Kaczka K, Fendler W, Borowiec M, Młynarski W, Celnik A, Pomorski L. Lymph node metastases in papillary thyroid cancer detected by quantitative real-time polymerase chain reaction for thyroglobulin and cytokeratine-19. *Pol J Pathol*. (2013) 64:90–5. doi: 10.5114/pjp.2013.36007

88. Allin DM, Shaikh R, Carter P, Thway K, Sharabiani MTA, Gonzales-de-Castro D, et al. Circulating tumour DNA is a potential biomarker for disease progression and response to targeted therapy in advanced thyroid cancer. *Eur J Cancer*. (2018) 103:165–75. doi: 10.1016/j.ejca.2018.08.013

89. Khatami F, Larijani B, Nasiri S, Tavangar SM. Liquid biopsy as a minimally invasive source of thyroid cancer genetic and epigenetic alterations. *Int J Mol Cell Med*. (2019) 8:19–29. doi: 10.22088/IJMCM.BUMS.8.2.19

90. Almubarak H, Qassem E, Alghofaili L, Alzahrani AS, Karakas B. Non-invasive molecular detection of minimal residual disease in papillary thyroid cancer patients. *Front Oncol*. (2020) 9:1510. doi: 10.3389/fonc.2019.01510

91. Sato A, Tanabe M, Tsuboi Y, Niwa T, Shinozaki-Ushiku A, Seto Y, et al. Circulating tumor DNA harboring the BRAFV600E mutation may predict poor outcomes of primary papillary thyroid cancer patients. *Thyroid*. (2021) 31:1822–8. doi: 10.1089/thy.2021.0267

92. Adam P, Kircher S, Sbiera I, Koehler VF, Berg E, Knösel T, et al. FGF-receptors and PD-L1 in anaplastic and poorly differentiated thyroid cancer: evaluation of the preclinical rationale. *Front Endocrinol (Lausanne)*. (2021) 12:712107. doi: 10.3389/fendo.2021.712107

93. O'Connell TJ, Dadafarin S, Jones M, Rodríguez T, Gupta A, Shin E, et al. Androgen activity is associated with PD-L1 downregulation in thyroid cancer. *Front Cell Dev Biol*. (2021) 9:663130. doi: 10.3389/fcell.2021.663130

94. Liotti F, Kumar N, Prevete N, Marotta M, Sorriento D, Ieranò C, et al. PD-1 blockade delays tumor growth by inhibiting an intrinsic SHP2/Ras/MAPK signalling in thyroid cancer cells. *J Exp Clin Cancer Res*. (2021) 40:22. doi: 10.1186/s13046-020-01818-1

95. Bastman JJ, Serracino HS, Zhu Y, Koenig MR, Mateescu V, Sams SB, et al. Tumor-infiltrating T cells and the PD-1 checkpoint pathway in advanced differentiated and anaplastic thyroid cancer. *J Clin Endocrinol Metab*. (2016) 101:2863–73. doi: 10.1210/jc.2015-4227

96. Chintakuntlawar AV, Rumilla KM, Smith CY, Jenkins SM, Foote RL, Kasperbauer JL, et al. Expression of PD-1 and PD-L1 in anaplastic thyroid cancer patients treated with multimodal therapy: results from a retrospective study. *J Clin Endocrinol Metab*. (2017) 102:1943–50. doi: 10.1210/jc.2016-3756

97. Lang M, Longerich T, Anamaterou C. Targeted therapy with vemurafenib in BRAF(V600E)-mutated anaplastic thyroid cancer. *Thyroid Res*. (2023) 16:5. doi: 10.1186/s13044-023-00147-7

98. Zhang Y, Xing Z, Liu T, Tang M, Mi L, Zhu J, et al. Targeted therapy and drug resistance in thyroid cancer. *Eur J Med Chem*. (2022) 238:114500. doi: 10.1016/j.ejmech.2022.114500

99. Silaghi H, Lozovanu V, Georgescu CE, Pop C, Nasui BA, Cătoi AF, et al. State of the art in the current management and future directions of targeted therapy for differentiated thyroid cancer. *Int J Mol Sci*. (2022) 23:3470. doi: 10.3390/ijms23073470

100. Valerio L, Giani C, Matrone A, Pontillo-Contillo B, Minaldi E, Agate L, et al. Adrenal insufficiency in thyroid cancer patients treated with tyrosine kinase inhibitors and detected by ACTH stimulation test. *J Endocrinol Invest*. (2023) 46:1663–71. doi: 10.1007/s40618-023-02025-3

101. Nervo A, Retta F, Ragni A, Piovesan A, Mella A, Biancone L, et al. Nephrotoxicity in advanced thyroid cancer treated with tyrosine kinase inhibitors: An update. *Crit Rev Oncol Hematol*. (2021) 168:103533. doi: 10.1016/j.critrevonc.2021.103533

102. Mahmood U, Lorch JH. Precision medicine in aggressive thyroid cancer: Moving beyond multitargeted tyrosine kinase inhibitors. *Cancer Cytopathol*. (2022) 130:8–11. doi: 10.1002/cncy.22516

103. Frasca F, Vigneri P, Vella V, Vigneri R, Wang JY. Tyrosine kinase inhibitor STI571 enhances thyroid cancer cell motile response to Hepatocyte Growth Factor. *Oncogene*. (2001) 20:3845–56. doi: 10.1038/sj.onc.1204531

104. Cabanillas ME, Patel A, Danysh BP, Dadu R, Kopetz S, Falchook G. BRAF inhibitors: experience in thyroid cancer and general review of toxicity. *Horm Cancer*. (2015) 6:21–36. doi: 10.1007/s12672-014-0207-9

105. Crispo F, Notarangelo T, Pietrafesa M, Lettini G, Storto G, Sgambato A, et al. BRAF inhibitors in thyroid cancer: clinical impact, mechanisms of resistance and future perspectives. *Cancers (Basel)*. (2019) 11:1388. doi: 10.3390/cancers11091388

106. Falchook GS, Millward M, Hong D, Naing A, Piha-Paul S, Waguespack SG, et al. BRAF inhibitor dabrafenib in patients with metastatic BRAF-mutant thyroid cancer. *Thyroid*. (2015) 25:71–7. doi: 10.1089/thy.2014.0123

107. Hińcza-Nowak K, Kowalik A, Walczyk A, Pałyga I, Gąsior-Perczak D, Płusa A, et al. CD276 as a candidate target for immunotherapy in medullary thyroid cancer. *Int J Mol Sci*. (2023) 24:10019. doi: 10.3390/ijms241210019

108. Hong K, Cen K, Chen Q, Dai Y, Mai Y, Guo Y. Identification and validation of a novel senescence-related biomarker for thyroid cancer to predict the prognosis and immunotherapy. *Front Immunol*. (2023) 14:1128390. doi: 10.3389/fimmu.2023.1128390

109. Gunda V, Frederick DT, Bernasconi MJ, Wargo JA, Parangi S. A potential role for immunotherapy in thyroid cancer by enhancing NY-ESO-1 cancer antigen expression. *Thyroid*. (2014) 24:1241–50. doi: 10.1089/thy.2013.0680

110. La Pietra V, Sartini S, Botta L, Antonelli A, Ferrari SM, Fallahi P, et al. Challenging clinically unresponsive medullary thyroid cancer: Discovery and pharmacological activity of novel RET inhibitors. *Eur J Med Chem*. (2018) 150:491–505. doi: 10.1016/j.ejmech.2018.02.080

111. Yokota T. Durable disease control by RET inhibitor selpercatinib in a heavily pre-treated RET fusion-positive papillary thyroid cancer. *Case Rep Oncol*. (2022) 15:833–40. doi: 10.1159/000526030

112. Contrera KJ, Gule-Monroe MK, Hu MI, Cabanillas ME, Busaidy NL, Dadu R, et al. Neoadjuvant selective RET inhibitor for medullary thyroid cancer: A case series. *Thyroid*. (2023) 33:129–32. doi: 10.1089/thy.2022.0506

113. Matrone A, Prete A, Sartini MS, Elisei R. Significant response of medullary thyroid cancer choroidal metastases to highly selective RET inhibitor selpercatinib: a case report. *Ann Oncol*. (2021) 32:1447–9. doi: 10.1016/j.annonc.2021.08.1987

114. Samadi AK, Mukerji R, Shah A, Timmermann BN, Cohen MS. A novel RET inhibitor with potent efficacy against medullary thyroid cancer. *vivo Surg*. (2010) 148:1228–36. doi: 10.1016/j.surg.2010.09.026

115. Enomoto K, Hirayama S, Kumashiro N, Jing X, Kimura T, Tamagawa S, et al. Synergistic effects of lenvatinib (E7080) and MEK inhibitors against anaplastic thyroid cancer in preclinical models. *Cancers (Basel)*. (2021) 13:862. doi: 10.3390/cancers13040862

116. Liu D, Xing J, Trink B, Xing M. BRAF mutation-selective inhibition of thyroid cancer cells by the novel MEK inhibitor RDEA119 and genetic-potentiated synergism with the mTOR inhibitor temsirolimus. *Int J Cancer*. (2010) 127:2965–73. doi: 10.1002/ijc.25304

117. Nucera C. A novel combined targeted therapy with bromodomain antagonist and MEK inhibitor in anaplastic thyroid cancer. *Oncotarget*. (2019) 10:686–7. doi: 10.18632/oncotarget.26591

118. Liu D, Xing M. Potent inhibition of thyroid cancer cells by the MEK inhibitor PD0325901 and its potentiation by suppression of the PI3K and NF-kappaB pathways. *Thyroid*. (2008) 18:853–64. doi: 10.1089/thy.2007.0357

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 22 May 2024
DOI 10.3389/fendo.2024.1400573



OPEN ACCESS

EDITED BY
Zili Zhang,
Nanjing University of Chinese Medicine, China

REVIEWED BY
Dafina Fondaj,
University of Bari Aldo Moro, Italy
Jia Li,
Fudan University, China

*CORRESPONDENCE
Hao Hu
lvye1025@163.com

†These authors have contributed equally to this work

RECEIVED 13 March 2024
ACCEPTED 22 April 2024
PUBLISHED 22 May 2024

CITATION
Yu L, Wang L, Xue Y, Ren Y, Liu T and Hu H (2024) Causal associations between platelet count, alcohol consumption, and the risk of liver hepatocellular carcinoma based on a Mendelian randomization study.
*Front. Endocrinol.* 15:1400573.
doi: 10.3389/fendo.2024.1400573



# Causal associations between platelet count, alcohol consumption, and the risk of liver hepatocellular carcinoma based on a Mendelian randomization study

Lihua Yu[1,3†], Leisheng Wang[2,3†], Yuzheng Xue[1], Yilin Ren[1], Tianhao Liu[1] and Hao Hu[2,3,4*]

[1]Department of Gastroenterology, Affiliated Hospital of Jiangnan University, Wuxi, China, [2]Hepatobiliary and Pancreatic Surgery, Affiliated Hospital of Jiangnan University, Wuxi, China, [3]School of Medicine, Jiangnan University, Wuxi, China, [4]Wuxi Institute of Hepatobiliary Surgery, Wuxi, China

**Background and aims:** Liver hepatocellular carcinoma (LIHC) exhibits a multifactorial etiology, insidious onset, and a significantly low 5-year survival rate. We aimed to evaluate the causal impact of exposure factors (Alzheimer's disease, platelet count, ambidextrousness, cigarettes smoked per day, alcohol consumption, and endocarditis) on the risk of LIHC using a two-sample Mendelian randomization (MR) study.

**Methods:** Independent single nucleotide polymorphisms (SNPs) strongly associated with Alzheimer's disease, platelet count, ambidextrousness, daily cigarette consumption, alcohol intake, and endocarditis were selected as instrumental variables (IVs) from the corresponding genome-wide association studies (GWAS). Genetic summary statistics for LIHC came from a GWAS that included 168 cases and 372,016 controls of European individuals. Multivariable MR analyses were performed to find the causal association between 6 exposure factors and LIHC risk. The inverse-variance weighted (IVW)-MR was employed as the primary analysis, and the MR-Egger regression, LASSO regression, and weighted Median approaches were performed as complementary analyses.

**Results:** Multivariable MR analysis showed causal association between Alzheimer's disease [Odds ratio (OR) = 0.9999, 95% confidence intervals (CI) = 0.9998-0.9999, p = 0.0010], platelet count (OR = 0.9997, 95% CI = 0.9995-0.9999, p = 0.0066), alcohol consumption (OR = 0.9994, 95% CI = 0.9990-0.9999, p = 0.0098) and the LIHC outcome. After IVW-MR, MR-Egger and LASSO tests, the results are still significant. Next, we used different MR Methods to analyze platelet count, alcohol consumption, and Alzheimer's disease separately. Moreover, both funnel plots and MR-Egger intercepts provided compelling evidence to refute the presence of directional pleiotropy in the association

between platelet count, alcohol consumption, Alzheimer's disease and the risk of LIHC. The IVW-MR analysis revealed a significant causal association between an elevated platelet count and a reduced risk of LIHC (OR = 0.9996, 95% CI= 0.9995-0.9998, p = 0.0005). Similarly, the analysis of weighted median revealed a negative correlation between platelet count and the risk of LIHC (OR = 0.9995, 95% CI = 0.9993-0.9999; p = 0.0160). Conversely, we observed a positive causal effect of alcohol consumption on the incidence of LIHC (OR = 1.0004, 95% CI = 0.9999-1.0009). However, no significant causal relationship was found between alcohol assumption, Alzheimer's disease, and LIHC susceptibility.

**Conclusions:** A significant causal relationship exists between platelet count, alcohol consumption, Alzheimer's disease, and an increased risk of LIHC. The study presents compelling evidence for a genetically predicted decreased susceptibility to LIHC based on platelet count. The research implies that elevated platelet count may serve as a protective mechanism against LIHC. These findings may inform clinical strategies for LIHC prevention

KEYWORDS

platelet count, alcohol consumption, Alzheimer's disease, liver hepatocellular carcinoma, Mendelian randomization

## 1 Introduction

Liver hepatocellular carcinoma (LIHC) is the most common primary liver cancer and ranks as the third leading cause of cancer-related deaths globally (1). LIHC typically occurs in individuals with chronic liver conditions, primarily resulting from viral hepatitis, alcohol-induced liver disease, or non-alcoholic fatty liver disease (2). Currently, there is a lack of established screening programs for early detection due to the difficulty in identifying symptoms during the initial stages of the disease (3). While understanding symptom profiles associated with LIHC may offer some potential for early diagnosis, it remains crucial to identify clinical and biochemical factors that can assist in identifying high-risk subpopulations for timely intervention through imaging or participation in screening studies (4). Enhancing prevention strategies and developing innovative therapies are essential to improve outcomes for patients with LIHC.

Platelets are increasingly being recognized for their role in inflammation and the progression of cancer, as they release various substances that contribute to tumor development (5). The use of anti-platelet medications such as aspirin holds promise in the treatment of cancer, as indicated by a systematic review and meta-analysis showing a decreased risk of liver cancer occurrence and improved survival rates related to liver health (6). In an external validation group comprising 525 patients with cirrhosis and liver cancer, individuals with low platelet count and high mean platelet volume demonstrated significantly prolonged overall survival (OS) based on both univariate and multivariate analysis (7); however, it is important to note that this study has a retrospective design with exploratory nature. Therefore, further confirmation through prospective randomized controlled trials is necessary to validate these findings.

Globally, cancer and dementia are prominent causes of mortality that tend to escalate with advancing age (8). Numerous epidemiological investigations have indicated a negative correlation between these two ailments, specifically in relation to Alzheimer's dementia (9, 10). No existing study has demonstrated a definitive cause-and-effect link between Alzheimer's dementia and LIHC. Furthermore, certain research findings have linked tobacco smoking (11–13), alcohol consumption (14), as well as streptococcus infection (15) to heightened susceptibility for fatality due to chronic liver disease; however, there is a scarcity of comprehensive analyses regarding their temporal patterns. Furthermore, it is important to mention that the majority of previous studies examining their correlation were primarily based on observation or cross-sectional analysis. This factor may have introduced potential confounding variables and thus produced inconclusive results. As a result, it is crucial to adopt a fresh investigative approach in order to uncover the precise impact of these 6 exposure factors on the risk of LIHC.

The Mendelian randomization (MR) approach was employed to establish causal links between exposures and outcomes by utilizing single nucleotide polymorphisms (SNPs) as instrumental variables

(IVs) (20). SNPs were randomly assigned from parents to offspring during conception, ensuring that the MR method remained unaffected by confounding or reverse causation, similar to the random assignment in randomized controlled trials (20). In this investigation, we conducted a two-sample MR analysis to explore the connection between exposure factors (Alzheimer's disease, platelet count, ambidextrousness, daily cigarette consumption, alcohol intake, and endocarditis) and the risk of LIHC.

# 2 Materials and methods

## 2.1 Study design

This MR investigation utilizes summary-level data from publicly accessible genome-wide association studies (GWAS). All of these studies have obtained approval from the appropriate institutional review boards, and participants have provided informed consent.

## 2.2 Exposure data sources

In this study, we selected six variables for investigation: Alzheimer's disease (dataset: ebi-a-GCST002245), platelet count (dataset: ebi-a-GCST004603), ambidextrousness (dataset: ebi-a-GCST90013420), daily cigarette consumption (dataset: ieu-b-4826), alcohol consumption (dataset: ieu-b-4834), and endocarditis (dataset: ieu-b-4972). The exposure data for late-onset Alzheimer's disease were obtained through genome-wide association studies (GWAS), which involved genotyping approximately 7,022,150 single nucleotide polymorphisms (SNPs). This study included a cohort of individuals with European ancestry comprising 17,008 cases and 37,154 control adults. The platelet count exposure data was derived from a GWAS analysis utilizing approximately 29,148,896 SNPs. The ambidextrousness exposure data was obtained through a GWAS using 11,683,993 SNPs in a cohort consisting of 47,637 cases and 1,422,823 controls. The data on daily cigarette consumption was obtained through a GWAS using 7,227,329 SNPs in a cohort consisting of both males and females, totaling 24,784 individuals. The data on alcohol consumption was obtained through a GWAS using 7,914,362 SNPs in a cohort comprising both males and females, totaling 83,626 individuals. The data on endocarditis exposure was obtained through a GWAS using 12,243,455 SNPs in a cohort consisting of 1,080 cases and 485,404 controls. The study population was limited to individuals of European descent.

## 2.3 Outcome sources

The UK Biobank is a prospective study that enrolled half a million volunteers aged 37 to 73 from various regions of the United Kingdom between 2006 and 2010. Detailed information regarding the involvement of patients and the public can be accessed online. All participants in this study provided written consent after being fully informed, and it received approval from the National Research Ethics Services Board, North-West-Haydock. The genetic summary statistics for LIHC were obtained from a GWAS involving individuals of European descent, consisting of 168 cases and 372,016 controls (ieu-b-4953). All research procedures strictly adhere to the ethical principles outlined in the Helsinki Declaration for Medical Research established by the World Medical Association.

## 2.4 Selection of instrumental variables

We selected IVs linked to LIHC at the genome-wide significance levels from GWAS with $p < 5.0\times10\text{-}8$. To ensure independence among the IVs, we utilized the "TwoSampleMR" package to set a linkage disequilibrium (LD) threshold of $R2 < 0.001$ for the 1000 Genomes European data and an aggregation distance of 10,000 kb. After extracting relevant information on each SNP's effect allele, including β value, standard error, and P-value, we calculated the variance explanation ratio (R2) and F statistic to quantify tool strength as follows: $R2 = 2 \times MAF \times (1 - MAF) \times \beta\text{^}2$ and $F = R2(n - k - 1)/(k(1 - R2))$, where MAF represents the minor allele frequency of SNPs used as IVs, n denotes sample size, and k signifies the number of IVs employed. The choice of statistical methods and instrumental variables in our study was guided by their ability to provide robust and reliable causal inferences. Specifically, we selected MR methods such as IVW and MR-Egger regression for their strengths in addressing different aspects of potential biases, and SNPs were chosen as instrumental variables based on stringent criteria to ensure their relevance and independence in relation to the exposure and outcome.

## 2.5 Statistical analyses

### MR analysis

The primary analysis selected was the inverse-variance-weighted Mendelian randomization (IVW-MR) method (16), while supplementary tools such as MR-Egger regression, weighted median (17), simple mode, and weighted mode methods were also utilized. Despite the inherent advantages of Mendelian randomization in mitigating confounding biases, we acknowledge the potential for residual confounding factors that could influence our observations. We have attempted to minimize this impact by using robust statistical methods and sensitivity analyses, yet the possibility of unmeasured confounders remains a limitation of our study. Initially, the IVW method utilizes the Wald estimator and Delta method to calculate rate estimates for individual SNPs, which are then combined to derive the primary causal estimate. When

10.3389/fendo.2024.1400573

heterogeneity was statistically significant, the random effect model was utilized. Otherwise, the fixed effect model was utilized. The MR-Egger regression technique was utilized to evaluate potential levels of pleiotropy, with a significance level of $P < 0.05$ indicating the presence of potential pleiotropy at the SNP level (18). The weighted median MR approach introduces a new methodology that provides a consistent estimator even in the presence of significant heterogeneity. This estimator effectively manages type I errors, improving the ability to identify causal effects and ensuring stability even when more than 50% of information is derived from invalid instrumental variables (17). The MR-Egger method can identify and correct potential pleiotropy and provide a relatively consistent estimate (18). For multivariable MR-IVW analyses, multivariable MR-IVW was performed as the primary analysis. The least absolute shrinkage and selection operator (LASSO) regression provides the best estimation for moderate-to-high levels of pleiotropy and valid inference (19).

### Sensitivity analysis

The heterogeneity among SNPs was assessed using Cochran's Q statistic (20). The MR-Egger intercept method was used to test whether genetic variants have pleiotropic effects on infections. To identify potential outlier SNPs, leave-one-out methods were applied (21). The effect size of individual SNPs on the risk of LIHC associated with exposure factors was visualized using a forest plot. Causal effects of exposure on LIHC were evaluated through scatter plots. Additionally, the symmetrical distribution of selected SNPs was demonstrated using a funnel plot. Statistical significance for sensitivity analysis was defined as $p<0.05$. R software version 4.1.0 was used for all data analyses.

# 3 Results

## 3.1 Multivariable MR analyses

We estimated mutually the effects of 6 exposure factors, including Alzheimer's disease, platelet count, ambidextrousness, daily cigarette consumption, alcohol consumption, and endocarditis on LIHC using multivariable MR analyses. We observed directly inverse effect of Alzheimer's disease (OR = 0.9999, 95% CI = 0.9998-0.9999, p = 0.0100), platelet count (OR = 0.9995, 95% CI = 0.9995-0.9999, p = 0.0066), and alcohol consumption (OR = 0.9990, 95% CI = 0.9997-0.9999, p = 0.0098) on LIHC (Figure 1; Table 1).

Using four distinct MR methods, we identified significant causal associations between Alzheimer's disease, platelet count, and alcohol consumption with the risk of LIHC in the IVW analysis. Similar conclusions were drawn from Egger analysis and LASSO analysis, while only a significant causal relationship between endocarditis and LIHC risk was observed using the Median method. Notably, LASSO-MR and IVW analyses yielded the most robust causal evidence for Alzheimer's disease, platelet count, and alcohol intake respectively (Figure 2; Table 2). To address the apparent contradiction in our findings regarding the relationship

TABLE 1 The results of multivariable Mendelian Randomization analyses.

| No. | id.exposure | exposure | id.outcome | outcome | nsnp | b | se | pval | lo_ci | up_ci | or | or_lci95 | or_uci95 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ebi-a-GCST002245 | Alzheimer's disease (late onset) | ieu-b-4953 | LIHC | 8 | -0.00013719 | 5.33E-05 | 0.01000504 | -0.0002 | ###### | 0.99986 | 0.99976 | 0.99997 |
| 2 | ebi-a-GCST004603 | Platelet count | ieu-b-4953 | LIHC | 172 | -0.00030326 | 0.00011171 | 0.00663247 | -0.0005 | ###### | 0.9997 | 0.99948 | 0.99992 |
| 3 | ebi-a-GCST90013420 | Ambidextrousness | ieu-b-4953 | LIHC | 0 | -0.01017807 | 0.01355612 | 0.45276695 | -0.0367 | 0.01639 | 0.98987 | 0.96392 | 1.01653 |
| 4 | ieu-b-4826 | Cigarettes smoked per day | ieu-b-4953 | LIHC | 3 | -4.39E-06 | 4.06E-05 | 0.91382739 | ###### | 7.51E-05 | 1 | 0.99992 | 1.00008 |
| 5 | ieu-b-4834 | Alcohol consumption | ieu-b-4953 | LIHC | 0 | -0.00057839 | 0.00022403 | 0.00983149 | -0.001 | -0.0001 | 0.99942 | 0.99898 | 0.99986 |
| 6 | ieu-b-4972 | Endocarditis | ieu-b-4953 | LIHC | 0 | -0.00014359 | 9.55E-05 | 0.13270298 | -0.0003 | 4.36E-05 | 0.99986 | 0.99967 | 1.00004 |

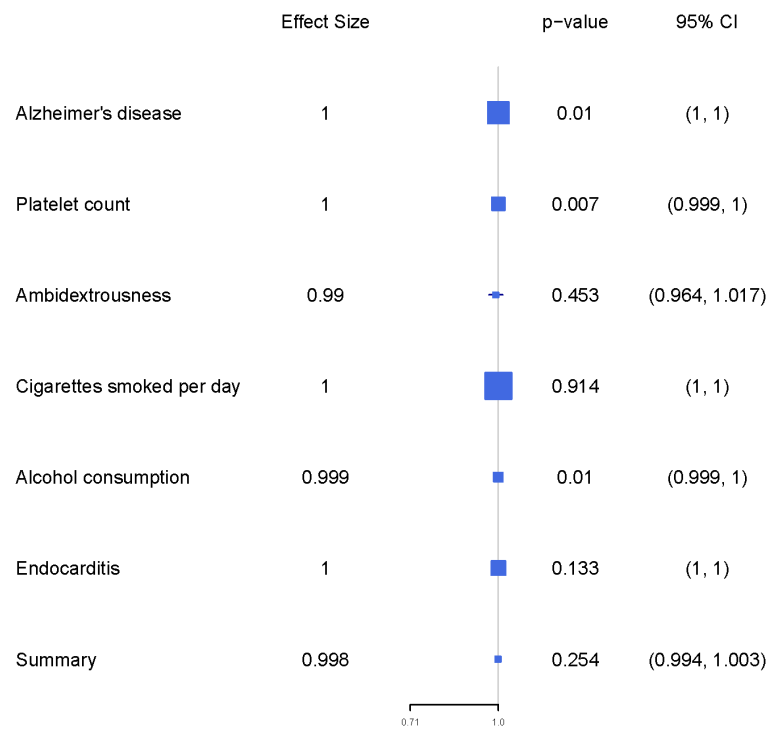



FIGURE 1
The forest plot presents the findings of a multivariable Mendelian Randomization (MR) analysis.

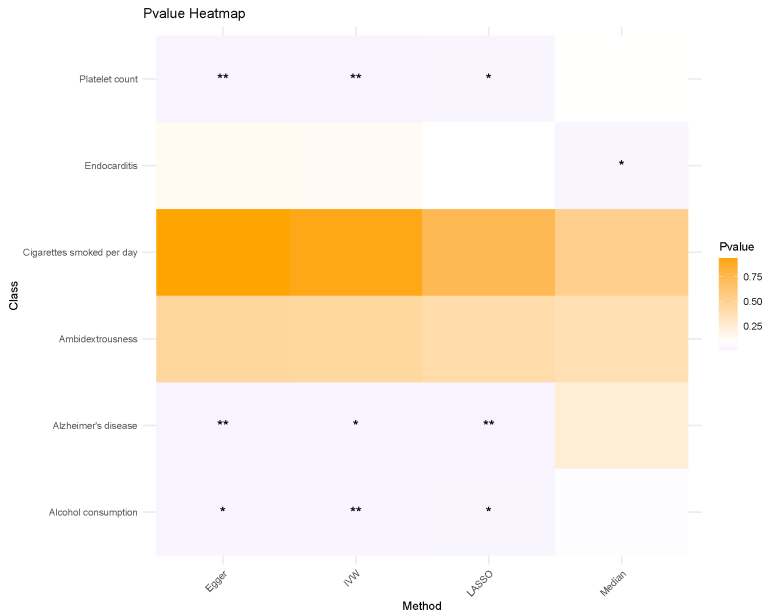



FIGURE 2
The heat map illustrates the impact of various MR methods on each exposure factor. * means p<0.05, ** means P<0.01.

TABLE 2 The effect of different MR Methods on each exposure factor.

| Method | Exposure | Estimate | StdError | CILower | CIUpper | Pvalue |
|---|---|---|---|---|---|---|
| IVW | exposure_1 | -0.0001372 | 5.33E-05 | -0.000242 | -3.28E-05 | 0.01001 |
| IVW | exposure_2 | -0.0003033 | 0.0001117 | -0.000522 | -8.43E-05 | 0.00663 |
| IVW | exposure_3 | -0.0101781 | 0.0135561 | -0.036748 | 0.01639143 | 0.45277 |
| IVW | exposure_4 | -4.39E-06 | 4.06E-05 | -8.39E-05 | 7.51E-05 | 0.91383 |
| IVW | exposure_5 | -0.0005784 | 0.000224 | -0.001017 | -0.0001393 | 0.00983 |
| IVW | exposure_6 | -0.0001436 | 9.55E-05 | -0.000331 | 4.36E-05 | 0.1327 |
| Egger | exposure_1 | -0.0001468 | 5.61E-05 | -0.000257 | -3.68E-05 | 0.0089 |
| Egger | exposure_2 | -0.0002981 | 0.0001123 | -0.000518 | -7.80E-05 | 0.00794 |
| Egger | exposure_3 | -0.0099459 | 0.0135892 | -0.03658 | 0.01668839 | 0.46423 |
| Egger | exposure_4 | -3.03E-06 | 4.07E-05 | -8.28E-05 | 7.68E-05 | 0.94067 |
| Egger | exposure_5 | -0.0005774 | 0.0002245 | -0.001017 | -0.0001375 | 0.0101 |
| Egger | exposure_6 | -0.0001428 | 9.57E-05 | -0.00033 | 4.48E-05 | 0.13573 |
| LASSO | exposure_1 | -0.0001422 | 5.09E-05 | -0.000242 | -4.24E-05 | 0.00524 |
| LASSO | exposure_2 | -0.0002545 | 0.0001071 | -0.000464 | -4.46E-05 | 0.0175 |
| LASSO | exposure_3 | -0.0105548 | 0.0129459 | -0.035928 | 0.01481859 | 0.4149 |
| LASSO | exposure_4 | 1.28E-05 | 3.89E-05 | -6.36E-05 | 8.91E-05 | 0.7433 |
| LASSO | exposure_5 | -0.0005061 | 0.000215 | -0.000927 | -8.48E-05 | 0.01856 |
| LASSO | exposure_6 | -0.0001595 | 9.20E-05 | -0.00034 | 2.08E-05 | 0.08294 |
| Median | exposure_1 | -0.0001052 | 8.98E-05 | -0.000281 | 7.09E-05 | 0.24167 |
| Median | exposure_2 | -0.000266 | 0.0001567 | -0.000573 | 4.11E-05 | 0.08959 |
| Median | exposure_3 | -0.0130053 | 0.0147294 | -0.041874 | 0.01586383 | 0.37727 |
| Median | exposure_4 | -3.27E-05 | 5.18E-05 | -0.000134 | 6.90E-05 | 0.52873 |
| Median | exposure_5 | -0.000457 | 0.0002477 | -0.000943 | 2.85E-05 | 0.06503 |
| Median | exposure_6 | -0.0002484 | 0.0001039 | -0.000452 | -4.48E-05 | 0.0168 |

TABLE 3 Causal effect of platelet count on LIHC risk using multiple MR analyses.

| id.exposure | outcome | method | nsnp | b | se | pval | lo_ci | up_ci | or | or_lci95 | or_uci95 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ebi-a-GCST004603 | Liver cell carcinoma \|\| id:ieu-b-4953 | MR Egger | 272 | -0.0002 | 0.0002 | 0.2879 | -0.0006 | 0.0002 | 0.9998 | 0.9994 | 1.0002 |
| ebi-a-GCST004603 | Liver cell carcinoma \|\| id:ieu-b-4953 | Weighted median | 272 | -0.0004 | 0.0002 | 0.016 | -0.0007 | -0.0001 | 0.9996 | 0.9993 | 0.9999 |
| ebi-a-GCST004603 | Liver cell carcinoma \|\| id:ieu-b-4953 | Inverse variance weighted | 272 | -0.0003 | 0.0001 | 0.0005 | -0.0005 | -0.0002 | 0.9997 | 0.9995 | 0.9998 |
| ebi-a-GCST004603 | Liver cell carcinoma \|\| id:ieu-b-4953 | Simple mode | 272 | -0.0005 | 0.0004 | 0.2544 | -0.0013 | 0.0003 | 0.9995 | 0.9987 | 1.0003 |
| ebi-a-GCST004603 | Liver cell carcinoma \|\| id:ieu-b-4953 | Weighted mode | 272 | -0.0002 | 0.0002 | 0.2642 | -0.0007 | 0.0002 | 0.9998 | 0.9993 | 1.0002 |

between alcohol consumption, Alzheimer's disease, and LIHC susceptibility, we conducted further sensitivity analyses. These analyses suggest that the initial non-significant association may be attributed to the limited power of our dataset to detect weaker causal relationships in the context of multiple testing and complex interactions among the studied variables.

## 3.2 Causal association between elevated platelet count and decreased risk of LIHC

Considering that multivariate MR Analyses showed significant causal relationships between platelet count, alcohol intake, and Alzheimer's disease and LIHC risk, we then used different MR Analyses to verify the relationship between these three exposure factors and LIHC susceptibility. Detailed information about the SNPs of LIHC on platelet count is shown in Supplementary Table 1. There is a causal relationship between reduced platelet count and increased risk of LIHC using the IVW-MR method (OR = 0.9997, 95% CI = 0.9995-0.9998, p=0.0005), and weighted median method (OR = 0.9996, 95% CI = 0.9993-0.9999, p = 0.0160) (Table 3). No heterogeneity (MR Egger, p=0.2196; IVW, p=0.2261) and no potential pleiotropy (MR-Egger, intercept= -5.05E-06, p=0.4927) were observed in the MR analysis. The scatter plot demonstrated the negative causal association between platelet count and the risk of LIHC (Figure 3A). The forest plot displays the effect size for every single SNP on the risk of LIHC and shows that causality existed between platelet count and the occurrence of LIHC (Figure 3B). The funnel plot showed the selected SNPs were distributed symmetrically. The leave-1-out suggested that no SNPs had an important impact on the estimated causal association.

## 3.3 MR analysis between causal association between alcohol assumption, Alzheimer's disease, and LIHC risk

Detailed information about SNPs of LIHC on alcohol assumption and Alzheimer's disease (Supplementary Tables 2, 3). The primary IVW-MR method showed no causality between alcohol assumption (Table 4), Alzheimer's disease, and LIHC risk. The other MR method results were consistent with IVWs (Table 5). The scatter plots, the forest plots, the funnel plots, and the leave-one-out plots of LIHC risk for alcohol assumption and Alzheimer's disease are displayed in Figures 4, 5, respectively.

# 4 Discussion

This study represents the first comprehensive MR analysis to systematically evaluate the causal relationship between 6 exposure (Alzheimer's disease, platelet count, ambidextrousness, daily cigarette consumption, alcohol intake, and endocarditis) and the risk of LIHC. Our findings reveal a significant inverse association between genetically predicted platelet count and the likelihood of developing LIHC. This negative correlation is further supported by robust sensitivity analyses. Overall, our MR investigation provides compelling evidence for a unidirectional causal link between platelet count and the risk of LIHC, suggesting that higher platelet count is associated with reduced susceptibility to LIHC.

By focusing on understanding how platelets contribute to carcinogenesis, previous research has suggested that low levels of platelets may increase the risk of developing LIHC (22, 23). Our

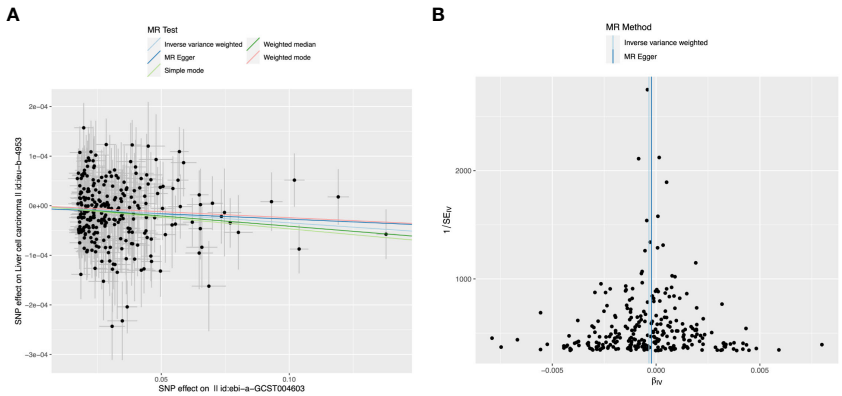



FIGURE 3
The causal relationship between platelet count and liver hepatocellular carcinoma (LIHC) risk. **(A)** The scatter plot visually represents the causal effect of platelet count on LIHC risk, with the slope of the line indicating the strength of the causal association. **(B)** The forest plot visually depicts the causal impact of individual single nucleotide polymorphisms (SNPs) of platelet count on susceptibility to LIHC. The funnel plots are used to visualize the overall heterogeneity of MR estimates for the effect of platelet count on the risk of LIHC. A leave-one-out plot is used to visualize the causal effect of platelet count on the risk of LIHC when one SNP is excluded.

TABLE 4 Causal effect of Alzheimer's disease on LIHC risk using multiple MR analyses.

| id.exposure | outcome | method | nsnp | b | se | pval | lo_ci | up_ci | or | or_lci95 | or_uci95 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ebi-a-GCST002245 | Liver cell carcinoma \|\| id:ieu-b-4953 | MR Egger | 24 | 0.00024701 | 0.000364733 | 0.505316629 | -0.000467866 | 0.000961886 | 1.00024704 | 0.999532243 | 1.000962348 |
| ebi-a-GCST002245 | Liver cell carcinoma \|\| id:ieu-b-4953 | Weighted median | 24 | -3.09E-05 | 0.000136677 | 0.820966198 | -0.000298817 | 0.000236956 | 0.99996907 | 0.999701228 | 1.000236984 |
| ebi-a-GCST002245 | Liver cell carcinoma \|\| id:ieu-b-4953 | Inverse variance weighted | 24 | -0.000102716 | 0.000100488 | 0.306701631 | -0.000299673 | 9.42E-05 | 0.999897289 | 0.999700372 | 1.000094246 |
| ebi-a-GCST002245 | Liver cell carcinoma \|\| id:ieu-b-4953 | Simple mode | 24 | 4.64E-05 | 0.000225734 | 0.838911365 | -0.000396027 | 0.000488849 | 1.000046412 | 0.999604051 | 1.000488969 |
| ebi-a-GCST002245 | Liver cell carcinoma \|\| id:ieu-b-4953 | Weighted mode | 24 | -6.05E-06 | 0.00017815 | 0.973208272 | -0.000355222 | 0.000343125 | 0.999993951 | 0.999644841 | 1.000343184 |

TABLE 5 Causal effect of alcohol assumption on LIHC risk using multiple MR analyses.

| id.exposure | outcome | method | nsnp | b | se | pval | lo_ci | up_ci | or | or_lci95 | or_uci95 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ieu-b-4834 | Liver cell carcinoma \|\| id:ieu-b-4953 | MR Egger | 13 | -0.001043507 | 0.00164626 | 0.539125649 | -0.004270176 | 0.002183162 | 0.998957037 | 0.995738928 | 1.002185546 |
| ieu-b-4834 | Liver cell carcinoma \|\| id:ieu-b-4953 | Weighted median | 13 | 0.000346529 | 0.000259117 | 0.181108965 | -0.000161339 | 0.000854398 | 1.000346589 | 0.999838674 | 1.000854763 |
| ieu-b-4834 | Liver cell carcinoma \|\| id:ieu-b-4953 | Inverse variance weighted | 13 | 0.000446162 | 0.000230073 | 0.052474688 | -4.78E-06 | 0.000897105 | 1.000446262 | 0.999995219 | 1.000897508 |
| ieu-b-4834 | Liver cell carcinoma \|\| id:ieu-b-4953 | Simple mode | 13 | 0.000204665 | 0.000477662 | 0.675897123 | -0.000731552 | 0.001140882 | 1.000204686 | 0.999268716 | 1.001141533 |
| ieu-b-4834 | Liver cell carcinoma \|\| id:ieu-b-4953 | Weighted mode | 13 | 0.000215491 | 0.000445877 | 0.637584107 | -0.000658429 | 0.00108941 | 1.000215514 | 0.999341788 | 1.001090004 |



10.3389/fendo.2024.1400573

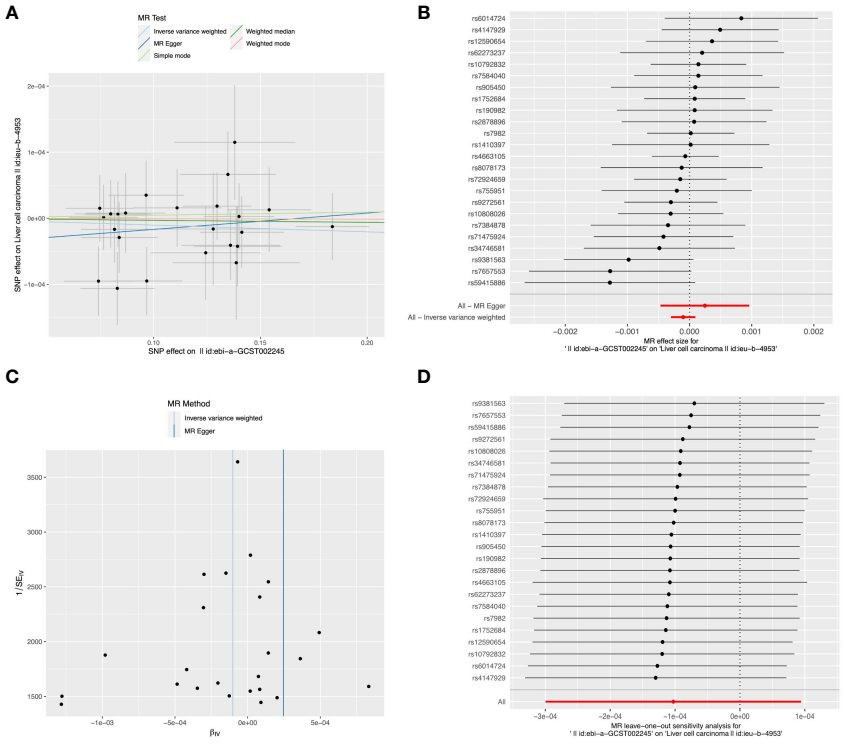



FIGURE 4
The causal relationship between Alzheimer's disease and LIHC risk. **(A)** The scatter plot visually represents the causal effect of Alzheimer's disease on LIHC risk, with the slope of the line indicating the strength of the causal association. **(B)** The forest plot visually depicts the causal impact of individual SNPs of Alzheimer's disease on susceptibility to LIHC. **(C)** The funnel plots are used to visualize the overall heterogeneity of MR estimates for the effect of Alzheimer's disease on the risk of LIHC. **(D)** A leave-one-out plot is used to visualize the causal effect of Alzheimer's disease on the risk of LIHC when one SNP is excluded.

own study supports this by demonstrating a direct link between reduced platelet count and higher LIHC risk. Nevertheless, it remains uncertain whether decreased platelet count acts as an independent risk factor for LIHC development or simply reflects advanced liver disease which coincides with an elevated incidence of LICH. Contrary to certain observational studies proposing an association between thrombocytosis and greater occurrence of distant metastasis across different types of cancer (24, 25), our current MR investigation does not provide evidence supporting these claims. Additionally, further exploration into the underlying cellular and molecular mechanisms responsible for this phenomenon is necessary. The protective effect of an increased platelet count against LIHC could be mediated through several biological pathways, including but not limited to, the modulation of inflammatory responses and the facilitation of tumor-suppressive immune surveillance. Similarly, the pathways through which alcohol consumption may exacerbate LIHC risk likely involve the induction of hepatic inflammation, oxidative stress, and direct DNA damage.

In our analysis using multiple variables, we discovered a noteworthy link between alcohol consumption and the risk of LIHC in individuals with Alzheimer's disease. Previous epidemiological studies have consistently reported an inverse correlation between dementia, specifically Alzheimer's dementia, and the occurrence of cancer (9). A recent meta-analysis also revealed that being diagnosed with cancer resulted in an 11% decrease in subsequent incidence rates of Alzheimer's disease (26). However, when conducting separate MR analyses on Alzheimer's disease and alcohol consumption as well as LIHC outcomes, we did not find any significant causal relationship. This lack of significance could potentially be attributed to biases within the study design or limitations associated with studying a European population.

One of the main strengths of this study was the utilization of the MR method to assess the causal relationship between genetically predicted platelet count and the risk of LIHC development within a European population. Moreover, by incorporating multiple samples, we were able to enhance both the overall sample size and precision in estimating causal effects. The implementation of a 2-sample summary MR approach also enabled us to leverage publicly available GWAS data instead of relying solely on individual-level data. In our analysis, summary estimates from individual-level data were obtained; however, researchers can easily apply these methods using the online platform MR-Base.

There are certain limitations that need to be acknowledged in this study. Firstly, the use of summary data restricts the exploration of potential non-linear relationships or stratification effects. Secondly, it is difficult to evaluate the absence of horizontal pleiotropic pathways, and any violation could introduce bias into estimates when using IVW regression. To investigate pleiotropic effects, we employed MR-Egger and weighted median approaches; however, it is important to note that both methods rely on assumptions that cannot currently be tested. Thirdly, due to

10.3389/fendo.2024.1400573

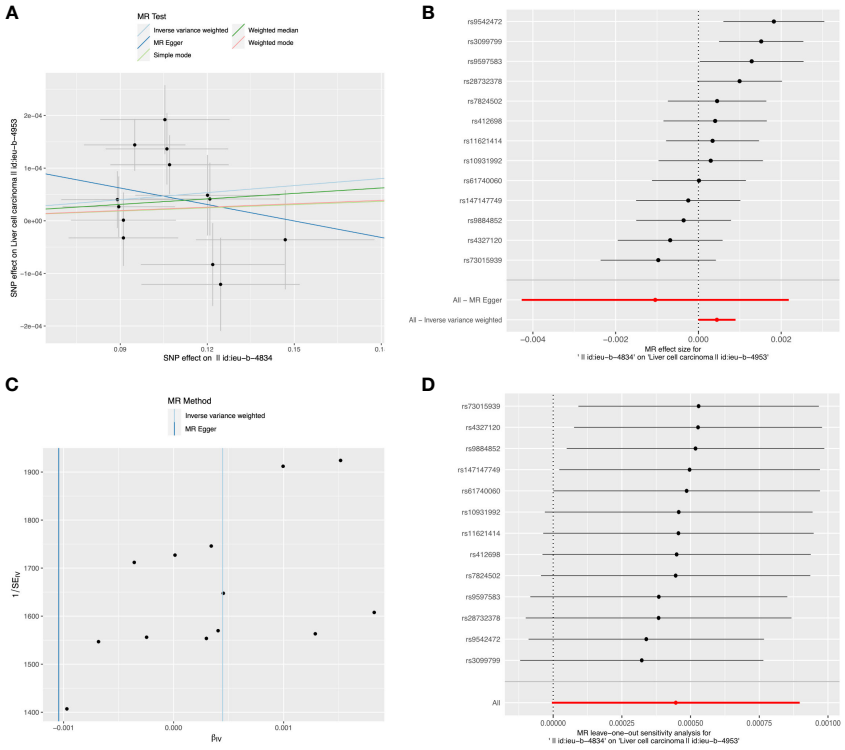



FIGURE 5
The causal relationship between alcohol assumption and LIHC risk. **(A)** The scatter plot visually represents the causal effect of alcohol assumption on LIHC risk, with the slope of the line indicating the strength of the causal association. **(B)** The forest plot visually depicts the causal impact of individual SNPs of alcohol assumption on susceptibility to LIHC. **(C)** The funnel plots are used to visualize the overall heterogeneity of MR estimates for the effect of alcohol assumption on the risk of LIHC. **(D)** A leave-one-out plot is used to visualize the causal effect of alcohol assumption on the risk of LIHC when one SNP is excluded.

limited available data for European populations, it is crucial to emphasize that since this study solely focuses on effects within a European population, further justification is required before generalizing these findings to other ethnicities. While our study provides valuable insights into the causal relationships between platelet count, alcohol consumption, Alzheimer's disease, and LIHC risk among individuals of European descent, the potential effects of population stratification warrant caution. Future research should aim to replicate these findings in diverse populations to ascertain the generalizability of our results.

## 5 Conclusions

In summary, our findings provide compelling evidence supporting a causal relationship between reduced platelet levels and heightened vulnerability to LIHC in the European population. Therefore, it is recommended to prioritize the management of individuals with lower platelet counts to minimize their risk of developing LIHC. As a result, this study contributes to an expanding body of literature indicating that targeting platelet-related agents holds promise as a potential therapeutic approach for early detection and treatment of LIHC.

## 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 authors.

## Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

## Author contributions

LY: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. LW: Investigation, Methodology, Writing – review & editing. YX: Project administration, Resources, Writing – review & editing. YR: Supervision, Writing

– review & editing. TL: Supervision, Writing – original draft. HH: Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

## Funding

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

## Conflict of interest

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

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1400573/full#supplementary-material

## References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. (2018) 68:394–424. doi: 10.3322/caac.21492

2. Vogel A, Meyer T, Sapisochin G, Salem R, Saborowski A. Hepatocellular carcinoma. *Lancet*. (2022) 400(10360):1345–62. doi: 10.1016/S0140-6736(22)01200-4

3. Huang DQ, Singal AG, Kanwal F, Lampertico P, Buti M, Sirlin CB, et al. Hepatocellular carcinoma surveillance - utilization, barriers and the impact of changing aetiology. *Nat Rev Gastroenterol Hepatol*. (2023) 20(12):797–809. doi: 10.1038/s41575-023-00818-8

4. Polyzos SA, Chrysavgis L, Vachliotis ID, Chartampilas E, Cholongitas E. Nonalcoholic fatty liver disease and hepatocellular carcinoma:Insights in epidemiology, pathogenesis, imaging, prevention and therapy. *Semin Cancer Biol*. (2023) 93:20–35. doi: 10.1016/j.semcancer.2023.04.010

5. Mezouar S, Frère C, Darbousset R, Mege D, Crescence L, Dignat-George F, et al. Role of platelets in cancer and cancer-associated thrombosis: Experimental and clinical evidences. *Thromb Res*. (2016) 139:65–76. doi: 10.1016/j.thromres.2016.01.006

6. Tan RZH, Lockart I, Abdel Shaheed C, Danta M. Systematic review with meta-analysis: The effects of non-steroidal anti-inflammatory drugs and anti-platelet therapy on the incidence and recurrence of hepatocellular carcinoma. *Aliment Pharmacol Ther*. (2021) 54(4):356–67. doi: 10.1111/apt.16515

7. Scheiner B, Kirstein M, Popp S, Hucke F, Bota S, Rohr-Udilova N, et al. Association of platelet count and mean platelet volume with overall survival in patients with cirrhosis and unresectable hepatocellular carcinoma. *Liver Cancer*. (2019) 8(3):203–17. doi: 10.1159/000489833

8. Wang J, Buto P, Ackley SF, Kobayashi LC, Graff RE, Zimmerman SC, et al. Association between cancer and dementia risk in the UK Biobank: evidence of diagnostic bias. *Eur J Epidemiol*. (2023) 38(10):1069–79. doi: 10.1101/2022.10.20.22281285

9. Freedman DM, Wu J, Chen H, Kuncl RW, Enewold LR, Engels EA, et al. Associations between cancer and Alzheimer's disease in a U.S. Medicare population. *Cancer Med*. (2016) 5(10):2965–76. doi: 10.1002/cam4.850

10. Musicco M, Adorni F, Di Santo S, Prinelli F, Pettenati C, Caltagirone C, et al. Inverse occurrence of cancer and Alzheimer disease: a population-based incidence study. *Neurology*. (2013) 81(4):322–8. doi: 10.1212/WNL.0b013e31829c5ec1

11. Marti-Aguado D, Clemente-Sanchez A, Bataller R. Cigarette smoking and liver diseases. *J Hepatol*. (2022) 77:191–205. doi: 10.1016/j.jhep.2022.01.016

12. Mumtaz H, Hameed M, Sangah AB, Zubair A, Hasan M. Association between smoking and non-alcoholic fatty liver disease in Southeast Asia. *Front Public Health*. (2022) 10:1008878. doi: 10.3389/fpubh.2022.1008878

13. Zhang Y, Li ZY, Shen QM, Tuo JY, Tan JY, Tan YT, et al. A prospective cohort study of cigarette smoking, alcohol drinking and liver cancer incidence in Chinese men. *J Dig Dis*. (2022) 23(8-9):527–34. doi: 10.1111/1751-2980.13136

14. Taniai M. Alcohol and hepatocarcinogenesis. *Clin Mol Hepatol*. (2020) 26:736–41. doi: 10.3350/cmh.2020.0203

15. Tripodi MF, Adinolfi LE, Ragone E, Durante Mangoni E, Fortunato R, Iarussi D , et al. Streptococcus bovis endocarditis and its association with chronic liver disease: an underestimated risk factor. *Clin Infect Dis*. (2004) 38:1394–400. doi: 10.1086/392503

16. Yavorska OO, Burgess S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. *Int J Epidemiol*. (2017) 46:1734–9. doi: 10.1093/ije/dyx034

17. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. (2016) 40(17):304–14. doi: 10.1002/gepi.21965

18. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur J Epidemiol*. (2017) 32:377–89. doi: 10.1007/s10654-017-0255-x

19. Grant AJ, Burgess S. Pleiotropy robust methods for multivariable Mendelian randomization. *Stat Med*. (2021) 40:5813–30. doi: 10.1002/sim.9156

20. Bowden J, Spiller W, Del Greco MF, Sheehan N, Thompson J, Minelli C, et al. Improving the visualization, interpretation and analysis of two-sample summary data Mendelian randomization via the Radial plot and Radial regression. *Int J Epidemiol*. (2018) 47:1264–78. doi: 10.1093/ije/dyy101

21. Stock M, Pahikkala T, Airola A, Waegeman W, De Baets B. Algebraic shortcuts for leave-one-out cross-validation in supervised network inference. *Brief Bioinform*. (2020) 21:262–71. doi: 10.1093/bib/bby095

22. Everson GT, Shiffman ML, Hoefs JC, Morgan TR, Sterling RK, Wagner DA, et al. Quantitative liver function tests improve the prediction of clinical outcomes in chronic hepatitis C: results from the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis Trial. *Hepatology*. (2012) 55:1019–29. doi: 10.1002/hep.24752

23. Realdi G, Fattovich G, Hadziyannis S, Schalm SW, Almasio P, Sanchez-Tapias J, et al. Survival and prognostic factors in 366 patients with compensated cirrhosis type B: a multicenter study. The Investigators of the European Concerted Action on Viral Hepatitis (EUROHEP). *J Hepatol*. (1994) 21:656–66. doi: 10.1016/S0168-8278(94)80115-0

24. Carr BI, Cavallini A, D'Alessandro R, Refolo MG, Lippolis C, Mazzocca A, et al. Platelet extracts induce growth, migration and invasion in human hepatocellular carcinoma in vitro. *BMC Cancer*. (2014) 14:43. doi: 10.1186/1471-2407-14-43

25. Bambace NM, Holmes CE. The platelet contribution to cancer progression. *J Thromb Haemost*. (2011) 9:237–49. doi: 10.1111/j.1538-7836.2010.04131.x

26. Ospina-Romero M, Glymour MM, Hayes-Larson E, Mayeda ER, Graff RE, Brenowitz WD, et al. Association between alzheimer disease and cancer with evaluation of study biases: A systematic review and meta-analysis. *JAMA Netw Open*. (2020) 3: e2025515. doi: 10.1001/jamanetworkopen.2020.25515

frontiers | Frontiers in Endocrinology

TYPE Methods
PUBLISHED 31 May 2024
DOI 10.3389/fendo.2024.1428433



OPEN ACCESS

EDITED BY
Min Tu,
Nanjing Medical University, China

REVIEWED BY
Yang Wu,
Nanjing Medical University, China
Tong Zhu,
LMU Munich University Hospital, Germany

*CORRESPONDENCE
Fanjie Liu
liufj198211@126.com
Ying Yin
563298098@qq.com
Meina Yang
meina861010@163.com

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

RECEIVED 06 May 2024
ACCEPTED 17 May 2024
PUBLISHED 31 May 2024

CITATION
Deng T, Lu X, Jia X, Du J, Wang L, Cao B, Yang M, Yin Y and Liu F (2024) Cathepsins and cancer risk: a Mendelian randomization study. *Front. Endocrinol.* 15:1428433. doi: 10.3389/fendo.2024.1428433



# Cathepsins and cancer risk: a Mendelian randomization study

Tingting Deng[1†], Xixue Lu[2†], Xuemin Jia[2], Jinxin Du[1], Lijuan Wang[1,2], Baorui Cao[1], Meina Yang[3,4*], Ying Yin[5*] and Fanjie Liu[2*]

[1]College of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China, [2]Bone Biomechanics Engineering Laboratory of Shandong Province, Shandong Medicinal Biotechnology Center (School of Biomedical Sciences), Neck-Shoulder and Lumbocrural Pain Hospital of Shandong First Medical University, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, China, [3]National Health Commission (NHC) Key Laboratory of Biotechnology Drugs (Shandong Academy of Medical Sciences), Biomedical Sciences College, Shandong First Medical University, Jinan, China, [4]Department of Endocrinology, The First Affiliated Hospital of Shandong First Medical University, Jinan, China, [5]Department of Acupuncture, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, China

**Background:** Previous observational epidemiological studies reported an association between cathepsins and cancer, however, a causal relationship is uncertain. This study evaluated the causal relationship between cathepsins and cancer using Mendelian randomization (MR) analysis.

**Methods:** We used publicly available genome-wide association study (GWAS) data for bidirectional MR analysis. Inverse variance weighting (IVW) was used as the primary MR method of MR analysis.

**Results:** After correction for the False Discovery Rate (FDR), two cathepsins were found to be significantly associated with cancer risk: cathepsin H (CTSH) levels increased the risk of lung cancer (OR = 1.070, 95% CI = 1.027–1.114, $P$ = 0.001, $P_{FDR}$= 0.009), and CTSH levels decreased the risk of basal cell carcinoma (OR = 0.947, 95% CI = 0.919–0.975, $P$ = 0.0002, $P_{FDR}$= 0.002). In addition, there was no statistically significant effect of the 20 cancers on the nine cathepsins. Some unadjusted low P-value phenotypes are worth mentioning, including a positive correlation between cathepsin O (CTSO) and breast cancer (OR = 1.012, 95% CI = 1.001–1.025, $P$ = 0.041), cathepsin S (CTSS) and pharyngeal cancer (OR = 1.017, 95% CI = 1.001–1.034, $P$ = 0.043), and CTSS and endometrial cancer (OR = 1.055, 95% CI = 1.012–1.101, $P$ = 0.012); and there was a negative correlation between cathepsin Z and ovarian cancer (CTSZ) (OR = 0.970, 95% CI = 0.949–0.991, $P$ = 0.006), CTSS and prostate cancer (OR = 0.947, 95% CI = 0.902–0.944, $P$ = 0.028), and cathepsin E (CTSE) and pancreatic cancer (OR = 0.963, 95% CI = 0.938–0.990, $P$ = 0.006).

**Conclusion:** Our MR analyses showed a causal relationship between cathepsins and cancers and may help provide new insights for further mechanistic and clinical studies of cathepsin-mediated cancer.

KEYWORDS

**cathepsins, cancers, Mendelian randomization, causality, single nucleotide polymorphisms (SNPs)**

# 1 Introduction

Cathepsins are a class of proteases found in various animal tissues intracellular (particularly in the lysosomal fraction). They finely regulate biological processes, such as proteolysis, metabolite storage, foreign body removal, immune response, and apoptosis, through efficient, highly selective, and limited specific substrate cleavage, thereby maintaining normal body homeostasis. However, irregularities in protein hydrolysis activity or "imbalances" of insufficient protease activity or excessive protein hydrolysis or dysregulation of signaling pathways are causative factors in diseases (1), including cancer, cardiovascular diseases, inflammatory and autoimmune diseases (2). A variety of catalytically active cathepsins act as potent effectors that alter the tumour microenvironment by remodeling the extracellular matrix (ECM) (at neutral pH), as well as the activation, processing, or degradation of chemokines, cytokines, and growth factors (3, 4). They also promote tissue invasion and metastasis by releasing cell adhesion molecules (5, 6) and are part of a dynamic response to anticancer therapy in the tumour microenvironment (7–9).

Recent studies have revealed the role of several cathepsins in promoting or inhibiting various cancers (e.g., lung (10), ovarian (11), thyroid (12), and colorectal (13)), including cathepsin B (CTSB) (14), cathepsin L (CTSL) (15), cathepsin G (CTSG) (16), and cathepsin S (CTSS) (17). However, few observational studies and clinical trials have investigated the relationship between cathepsins and cancer. Previous studies reported the high CTSB expression in pancreatic ductal adenocarcinoma (PDAC) cells in serum samples from patients with PDAC (18). One study found that the serum cystatin/CTSB ratio was a prognostic indicator of survival in patients with esophageal cancer (19). CTSS levels are significantly elevated in the sera of patients with gastric, esophageal, liver, colorectal, nasopharyngeal, and lung cancers (20). Despite extensive research, no uniform or conclusive study has been conducted on the correlation between cathepsins and cancer. Therefore, there is a need for further research on the causal relationship between the different types of cathepsins and cancer risk.

Mendelian randomization (MR) uses exposure-related genetic variants as instrumental variables (IVs) to robustly assess causality between exposure and outcome (21, 22). As alleles are randomly assigned and do not change in response to disease onset, MR analyses effectively reduce the influence of confounding factors, avoid reverse causation bias, and yield more reliable causal effects than observational studies (23, 24). MR analysis is now widely used to explore causal associations between exposure factors and cancer (25, 26). In oncology, MR analysis can provide insight into the complex relationship between exposure factors and cancer development, providing a basis for prevention and treatment in clinical research (27). Therefore, this study collected data on nine cathepsins and cancers from a large-scale genome-wide association study (GWAS), performed two-sample MR, followed by inverse MR to adjust for the pleiotropic effects of genetic tools and potential confounders, and assessed potential genetic-causal associations between cathepsins and cancers to provide a basis for future prevention and treatment strategies.

# 2 Materials and methods

## 2.1 Study design

A GWAS was performed for nine cathepsins and 20 cancers from the IEU GWAS database (https://gwas.mrcieu.uk/) at the University of Bristol, UK. Cathepsin data were obtained from an INTERVAL study, which included 3,301 Europeans (28). All donors completed a trial consent form, and the INTERVAL study was approved by the US National Research Ethics Committee (11/EE/0538). Considering the effect of linkage disequilibrium (LD) among SNPs, we screened for SNPs that were independent of each other and had genome-wide significance in the strength of association with cathepsin from the pooled GWAS data of cathepsin using the following screening criteria (29): (1)$P < 5\times10^{-6}$ of the correlation effect between cathepsin and IVs; (2) the physical distance between every two genes > 10,000 kb; and (3) $R^2 < 0.001$ for LD between genes.

## 2.2 Data source

The GWAS summary statistics for a wide range of cancers were obtained from publicly available databases from the MRC IEU OpenGWAS (MR-base) database. We identified 20 cancer outcomes: bladder, lung, anal, testicular, thyroid, colorectal, ovarian, prostate, breast, esophageal, pharyngeal, endometrial, pancreatic, cecum, sialadenitis, hepatocellular, vulvar, gastric, basal cell, and bronchogenic carcinomas. The number of cases ranged from 105 to 122,188 (Supplementary Table S1).

## 2.3 Selection of IVs

We refer to the three core assumptions of association, independence, and exclusivity, which must be fulfilled in MR analyses. Single nucleotide polymorphisms (SNPs) with the genome-wide significance of association strength with cathepsins were selected as IVs. Weak IV bias was determined using the F-test statistic, and no weak IV bias was considered to exist if F > 10. The F statistic was calculated as $F = [(N-K-1)/K]\times[R^2/(1-R^2)]$, where N is the sample size, K is the number of IVs, and $R^2$ denotes the variance of the exposure explained by each IV alone (30). A flowchart of the study is shown in Figure 1.

## 2.4 Statistical analysis

Determination of the causal relationship between cathepsins and cancer risk was carried out in two-sample Mendelian randomization using five methods: Inverse variance weighting (IVW) (31), MR-Egger (32), Weighted Median (33), Simple Mode (34), and Weighted Mode (35). Odds ratios (OR) and 95% confidence intervals (CI) were used to determine whether a causal relationship existed between cathepsins and cancer risk. According to previous studies, the IVW method is superior to other tests (36, 37), and is used as the main MR analysis

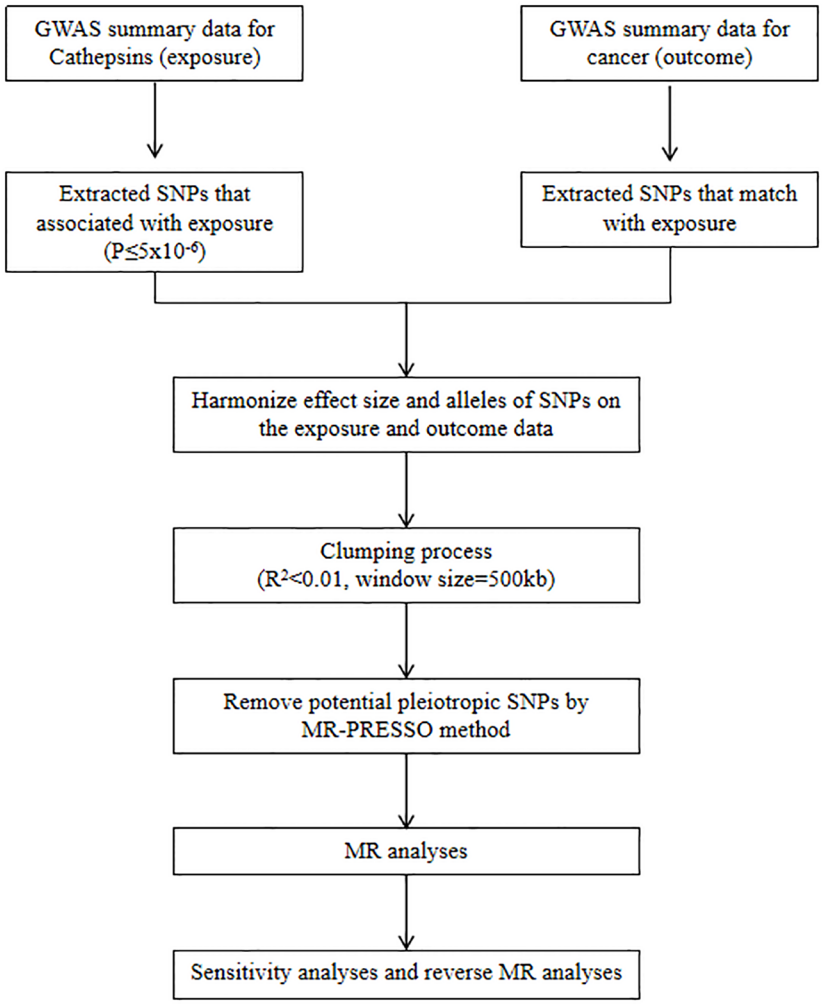



FIGURE 1
Study design and workflow.

method (38–40). Among these, the IVW was used as the primary method of analysis. Because of the multiple exposures and outcomes in this study, multiple test corrections were performed using the FDR method (41), and it was necessary to report whether the *P*-values tested by the IVW method reached nominal significance ($P < 0.05$) and statistical significance ($P_{FDR} < 0.05$). The MR-Egger intercept was used to assess the relationship between IVs and other potential confounders and to ensure that the selected IVs did not influence the outcome variables through pathways other than exposure factors. Horizontal pleiotropy (27) is indicated if the MR-Egger intercept analysis shows a statistically significant relationship ($P < 0.05$). At $P < 0.05$, an outlier test was used to eliminate horizontal pleiotropy using the MR-PRESSO global test (42). An OR less than 1 indicates that exposure plays a protective role in predicting the occurrence of an outcome event. In other words, exposure played a positive role in preventing or reducing the occurrence of outcome events. Conversely, if the OR is greater than 1, the exposure is categorized as a risk factor for the outcome, and exposure can promote the occurrence of the outcome. Cochran's Q statistic was used to perform the heterogeneity test. Statistically significant ($P < 0.05$) Cochran's Q test proves that the analyses were significantly heterogeneous (43).

We performed a reverse MR analysis (20 cancers as exposures and cathepsins as outcomes) to explore whether cancer has a causal effect on cathepsins identified in the forward MR analysis. The analysis procedure was consistent with that of the forward MR analysis.

MR analyses were performed using "TwoSampleMR" (version 0.5.6) in R (version 4.2.3), Mendelian Randomization (0.7.0), and TwoSample MR (0.5.6). $P < 0.05$ indicates that the results are statistically significant.

# 3 Results

## 3.1 IVs selection

Based on the screening criteria, nine IVs for cathepsin were included in this study. The F-statistic for each IV was > 10, indicating low evidence of weak IV bias (Supplementary Data 1).

## 3.2 MR main analysis results

The IVW approach revealed significant evidence of a causal relationship between cathepsins and different cancer risks. Our pooled analysis identified nine cathepsins that exhibited potential causal associations with 20 cancers (Figure 2). Of the 180 associations included (9 exposures x 20 outcomes), six were statistically significant in the IVW analysis (Figure 2). Cathepsin H (CTSE) levels reduced the risk of vulvar carcinoma (OR = 0.483, 95% CI = 0.241–0.966, $P$ = 0.039), and cathepsin H (CTSH) levels reduced basal cell carcinoma risk (OR = 0.947, 95% CI = 0.919–0.975, $P$ = 0.0002); CTSF levels increased the risk of vulvar carcinoma (OR = 1.736, 95% CI = 1.026–2.937, $P$ = 0.040), CTSS levels increased the risk of colorectal cancer (OR = 1.051, 95% CI = 1.008–1.097, $P$ = 0.02), CTSZ levels increased the risk of thyroid cancer (OR = 1.157, 95% CI = 1.017–1.317, $P$ = 0.026), CTSH levels increased the risk of lung cancer (OR = 1.070, 95% CI = 1.027–1.114, $P$ = 0.001).

Two associations were based on the number of exposure-outcome pairs showing FDR-corrected significance ($P$ < 0.05). CTSH levels increased the risk of lung cancer (OR = 1.070, 95% CI = 1.027–1.114, $P$ = 0.001, $P_{FDR}$ = 0.009), and CTSH levels decreased the risk of basal cell carcinoma (OR = 0.947, 95% CI = 0.919–0.975, $P$ = 0.0002, $P_{FDR}$ = 0.002). These two associations had 10 and 8 IVs, respectively, and the robustness of these causal relationships was further supported by combined data from multiple sensitivity analyses (Supplementary Data 2). Specifically, our analyses by Cochran's Q did not reveal any signs of heterogeneity ($P$ = 0.729 > 0.05, $P$ = 0.065 > 0.05). The MR-Egger intercept assessment did not provide evidence of horizontal pleiotropy ($P$ = 0.236 > 0.05, $P$ = 0.969 > 0.05).

## 3.3 Reverse MR analysis results

We used cancer as the exposure factor, cathepsins as the outcome, and cancer-associated SNPs ($P < 5 \times 10^{-5}$) as the IVs to explore whether there was reverse causality for the significant results obtained. Figure 3 shows the six cathepsin immunophenotypes potentially affected by cancer. After reverse analysis, six were statistically significant: a positive correlation between CTSO and breast cancer (OR = 1.012, 95% CI = 1.001–1.025, $P$ = 0.041), CTSS (OR = 1.017, 95% CI = 1.001–1.034, $P$ = 0.043) and pharyngeal cancer, and CTSS (OR = 1.055, 95% CI = 1.012–1.101, $P$ = 0.012) and endometrial cancer; There was a negative correlation between CTSZ and ovarian cancer (OR = 0.970, 95% CI = 0.949–0.991, $P$ = 0.006), CTSS and prostate cancer (OR = 0.947, 95% CI = 0.902–0.944, $P$ = 0.028), CTSE and pancreatic cancer (OR = 0.963, 95% CI = 0.938–0.990, $P$ = 0.006). The Cochrane Q-test provided no evidence of heterogeneity ($P$ = 0.388 > 0.05, $P$ = 0.837 > 0.05; $P$ = 0.909 > 0.05, $P$ = 0.221 > 0.05, $P$ = 0.667 > 0.05, $P$ = 0.667 > 0.05, $P$ = 0.832 > 0.05). SNP pleiotropy was not detected for the MR-Egger test intercept ($P$ = 0.872 > 0.05, $P$ = 0.393 > 0.05, $P$ = 0.695 > 0.05; $P$ = 0.200 > 0.05, $P$ = 0.558 > 0.05, $P$ =

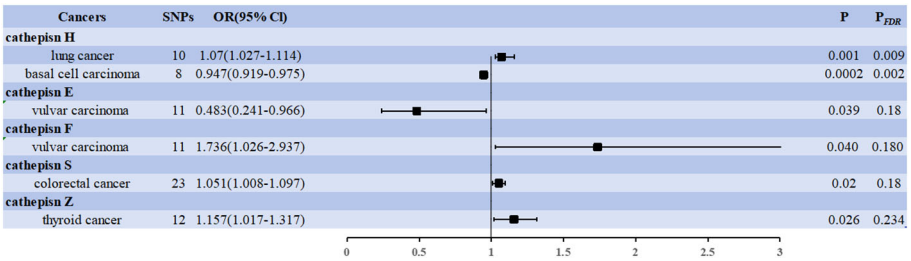



FIGURE 2
Forest plots showed the causal associations between cathepsins and cancers. IVW, inverse variance weighting; CI, confidence interval; FDR, false discovery rate.

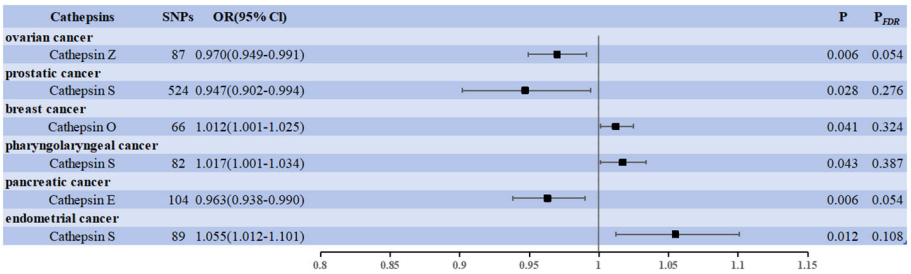



FIGURE 3
Forest plots showed the causal associations between cancers and cathepsins. IVW, inverse variance weighting; CI, confidence interval; FDR, false discovery rate.

0.290 > 0.05). These associations, based on the number of exposure-outcome pairs, did not show FDR-corrected significance ($P > 0.05$) (Figure 3). The results of the heterogeneity and pleiotropy tests are presented in Supplementary Data 3.

# 4 Discussion

This study investigated the causal association between cathepsin levels and cancer. The causal effects of nine cathepsins on 20 cancers were comprehensively evaluated by MR analysis. The results showed a causal association between certain cathepsins and cancers, suggesting that cathepsins may have an essential influence on cancer and play an important role in cancer development.

In recent decades, the incidence of various types of cancer has increased; cancer has become a significant public health problem worldwide. It is the second leading cause of death in humans, after cardiovascular diseases (44). CTSH acts as an aminopeptidase and endopeptidase with endo protein hydrolytic activity and can hydrolyze a wide range of proteins (45). CTSH has been detected in type II pneumocytes and alveolar macrophages in the lung (46, 47). It is located in lamellipodia, dense multivesicular vesicles, and type II complex vesicle pneumocytes, which constitute sites of surfactant maturation (48, 49). Microarray analysis studies have shown that CTSH expression is lower in non-small cell lung cancer than in normal lung tissue (50) and that CTSH is involved in SP-B maturation by cleaving the peptide bond between pro-SP-B residues 279 and 280 (51, 52). Some studies have also found that silencing of CTSH significantly reduces SP-B maturation and subsequently reduces SP-B secretion (53). CTSH progression in lung cancer may regulate the sPLA2-PKCδ-MAPKs-cPLA2α pathway by modulating SP-B maturation, thereby regulating lipid metabolism in the lungs (54, 55). CTSH is highly expressed in small cells and in adenocarcinomas (56, 57). Luyapan et al. (58) conducted a transcriptome-wide association study using expression weights from a quantitative trait locus study of lung expression and found that the gene most strongly associated with lung cancer was CTSH.

The epidermis of the skin constantly undergoes cell renewal and differentiation to maintain its normal structure and function. However, when the balance between renewal and differentiation is disrupted, uncontrolled cell proliferation and cancer can result (59). Basal cell carcinoma, the most common form of skin cancer, originates in the basal layer of the epidermis and appendages. The tumor grows slowly, rarely metastasizes, and generally infiltrates the surrounding tissues slowly (60). The interplay between various environmental, genetic, phenotypic, and genetic risk factors contributes to the development of basal cell carcinomas. Cathepsin is an essential protease required for invasion. It has been found that CTSH is mainly localized in the lowermost basal cell layer (61). Basal cells are undifferentiated and can grow and divide. CTSH is a lysosomal cysteine protease involved in the degradation of extracellular matrix components and has been found to be more active in basal cell carcinoma tumors than in normal skin tissue (62). The mechanism underlying the involvement of CTSH in the development of basal cell carcinoma has not yet been investigated. However, CTSH activity is dysregulated in tissues surrounding basal cell carcinoma tumors, leading to its overexpression and secretion into the extracellular space to degrade structural proteins such as collagen and fibronectin (8, 63–67), thereby regulating the structure and stability of the extracellular matrix and promoting tumor cell invasion (68, 69).

It is also worth noting that breast cancer was associated with elevated CTSO, pharyngeal and endometrial cancers with elevated CTSS, ovarian cancer with decreased CTSZ, prostate cancer with decreased CTSS, and pancreatic cancer with decreased CTSE. CTSO was found to be significantly overexpressed in T47D, CAMA-1, and ZR75–1 cells, reducing BRCA1 levels and promoting cell proliferation by promoting the cysteine protease-mediated degradation of metadherin, polyadenylate-binding protein 4-like, recombinant lamin A/C, and recombinant eukaryotic translation elongation factor 1 alpha 1 protein levels (70–72). However, CTSS and CTSE are overexpressed in prostate cancer (73) and pancreatic cancer (74), respectively; this is contrary to the results of the present study and needs to be verified by more clinical and experimental studies in the future.

Previous studies did not comprehensively analyze the causal relationship between cathepsins and cancer. This study used two samples of MR studies and obtained reliable results: firstly, MR analysis has the advantage of avoiding reverse causal associations and confounders and saving time and resources compared to observational studies; secondly, according to our analysis, multiple cathepsins are risk and protective factors for cancers, and this study did not reveal potential horizontal pleiotropy, thus confirming the reliability of the conclusions. However, there are some limitations to this study. First, this study only observed a causal effect of cathepsins on the risk of multiple cancers at the gene level. Future MR studies with larger sample sizes and randomized controlled trials are required to validate these results. Second, the study was limited to the European population, and it is not possible to demonstrate whether the findings can be extended to other populations. Furthermore, as with all published MR studies, the possibility that unobserved pleiotropy affects the results cannot be ruled out, even if measures are taken to identify and eliminate aberrant variants (21); the study was unable to infer a non-linear correlation between cathepsins and cancers. Lastly, the cathepsins and cancer GWAS data were obtained from publicly available databases, and subgroup analyses were not possible due to the lack of detailed clinical patient information. In summary, the results of this study, using two-sample and inverse MR methods, suggest a causal relationship between cathepsins and various cancers. The results of this study should be interpreted with caution. More investigative studies should be conducted to validate the results and consider their application in clinical trials.

# 5 Conclusion

In conclusion, these results suggest a potential causal relationship between cathepsins and cancer. These findings

provide new insights for further mechanistic studies on cathepsin-mediated cancers, potential targets, and new biomarkers for the early diagnosis and interventional therapy of cancers.

## 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 authors.

## Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

## Author contributions

TD: Conceptualization, Methodology, Writing – original draft. XL: Visualization, Writing – original draft. XJ: Data curation, Project administration, Writing – original draft. JD: Data curation, Project administration, Writing – original draft. LW: Software, Writing – original draft. BC: Formal Analysis, Investigation, Writing – original draft. MY: Funding acquisition, Writing – review & editing. YY: Resources, Supervision, Writing – review & editing. FL: Writing – review & editing.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was financially supported by the National Natural Science Foundation of China (No. 82004212), the State Administration of Traditional Chinese Medicine Science and technology department co-build traditional Chinese medicine science and technology project (No.GZY-KJS-SD-2023-084), the Shandong Province Medical Health Science and Technology Development Plan Project (No. 202204070951), the TCM Science and Technology Project of Shandong Province (No. 2021M175), and the Shandong Traditional Chinese medicine classic famous collaborative innovation center open subject (No.2019KFY07).

## Acknowledgments

We would like to express our gratitude to all the researchers and contributors to the GWASs. We would also like to express our gratitude to all the GWASs for opening up their summary data to the public.

## Conflict of interest

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

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1428433/full#supplementary-material

## References

1. Ruiz-Blázquez P, Pistorio V, Fernández-Fernández M, et al. The multifaceted role of cathepsins in liver disease. *J Hepatol.* (2021) 75:1192–202. doi: 10.1016/j.jhep.2021.06.031

2. Smyth P, Sasiwachirangkul J, Williams R, Cathepsin S. (CTSS) activity in health and disease - A treasure trove of untapped clinical potential. *Mol Aspects Med.* (2022) 88:101106. doi: 10.1016/j.mam.2022.101106

3. Soond SM, Kozhevnikova MV, Zamyatnin AA Jr. 'Patchiness' and basic cancer research: unravelling the proteases. *Cell Cycle.* (2019) 18:1687–701. doi: 10.1080/15384101.2019.1632639

4. Gocheva V, Wang HW, Gadea BB, et al. IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. *Genes Dev.* (2010) 24:241–55. doi: 10.1101/gad.1874010

5. Sevenich L, Bowman RL, Mason SD, et al. Analysis of tumour- and stroma-supplied proteolytic networks reveals a brain-metastasis-promoting role for cathepsin S. *Nat Cell Biol.* (2014) 16:876–88. doi: 10.1038/ncb3011

6. Joyce JA, Baruch A, Chehade K, et al. Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis. *Cancer Cell.* (2004) 5:443–53. doi: 10.1016/S1535-6108(04)00111-4

7. Verbovšek U, Van Noorden CJ, Lah TT. Complexity of cancer protease biology: Cathepsin K expression and function in cancer progression. *Semin Cancer Biol.* (2015) 35:71–84. doi: 10.1016/j.semcancer.2015.08.010

8. Palermo C, Joyce JA. Cysteine cathepsin proteases as pharmacological targets in cancer. *Trends Pharmacol Sci.* (2008) 29:22–8. doi: 10.1016/j.tips.2007.10.011

9. Soond SM, Kozhevnikova MV, Frolova AS, et al. Lost or Forgotten: The nuclear cathepsin protein isoforms in cancer. *Cancer Lett.* (2019) 462:43–50. doi: 10.1016/j.canlet.2019.07.020

10. Xiao Y, Cong M, Li J, et al. Cathepsin C promotes breast cancer lung metastasis by modulating neutrophil infiltration and neutrophil extracellular trap formation. *Cancer Cell.* (2021) 39:423–437.e7. doi: 10.1016/j.ccell.2020.12.012

11. Pranjol MZ, Gutowski N, Hannemann M, Whatmore J. The potential role of the proteases cathepsin D and cathepsin L in the progression and metastasis of epithelial ovarian cancer. *Biomolecules*. (2015) 5:3260–79. doi: 10.3390/biom5043260

12. Kim EK, Song MJ, Jang HH, Chung YS. Clinicopathologic analysis of cathepsin B as a prognostic marker of thyroid cancer. *Int J Mol Sci*. (2020) 21:9537. doi: 10.3390/ijms21249537

13. Wang Z, Chen K, Zhang K, He K, Zhang D, Guo X, et al. Agrocybe cylindracea fucoglucogalactan induced lysosome-mediated apoptosis of colorectal cancer cell through H3K27ac-regulated cathepsin D. *Carbohydr Polym*. (2023) 319:121208. doi: 10.1016/j.carbpol.2023.121208

14. Xu LB, Qin YF, Su L, Huang C, Xu Q, Zhang R, et al. Cathepsin-facilitated invasion of BMI1-high hepatocellular carcinoma cells drives bile duct tumor thrombi formation. *Nat Commun*. (2023) 14:7033. doi: 10.1038/s41467-023-42930-y

15. Liu P, Ju M, Zheng X, Jiang Y, Yu X, Pan B, et al. Methyltransferase-like 3 promotes cervical cancer metastasis by enhancing cathepsin L mRNA stability in an N6-methyladenosine-dependent manner. *Cancer Sci*. (2023) 114:837–54. doi: 10.1111/cas.15658

16. Tanigawa K, Kiriya M, Hayashi Y, Shinden Y, Kijima Y, Natsugoe S, et al. Cathepsin G-induced Malignant progression of MCF-7 cells involves suppression of PAF signaling through induced expression of PAFAH1B2. *Biochim Biophys Acta Mol Cell Biol Lipids*. (2022) 1867:159164. doi: 10.1016/j.bbalip.2022.159164

17. Small DM, Burden RE, Jaworski J, Hegarty SM, Spence S, Burrows JF, et al. Cathepsin S from both tumor and tumor-associated cells promote cancer growth and neovascularization. *Int J Cancer*. (2013) 133:2102–12. doi: 10.1002/ijc.28238

18. Jiang Y, Han L, Xue M, Wang T, Zhu Y, Xiong C, et al. Cystatin B increases autophagic flux by sustaining proteolytic activity of cathepsin B and fuels glycolysis in pancreatic cancer: CSTB orchestrates autophagy and glycolysis in PDAC. *Clin Transl Med*. (2022) 12:e1126. doi: 10.1002/ctm2.1126

19. Yan Y, Zhou K, Wang L, Wang F, Chen X, Fan Q. Clinical significance of serum cathepsin B and cystatin C levels and their ratio in the prognosis of patients with esophageal cancer. *Onco Targets Ther*. (2017) 10:1947–54. doi: 10.2147/OTT

20. Liu WL, Liu D, Cheng K, Liu YJ, Xing S, Chi PD, et al. Evaluating the diagnostic and prognostic value of circulating cathepsin S in gastric cancer. *Oncotarget*. (2016) 7:28124–38. doi: 10.18632/oncotarget.v7i19

21. Birney E. Mendelian randomization. *Cold Spring Harb Perspect Med*. (2022) 12: a041302. doi: 10.1101/cshperspect.a041302

22. Sekula P, Del Greco MF, Pattaro C, Köttgen A. Mendelian randomization as an approach to assess causality using observational data. *J Am Soc Nephrol*. (2016) 27:3253–65. doi: 10.1681/ASN.2016010098

23. Carter AR, Sanderson E, Hammerton G, Richmond RC, Davey Smith G, Heron , et al. Mendelian randomisation for mediation analysis: current methods and challenges for implementation. *Eur J Epidemiol*. (2021) 36:465–478. doi: 10.1007/s10654-021-00757-1

24. Pang Y, Kartsonaki C, Lv J, Fairhurst-Hunter Z, Millwood IY, Yu C, et al. Associations of Adiposity, Circulating Protein Biomarkers, and Risk of Major Vascular Diseases [published correction appears in JAMA Cardiol. *2021 Feb 1;6(2):246]. JAMA Cardiol*. (2021) 6:276–86. doi: 10.1001/jamacardio.2020.6041

25. Yu N, Qi H, Guo Y, Wu L, Su J, Huang K, et al. Associations between rheumatoid arthritis and skin cancer: A bidirectional two-sample Mendelian randomization study. *J Am Acad Dermatol*. (2024) 90:198–200. doi: 10.1016/j.jaad.2023.09.046

26. Chen Y, Xie Y, Ci H, Cheng Z, Kuang Y, Li S, et al. Plasma metabolites and risk of seven cancers: a two-sample Mendelian randomization study among European descendants. *BMC Med*. (2024) 22:90. doi: 10.1186/s12916-024-03272-8

27. Gala H, Tomlinson I. The use of Mendelian randomisation to identify causal cancer risk factors: promise and limitations. *J Pathol*. (2020) 250:541–54. doi: 10.1002/path.5421

28. Sun BB, Maranville JC, Peters JE, Stacey D, Staley JR, Blackshaw J, et al. Genomic atlas of the human plasma proteome. *Nature*. (2018) 558:73–9. doi: 10.1038/s41586-018-0175-2

29. Noyce AJ, Kia DA, Hemani G, Nicolas A, Price TR, De Pablo-Fernandez E, et al. Estimating the causal influence of body mass index on risk of Parkinson disease: A Mendelian randomisation study. *PloS Med*. (2017) 14:e1002314. doi: 10.1371/journal.pmed.1002314

30. Levin MG, Judy R, Gill D, Vujkovic M, Verma SS, Bradford Y, et al. Genetics of height and risk of atrial fibrillation: A Mendelian randomization study. *PloS Med*. (2020) 17:e1003288. doi: 10.1371/journal.pmed.1003288

31. Mounier N, Kutalik Z. Bias correction for inverse variance weighting Mendelian randomization. *Genet Epidemiol*. (2023) 47:314–31. doi: 10.1002/gepi.22522

32. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger. *Eur J Epidemiol*. (2017) 32:377–89. doi: 10.1007/s10654-017-0255-x

33. Li P, Wang H, Guo L, Gou X, Chen G, Lin D, et al. Association between gut microbiota and preeclampsia-eclampsia: a two-sample Mendelian randomization study. *BMC Med*. (2022) 20:443. doi: 10.1186/s12916-022-02657-x

34. Huang D, Lin S, He J, Wang Q, Zhan Y. Association between COVID-19 and telomere length: A bidirectional Mendelian randomization study. *J Med Virol*. (2022) 94:5345–53. doi: 10.1002/jmv.28008

35. Wu F, Huang Y, Hu J, Shao Z. Mendelian randomization study of inflammatory bowel disease and bone mineral density. *BMC Med*. (2020) 18:312. doi: 10.1186/s12916-020-01778-5

36. Lin Z, Deng Y, Pan W. Combining the strengths of inverse-variance weighting and Egger regression in Mendelian randomization using a mixture of regressions model. *PloS Genet*. (2021) 17:e1009922. doi: 10.1371/journal.pgen.1009922

37. Bowden J, Davey Smith G, Haycock PC, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. (2016) 40:304–14. doi: 10.1002/gepi.21965

38. Cui K, Song N, Fan Y, Zeng L, Shi P, Wang Z, et al. A two-sample Mendelian randomization analysis: causal association between chemokines and pan-carcinoma. *Front Genet*. (2023) 14:1285274. doi: 10.3389/fgene.2023.1285274

39. Tang Y, Zhang L, Ye D, Zhao A, Liu Y, Zhang M, et al. Causal relationship between Type 1 diabetes and osteoporosis and fracture occurrence: a two-sample Mendelian randomization analysis. *Osteoporos Int*. (2023) 34:1111–7. doi: 10.1007/s00198-023-06734-6

40. Yang H, Shi P, Li M, Kong L, Liu S, Jiang L, et al. Mendelian-randomization study reveals causal relationships between nitrogen dioxide and gut microbiota. *Ecotoxicol Environ Saf*. (2023) 267:115660. doi: 10.1016/j.ecoenv.2023.115660

41. Glickman ME, Rao SR, Schultz MR. False discovery rate control is a recommended alternative to Bonferroni-type adjustments in health studies. *J Clin Epidemiol*. (2014) 67:850–7. doi: 10.1016/j.jclinepi.2014.03.012

42. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases [published correction appears. *Nat Genet*. (2018) 50:693–8. doi: 10.1038/s41588-018-0099-7

43. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan N, Thompson J, et al. A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization. *Stat Med*. (2017) 36:1783–802. doi: 10.1002/sim.7221

44. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. (2023) 73:17–48. doi: 10.3322/caac.21763

45. Wang Y, Zhao J, Gu Y, Wang H, Jiang M, Zhao S, et al. Cathepsin H: Molecular characteristics and clues to function and mechanism. *Biochem Pharmacol*. (2023) 212:115585. doi: 10.1016/j.bcp.2023.115585

46. Ishii Y, Hashizume Y, Kominami E, Uchiyama Y. Changes in immunoreactivity for cathepsin H in rat type II alveolar epithelial cells and its proteolytic activity in bronchoalveolar lavage fluid over 24 hours. *Anat Rec*. (1991) 230:519–23. doi: 10.1002/ar.1092300411

47. Brasch F, Ten Brinke A, Johnen G, Ochs M, Kapp N, Müller KM, et al. Involvement of cathepsin H in the processing of the hydrophobic surfactant-associated protein C in type II pneumocytes. *Am J Respir Cell Mol Biol*. (2002) 26:659–70. doi: 10.1165/ajrcmb.26.6.4744

48. Ishii Y, Hashizume Y, Watanabe T, Waguri S, Sato N, Yamamoto M, et al. Cysteine proteinases in bronchoalveolar epithelial cells and lavage fluid of rat lung. *J Histochem Cytochem*. (1991) 39:461–8. doi: 10.1177/39.4.2005374

49. Woischnik M, Bauer A, Aboutaam R, Pamir A, Stanzel F, de Blic J, et al. Cathepsin H and napsin A are active in the alveoli and increased in alveolar proteinosis. *Eur Respir J*. (2008) 31:1197–204. doi: 10.1183/09031936.00081207

50. Ueno T, Linder S, Na CL, Rice WR, Johansson J, Weaver TE, et al. Processing of pulmonary surfactant protein B by napsin and cathepsin H. *J Biol Chem*. (2004) 279:16178–84. doi: 10.1074/jbc.M312029200

51. Guttentag S, Robinson L, Zhang P, Brasch F, Bühling F, Beers M, et al. Cysteine protease activity is required for surfactant protein B processing and lamellar body genesis. *Am J Respir Cell Mol Biol*. (2003) 28:69–79. doi: 10.1165/rcmb.2002-0111OC

52. Bühling F, Kouadio M, Chwieralski CE, Kern U, Hohlfeld JM, Klemm N , et al. Gene targeting of the cysteine peptidase cathepsin H impairs lung surfactant in mice. *PloS One*. (2011) 6:e26247. doi: 10.1371/journal.pone.0026247

53. Lee S, Kim D, Kang J, Kim E, Kim W, Youn H, et al. Surfactant protein B suppresses lung cancer progression by inhibiting secretory phospholipase A2 activity and arachidonic acid production. *Cell Physiol Biochem*. (2017) 42:1684–700. doi: 10.1159/000479418

54. Han WK, Sapirstein A, Hung CC, Alessandrini A, Bonventre JV. Cross-talk between cytosolic phospholipase A2 alpha (cPLA2 alpha) and secretory phospholipase A2 (sPLA2) in hydrogen peroxide-induced arachidonic acid release in murine mesangial cells: sPLA2 regulates cPLA2 alpha activity that is responsible for arachidonic acid release. *J Biol Chem*. (2003) 278:24153–63. doi: 10.1074/jbc.M300424200

55. Hite RD, Grier BL, Waite BM, Veldhuizen RA, Possmayer F, Yao LJ, et al. Surfactant protein B inhibits secretory phospholipase A2 hydrolysis of surfactant phospholipids. *Am J Physiol Lung Cell Mol Physiol*. (2012) 302:L257–65. doi: 10.1152/ajplung.00054.2011

56. Schweiger A, Staib A, Werle B, Krasovec M, Lah TT, Ebert W, et al. Cysteine proteinase cathepsin H in tumours and sera of lung cancer patients: relation to prognosis and cigarette smoking. *Br J Cancer*. (2000) 82:782–8. doi: 10.1054/bjoc.1999.0999

57. Linnerth NM, Sirbovan K, Moorehead RA. Use of a transgenic mouse model to identify markers of human lung tumors. *Int J Cancer*. (2005) 114:977–82. doi: 10.1002/ijc.20814

58. Luyapan J, Bossé Y, Li Z, Xiao X, Rosenberger A, Hung RJ. Candidate pathway analysis of surfactant proteins identifies CTSH and SFTA2 that influences lung cancer risk. *Hum Mol Genet*. (2023) 32:2842–55. doi: 10.1093/hmg/ddad095

59. Hsu YC, Fuchs E. Building and maintaining the skin. *Cold Spring Harb Perspect Biol*. (2022) 14:a040840. doi: 10.1101/cshperspect.a040840

60. Heath MS, Bar A. Basal cell carcinoma. *Dermatol Clin*. (2023) 41:13–21. doi: 10.1016/j.det.2022.07.005

61. Rinne A, Kirschke H, Järvinen M, Hopsu-Havu VK, Wieranders B, Bohley P. Localization of cathepsin H and its inhibitor in the skin and other stratified epithelia. *Arch Dermatol Res*. (1985) 277:190–4. doi: 10.1007/BF00404315

62. Fröhlich E, Möhrle M, Klessen C. Cathepsins in basal cell carcinomas: activity, immunoreactivity and mRNA staining of cathepsins B, D, H and L [published correction appears. *Arch Dermatol Res*. (2004) 295:411–21. doi: 10.1007/s00403-003-0449-9

63. Villalobo E, Moch C, Fryd-Versavel G, Fleury-Aubusson A, Morin L, et al. Cysteine proteases and cell differentiation: excystment of the ciliated protist Sterkiella histriomuscorum. *Eukaryot Cell*. (2003) 2:1234–45. doi: 10.1128/EC.2.6.1234-1245.2003

64. Jokimaa V, Oksjoki S, Kujari H, Anttila L. Expression patterns of cathepsins B, H, K, L and S in the human endometrium. *Mol Hum Reprod*. (2001) 7:73–8. doi: 10.1093/molehr/7.1.73

65. Sevenich L, Joyce JA. Pericellular proteolysis in cancer. *Genes Dev*. (2014) 28:2331–47. doi: 10.1101/gad.250647.114

66. Gocheva V, Joyce JA. Cysteine cathepsins and the cutting edge of cancer invasion. *Cell Cycle*. (2007) 6:60–4. doi: 10.4161/cc.6.1.3669

67. Tsushima H, Ueki A, Matsuoka Y, Mihara H, Hopsu-Havu VK. Characterization of a cathepsin-H-like enzyme from a human melanoma cell line. *Int J Cancer*. (1991) 48:726–32. doi: 10.1002/ijc.2910480516

68. Pickup MW, Mouw JK, Weaver VM. The extracellular matrix modulates the hallmarks of cancer. *EMBO Rep*. (2014) 15:1243–53. doi: 10.15252/embr.201439246

69. Wu SM, Huang YH, Yeh CT, Tsai M-M, Liao C-H, Cheng W-L, et al. Cathepsin H regulated by the thyroid hormone receptors associate with tumor invasion in human hepatoma cells. *Oncogene*. (2011) 30:2057–69. doi: 10.1038/onc.2010.585

70. Cairns J, Ingle JN, Wickerham LD, Weinshilboum R, Liu M, Wang L, et al. SNPs near the cysteine proteinase cathepsin O gene (CTSO) determine tamoxifen sensitivity in ERα-positive breast cancer through regulation of BRCA1. *PloS Genet*. (2017) 13: e1007031. doi: 10.1371/journal.pgen.1007031

71. Somasundaram K, Zhang H, Zeng YX, Houvras Y, Peng Y, Zhang H, et al. Arrest of the cell cycle by the tumour-suppressor BRCA1 requires the CDK-inhibitor p21WAF1/CiP1. *Nature*. (1997) 389:187–90. doi: 10.1038/38291

72. Ouchi T, Monteiro AN, August A, Aaronson SA, Hanafusa H. BRCA1 regulates p53-dependent gene expression. *Proc Natl Acad Sci U.S.A*. (1998) 95:2302–6. doi: 10.1073/pnas.95.5.2302

73. Fernández PL, Farré X, Nadal A, Fernández E, Peiró N, Sloane BF, et al. Expression of cathepsins B and S in the progression of prostate carcinoma. *Int J Cancer*. (2001) 95:51–5. doi: 10.1002/(ISSN)1097-0215

74. Cruz-Monserrate Z, Abd-Elgaliel WR, Grote T, Deng D, Ji B, Arumugam T, et al. Detection of pancreatic cancer tumours and precursor lesions by cathepsin E activity in mouse models. *Gut*. (2012) 61:1315–22. doi: 10.1136/gutjnl-2011-300544

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 18 June 2024
DOI 10.3389/fonc.2024.1378563



OPEN ACCESS

EDITED BY
Zili Zhang,
Faculty of Medicine, Dokuz Eylül University Izmir, Türkiye

REVIEWED BY
Fiona Huanhuan Zhou,
Nanjing University of Chinese Medicine Nanjing, China
Kathleen Degen,
Garvan Institute of Medical Research Darlinghurst, Australia

*CORRESPONDENCE
Merve Keskinkilic
mervekeskinkilic90@gmail.com

RECEIVED 29 January 2024
ACCEPTED 03 June 2024
PUBLISHED 18 June 2024

CITATION
Keskinkilic M, Semiz HS, Yavuzsen T and Oztop I (2024) Is the percentage of hormone receptor positivity in HR+ HER2-metastatic breast cancer patients receiving CDK 4/6 inhibitor with endocrine therapy predictive and prognostic?.
*Front. Oncol.* 14:1378563.
doi: 10.3389/fonc.2024.1378563



# Is the percentage of hormone receptor positivity in HR+ HER2-metastatic breast cancer patients receiving CDK 4/6 inhibitor with endocrine therapy predictive and prognostic?

Merve Keskinkilic [1,2*], Huseyin Salih Semiz [3], Tugba Yavuzsen [3] and Ilhan Oztop [3]

[1]Department of Hematology and Medical Oncology, Emory Winship Cancer Institute, Atlanta, GA, United States, [2]Department of Medical Oncology, Dokuz Eylul University Faculty of Medicine, Izmir, Türkiye, [3]Department of Medical Oncology, Institute of Oncology, Dokuz Eylul University, Izmir, Türkiye

**Purpose:** There is no clear information in the literature about the relationship between the efficacy of CDK 4/6i combined with ET and HR positivity. However, we know that the longest overall survival was in the ER-strong positive/PR intermediate or strong positive groups. Therefore, we aimed to investigate CDK4/6i treatments that create positivity in HR.

**Methods:** Patients with the diagnosis of HR+/HER2- MBC who were treated with CDK 4/6i and HR >10% were retrospectively evaluated. To analyze the role of HR positivity, ER was moderately positive (10-49%) and ER was strongly positive (50-100%); PR was grouped as moderately positive (10-49%) and PR strongly positive (50-100%).

**Results:** Median follow-up of 150 patients included in the study was 15.2 months (95% CI, 2.1-40.9 months). The highest response in the whole group was obtained in the ER-strong positive/PR moderate or strong positive group, and the ER moderate positive/PR moderate or strong group. This was followed by the ER strong positive/PR negative group, and then the ER moderate positive/PR negative group. Although these advantages were not statistically significant, they were numerically higher (ORR: 83.8% vs. 83.3% vs. 77.4% vs. 62.5%, p=0.488, respectively). The highest survival in the whole group was achieved in the ER strong positive/PR moderate or strongly positive group, followed by the ER moderately positive/PR moderate or strongly positive group, the ER strongly positive/PR negative group followed by the ER moderate positive/PR negative group, respectively(p=0.410). However, these advantages were not statistically significant.

**Conclusion:** As a result, HR+/HER2- MBC patients receiving CDK 4/6i combined with ET suggest that the percentage of HR positivity may have a predictive and prognostic role.

KEYWORDS

CDK 4/6 inhibitor, hormone receptor positivity, metastatic breast cancer, palbociclib, ribociclib

# Introduction

Breast cancer is the most common type of cancer in women and is an important public health problem worldwide (1). Although significant improvements in survival have been achieved in recent years thanks to the advances in systemic treatments, metastatic disease still remains a serious problem affecting prognosis. The main treatment options for metastatic disease have historically been chemotherapy, targeted therapies, and hormone therapy, but in recent years cyclin-dependent kinase 4/6 inhibitor (CDK 4/6i) and immunotherapy have been added to these (2, 3). Breast cancer is classified into three main subgroups according to hormone receptor (HR) (estrogen receptor (ER) and progesterone receptor (PR)) status and human epidermal growth factor 2 (HER2) status: HR+ group, HER2+ group and triple negative group (4). The HR+/HER2- group constitutes approximately two-thirds of patients with metastatic breast cancer (MBC) (5). In this group, endocrine therapy (ET) constitutes the main framework of treatment (6). Aromatase inhibitors (AI) (Anastrozole, Letrozole, Exemestan, etc.), selective estrogen modulators (Tamoxifen), and selective estrogen degraders (Fulvestrant) are widely used as ET (7–9). However, intrinsic or acquired resistance is also encountered in endocrine treatments (10). In order to overcome this problem, combinations with targeted agents, especially CDK 4/6i, have been sought in recent years.

By inactivating the CDK-D-cyclins (CCND) complex, CDK 4/6i increase retinoblastoma protein (pRb), which negatively affects the E2F transcriptional factor, and ultimately induces tumor cell apoptosis by inhibiting cell cycle progression (11). In the vast majority of phase III trials combining CDK 4/6i and ET (AI (Anastrozole, Letrozole) and selective estrogen degraders (Fulvestrant)), progression free survival (PFS) and overall survival (OS) have been significantly improved in the front line as well as in subsequent lines of therapy. Thus, CDK 4/6i have become the main treatment model in the HR+ HER2- patient group with their unique mechanism of action, consistent survival advantages in phase III studies, and different toxicity characteristics (12–14).

It is known that the estrogen signal, which has a fundamental role in breast cancer, has a significant effect on the Cyclin D1-CDK4/6-RB1 complex. This constitutes the rationale for combination therapy based on inhibition of this interaction by combined with ET and CDK 4/6i (15, 16). In this direction, the first studies were carried out in patients with HR+/HER2- MBC (12–14). In general, those with ER positive staining percentage >1% in the pathology material are defined as endocrine sensitive, but those with 1-9% ER+ are called ERlow positive according to the American Society of Clinical Oncology/College of American Pathologists Guideline (ASCO/CAP). However it is well known that these tumors often gain little benefit from ET (17–20). Therefore, in studies evaluating the combination of ET and CDK 4/6i, patients with an ER+ of 10% and above were included (12–14). In these studies, patients were grouped as ER+/PR+ and ER+/PR- according to their HR+, but the percentage of HR positivity was not further categorized in terms of endocrine sensitivity.

On the other hand, when the meta-analysis of the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) is evaulated it is observed that ER positivity is categorized and the benefit from ET is highest especially in the group with ER+>50% (18). In different studies, it has been reported that the therapeutic effect and survival are correlated with the rate of ER positivity in breast cancer patients who is receiving ET (19–22). Similarly, there is information regarding the predictive and prognostic role of a high percentage of predictive biomarker positivity in other tumor groups. For example, in the TOGA study, in which the addition of trastuzumab to systemic chemotherapy in metastatic gastric cancer was investigated the most benefit was observed in the group with immunohistochemically (IHC) HER2-positive 3+/*in-situ* hybridization (ISH)+ with proportionately less benefit in the IHC 2+/ISH+ and IHC1+/ISH+ subgroups (23). Similarly, it has been reported that the percentage of ALK-positivity in anaplastic lymphoma kinase (ALK)-positive non-small cell lung cancer (NSCLC) is correlated with response and survival (24).

Therefore, when all these results were evaluated together, we suggested that the percentage of hormone positivity in patients receiving CDK 4/6i together with ET may affect the response and overall outcome.

# Methods

## Patient characteristics

In this study, patients who were followed up and treated in Dokuz Eylul University Faculty of Medicine, Department of Medical Oncology between January 01, 2020 and January 01, 2023, with the

diagnosis of HR+ HER-2- MBC, who received ET plus CDK 4/6i, were retrospectively evaluated. Demographic characteristics of the patients, complete blood count, biochemical laboratory parameters, clinicopathological features of the tumor were recorded from the hospital database. Patients were included on the basis of the following criteria: (1) patients with breast cancer based on core needle biopsy before treatment; (2) having diagnosed with HR-positive HER2-negative MBC; (3) patients who is receiving CDK 4/6i with (palbociclib or ribociclib) ET a for at least 2 months, (4) performance status (ECOG-PS) ranging from 0 to 2; (5) having complete medical record and follow-up information; (6) be 18 years or older; (7) to be survived more than 3 months. Patients were excluded on the basis of the following criteria: (1) Patients with synchronous and metachronous tumors; (2) having diagnosed with HR-negative HER2-positive and triple-negative breast cancer.

## Ethics committee approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by Non-Invasive Research Ethics Committee of Dokuz Eylul University Faculty of Medicine (Date: 09.02.2022/No: 2022/05-09).

## Endocrine therapy + CDK 4/6 inhibitor therapy

Patients who were started on CDK 4/6i combined with ET with the diagnosis of HR+ HER2- MBC were included in the study. Those receiving endocrine therapy were categorized as either AI (anastrozole or letrozole) or selective estrogen degrader (fulvestrant). Those receiving CDK 4/6i therapy were also grouped as those receiving palbociclib or ribociclib. Then, the patients were subcategorized as two groups by receipt of AI plus CDK4/6i or fulvestrant plus CDK4/6i.

## Hormone receptor status

ER and PR analyzes of tumor materials of the patients were performed by IHC, based on the American Society of Clinical Oncology/College of American Pathologists Guideline (ASCO/CAP) (17). The results obtained according to the immune reactivity status in the tumor cell nucleus were categorized as follows for both ER and PR: ERnegative: 0% or <1%, ERlow: 1-9%, ERpositive: 10-100%; PRnegative: 0% or 1%, PRlow: 1-9%, PRpositive: 10-100%. To analyze the role of high hormone receptor positivity in this study, ERpositive (10-100%) and PRpositive (10-100%) groups were also categorized as an ERmoderately positive (10-49%) and ERstrongly positive (50-100%); PR moderately positive (10-49%) and PR strongly positive (50-100%). The status of the aforementioned ER and PR analyzes from primary tumor or metastasis was recorded. ER and PR analyzes of all patients were evaluated and recorded before starting CDK 4/6i combined with ET.

## Response and toxicity assessment

Tumor staging was performed according to "Eighth Edition of American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC) TNM stage classification" (25). Response assessments were made according to the "Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 guidelines" (26). Toxicity assessments were made according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) (27).

## Statistical analysis

Demographic characteristics, clinicopathological features, and blood sample results were collected from the hospital database. Since our study was a retrospective, cross-sectional study, the sample size was not calculated. In addition to descriptive statistics, Chi-square and Fisher's exact tests were used for categorical variables in the evaluation of the data. The effect of ER and PR positivity percentage and clinicopathological features of breast cancer on treatment response and survival were analyzed with Chi-square and Fisher's Exact tests. Student's t test, Mann-Whitney U test and Kruskal Wallis test were used to determine the differences between the measured variables according to their suitability. As progression-free survival time (PFS), the time from the start of ET plus CDK 4/6i therapy to the date of progression; The overall survival time (OS) was taken as the time from the start of ET plus CDK 4/6i therapy to death/last follow-up date. Kaplan-Meier method and Log-rank test were used for survival analysis. The suitability of the data for normal distribution was evaluated with the Kolmogorov Smirnov test and it was found that it did not have a normal distribution. Therefore, median values were used when reporting OS and PFS data. Therefore, mean values were used when reporting OS and PFS data. The prognostic and predictive effect of ER and PR positivity percentage was analyzed with univariate and multivariate Cox Regression model. The median follow-up time in the study was calculated using the reverse Kaplan-Meier. IBM SPSS for analysis of all data *(Sciences Statistical Package for the Social, version* 24.0) package program was used. Statistical significance was determined as $p<0.05$.

# Results

## Patient characteristics

A total of 150 patients with HR+ HER2- MBC who received CDK 4/6i combined with ET were evaluated. The median age of the patients was 55.0 years (26.2-90.2), of which 147 (98%) were female and 3 (2%) were male. Of the 147 female patients, 109 (74.1%) were postmenopausal. The most common site of metastasis was bone (n=119, 79.3%), followed by lymph nodes (n=91, 60.7%) and liver (n=42, 28%). The characteristics of the patients are shown in Table 1.

10.3389/fonc.2024.1378563

TABLE 1 Sociodemographic and clinicopathologic characteristics of patients.

| Characteristics | n (%) |
|---|---|
| **Sex** | |
| Female | 147 (98%) |
| Male | 3 (2%) |
| **Comorbidity** | |
| None | 75 (50%) |
| One | 47 (31.3%) |
| Two or more | 28 (18.7%) |
| **Menopause Status** | |
| Postmenopausal | 109 (74.1%) |
| Pre/peri menopausal | 38 (25.9%) |
| **Histological Subtype** | |
| Invasive ductal carcinoma (IDC) | 51 (34.0%) |
| Invasive carcinoma | 38 (25.3%) |
| Invasive lobular carcinoma (ILC) | 24 (16.0%) |
| Mixed type (IDC+ ILC) | 17 (11.3%) |
| Mucinous | 5 (3.3%) |
| Tubuloalveoler | 1 (0.7%) |
| Unknown | 14 (9.4%) |
| **Metastasis Site** | |
| Bone | 119 (79.3%) |
| Lymph node | 91 (60.7%) |
| Liver | 42 (28%) |
| Lung | 37 (24.7%) |
| Brain | 5 (3.3%) |
| Others | 32 (21.3%) |

## CDK 4/6 inhibitor and endocrine therapy

Seventy five (50.0%) of 150 patients received AI plus CDK 4/6i treatment and 75 (50.0%) received fulvestrant plus CDK 4/6i. Patients receiving AI plus CDK 4/6i receive this treatment as first-line therapy while those receiving fulvestrant + CDK 4/6i were being treated in second-line therapy after progression on AI treatment. Median duration of treatment for AI plus CDK 4/6i was 14.0 months; for fulvestrant plus CDK 4/6i was 16.1 months.

## Hormone receptor status

All 150 patients in total were HR+/HER2-, and their distribution according to the percentage of ER and PR positivity was as follows in Table 2.

TABLE 2 Hormone receptor status of study population.

| Characteristics | Total n (%) |
|---|---|
| ER $^{\text{strong positive}}$/PR $^{\text{strong positive}}$ | 49 (32.7%) |
| ER $^{\text{strong positive}}$/PR $^{\text{moderate positive}}$ | 31 (20.7%) |
| ER $^{\text{strong positive}}$/PR $^{\text{low positive}}$ | 19 (12.7%) |
| ER $^{\text{strong positive}}$/PR $^{\text{negative}}$ | 31 (20.7%) |
| ER $^{\text{moderate positive}}$/PR $^{\text{strong positive}}$ | 2 (1.3%) |
| ER $^{\text{moderate positive}}$/PR $^{\text{moderate positive}}$ | 4 (2.7%) |
| ER $^{\text{moderate positive}}$/PR $^{\text{low positive}}$ | 6 (4%) |
| ER $^{\text{moderate positive}}$/PR $^{\text{negative}}$ | 8 (5.3%) |

## Response evaluation

Among all patients, 86 (57.3%) obtained partial response, 38 (25.3%) had stable disease and 22 (14.7%) had progression. Response assessment could not be performed in four patients (2.7%) at 3 months.

When the response rates according to use of endocrine partner (AI vs fulvestrant respectively) were examined, partial responses were seen in 45 (60%) vs 41 (54.7)%), stable disease in 18 (24%) vs 20 (26.7%), and progression in 9 (12%) vs 13 (17.3%), respectively.

## Toxicity

Grade 3 toxicity was observed in 57 (38%) patients with the most common adverse effect being neutropenia. While no dose reduction was required for ET in the whole group, CDK 4/6i dose reduction was performed in 17 (53.1%) patients in the group receiving AI plus CDK 4/6i and in 15 (46.9%) patients in the group receiving fulvestrant plus CDK 4/6i. Treatment was discontinued in 2 (2.8%) patients in the group receiving AI plus CDK 4/6i due to side effects.

## Survival analysis

Median follow-up was 15.2 months (95% CI, 2.1-40.9 months). The median PFS obtained with ET plus CDK 4/6i among all patients was 23.4 months (95% CI, 21.2-25.6) and the median OS was 29.4 months (95% CI, 26.3-32.3) (Figures 1A, B).

When outcomes were examined based on endocrine partner (AI vs fulvestrant, respectively) median PFS was 24.4 months (95% CI, 21.8-26.9) vs 22.5 months (95% CI, 20.2-25.8) and medial OS was 25.4 months (95% CI, 22.7-28.2) vs 28.8 (95% CI, 25.0-32.6).

## Effect of hormone receptor positivity percentage on treatment response

The highest response rates were seen in the group with ER$^{\text{-strong positive}}$/PR $^{\text{moderate or strong positive}}$, ER $^{\text{moderate positive}}$/PR $^{\text{moderate or}}$

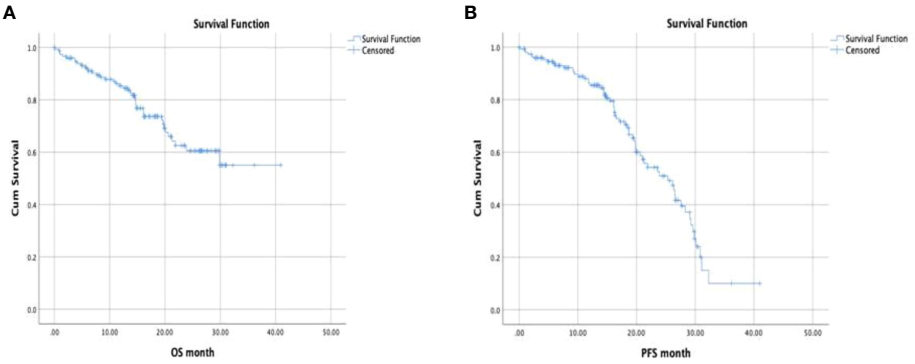



FIGURE 1
**(A, B)** Progression free survival and overall survival (Kaplan Meier Test).

strong positive group, ER strong positive/PR negative group, and then ER moderate positive/PR negative group followed in descending order. Although these advantages were not statistically significant, they were numerically higher (ORR: 83.8% vs. 83.3% vs. 77.4% vs. 62.5% p=0.488, respectively). Responses were similar for the first three subgroups, but significantly lower for the ERmoderate positive/PRnegative group (Table 3).

## Effect of hormone receptor positivity percentage on survival

When the effect of ER and PR positivity percentage on survival in the whole group was analyzed, it was observed that the highest survival was obtained in the ER-strong positive/PR moderate or strongly positive group before treatment, followed by the ER moderate positive/PR moderate or strongly positive group, the ER strongly positive/PR negative group and followed by ERmoderate positive/PRnegative group (mPFS 24.5 vs 23.3 vs 22.6 months vs 17.8 months, p=0.469; mOS 29.6 vs 27.0 months vs 24.7 months vs 18.5 months, p=0.410, respectively). However, these advantages were not statistically significant. Overall survival was similar for the first three subgroups, but significantly shorter for the ER moderate-positive/PRnegative group (Figure 2). The survival advantage observed in the ER strongly positive/PR moderately or strongly positive group was similar in both the AI plus CDK 4/6i group and the fulvestrant plus CDK 4/6i group.

TABLE 3 The relationship between hormone positivity and treatment response.

| Characteristics | Response Rate | P value |
|---|---|---|
| ER-strong positive/PR moderate or strong positive, | 83.8% | P= 0.488 |
| ER moderate positive/PR moderate or strong positive | 83.3% | |
| ER strong positive/ PR negative | 77.4% | |
| ER moderate positive/ PR negative | 62.5% | |

Clinicopathological and therapeutic features that may have an impact on survival, and the role of ER and PR positivity percentage were evaluated in univariate and multivariate analysis. The effects of ECOG Performance status, type of endocrine therapy, lung metastases, liver metastases, presence of bone metastases, and percentage of ER and PR positivity on survival in both univariate and multivariate analysis were evaluated. However, no statistical significance was found.

# Discussion

In this study, it was determined that the percentage of baseline HR positivity affected the prognosis in patients with HR+ HER2- MBC treated with CDK 4/6i in combination with an ET. It was observed that the group with ER strong positive/PR moderate or strong positive before treatment was the best prognostic group, followed by the ER moderate positive/PR moderate or strong positive group, followed by the ER moderate positive/PR negative group with a decreasing rate.

One of the most basic features of cancer is the loss of control in cell cycle regulation (28). Normally, the transition from G1 to S phase in the cell cycle is controlled by the Rb gene, through the sequestration of the E2F family transcriptional factor. CDK 4/6, on the other hand, inactivates the Rb gene by forming a complex with D-type cyclins, thus inducing the transition from G1 to S phase (11). Since Cyclin D1 is a direct transcriptional target of estrogen, it has been reported that ER+ tumor cells are particularly dependent on CDK 4/6 activation in cell proliferation (29). In addition, it has been reported that Cyclin D1 amplification is widely observed (29 to 58%) in ER+ breast cancer (30). All these features have paved the way for clinical trials with CDK 4/6i in patients with HR+ HER2- breast cancer. In this sense, studies were conducted in which palbociclib, ribociclib and abemaciclib were combined with ET (12–14). It is observed that these studies were primarily carried out in postmenopausal patients with HR+ HER2- MBC, followed by premenopausal patients, and also studies on its use in the adjuvant setting. In studies in the postmenopausal group, AI or Fulvestrant was used as ET in both metastatic and adjuvant periods; In studies in the premenopausal group, it is seen that tamoxifen or AI is used together with the LHRH analogue. In all these studies, the addition of

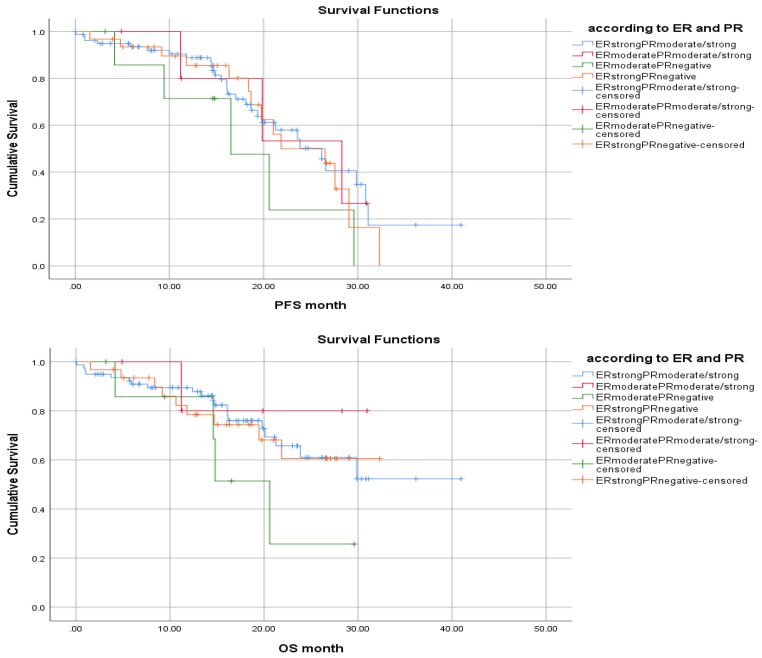



FIGURE 2
According to status of HR, PFS and OS (Kaplan Meier Test, Log-Rank Analysis).

CDK 4/6i to ET has been shown to contribute significantly to survival and has become one of the main treatment models for MBC.

To date studies of CDK4/6i with ET have been conducted in postmenopausal and premenopausal patients, both in the metastatic and adjuvant settings. In postmenopausal women, CDK4/6i is generally combined with AI or fulvestrant while in premenopausal women, CDK4/6i can be partnered with tamoxifen or AI with LHRH analogue. Across most studies CKD4/6i combined with ET has been shown to improve outcomes. Most Phase III studies with CDK4/6i have included patients with ER+ ≥ 10% and PR+/- (12–14). As a result, the benefit of adding CDK 4/6i to ET was demonstrated in the whole group, and subgroup analysis was also performed according to PR positivity versus negativity. And although there are differences in both analysis and results between studies, the ER+/PR+ group appears to benefit more than the ER+/PR- group. And also in the Paloma-2 study, patients were not stratified by PR positivity however they were in the Monaleesa-2 study (31, 32). In the latter study, a smaller benefit was seen in the PR+ patients when compared to the PR- patients, though the small PR- sample size may have confounded these results. On the other hand, in the Monarch-3 study no significant difference was observed in terms of PR status (33).

We also reviewed the impact of PR positivity or negative in second lines studies of CDK4/6i paired with fulvestrant. In the Paloma-3 study, the role of PR +/- was not found to impart a significant difference.

And neither the efficacy of fulvestrant plus palbociclib nor the likelihood of disease progression more than 6 months after study entry were significantly associated with the level of expression of estrogen or progesterone receptors (HR: 0.32 vs 0.54, respectively) (34). And also in the Paloma-3 study, it was also stated that endocrine sensitivity was a prognostic factor in favor of CDK 4/6i in patients (35). In the Monaleesa-3 and Monarch-2 studies, no significant difference was found between the ER+/PR+ group and the other group (including PR- patients), as well as between the PR+ group and the PR- group, respectively (36, 37). In the Monaleesa-7 study in premenopausal patients receiving CDK4/6i with ET and ovarian function suppression (OFS) no difference was observed between the ER+/PR+ group and the other group (38). In a meta-analysis that included of all phase III studies on CDK 4/6i in metastatic disease, no significant difference was found between the groups in terms of PR status (39). On the other hand, in adjuvant studies of CDK4/6i, such as the MONARCH-E study, benefit was found independent of PR status, although more benefit was obtained in the PR+ group than the PR- group (HR 0.73 vs 0.81) (40).

Many studies in the literature suggest that patients with ER +/PR- tumors have a worse prognosis and higher risk of recurrence than ER+/PR+ tumors (41). In Gharib KE, et al. (42), predictive and prognostic factors were investigated in patients with MBC receiving palbocicilib and letrozole. They reported negative prognostic and predictive features including liver metastases, line of treatment, and absence of PR. The median PFS in PR+ vs PR- groups was 20.05 months vs 12.99 months (p= 0.046) (42).

Similarly, Canino F, et al. (43) evaluated the prognostic role of the intrinsic subtype detected by PAM50 in patients with HR +/HER2- MBC. As a result, it has been reported that the response to ET is low and the prognosis is worse in non-luminal subtypes. In addition, they stated that the response to endocrine therapy was significantly lower in patients whose non-luminal subtype was detected not from the primary tumor but from the metastatic area (43). In a biomarker study conducted in the intrinsic subtypes of the Monaleesa studies, it was stated that the addition

of ribociclib to endocrine therapy contributed significantly in all subgroups except the basal-like group. Compared to the luminal A group, the risk of disease progression was found to be 1.44, 2.31 and 3.96 times higher in the Luminal B, HER2-enriched and basal-like groups, respectively (44). Although no separate analysis was performed in the Luminal B group according to PR percentage or Ki-67 index in this analysis, the high risk of progression in this group compared to Luminal A may be an indirect indicator of the importance of hormone receptor positivity.

It has also been postulated that the level of ER expression has a prognostic role in patients with breast cancer undergoing ET (19–22). In Yoon KH et al. patients with ERlow (ER 1-9%) benefitted less fromendocrine therapy and had a significantly higher risk of recurrence compared to the ERhigh group (20). Apart from this, the percentage of hormone receptor positivity as well as its presence or absence have prognostic importance, and in a study conducted by Bae et al., single HR+ tumors without HER2 overexpression (ER + PR-HER2- or ER-PR + HER2-) were found to be of prognostic importance. has been shown to have a poorer survival rate than triple-positive tumors, and this group even has a poorer prognosis comparable to triple-negative breast cancer (45). On the other hand, in the EBCTCG meta-analysis, it was observed that ER positivity was categorized and the benefit from endocrine therapy was highest especially in the group with ER+>50% (18). Similarly, in the P024 study, in which Letrozole and TMX were compared in neoadjuvant therapy in patients with ER+/PR+ breast cancer, patients with ER≥10% positive were included and a linear correlation was reported between ER expression levels and response (46). However, studies investigating the role of hormone receptor levels in the effectiveness of CDK 4/6i are limited in the literature.

Of these, Shikanaj A et al. (47), clinicopathological factors associated with efficacy in patients with MBC were analyzed. As a result, it was reported that tumor grade in the primary lesion and initial neutrophil/lymphocyte ratio (NLR) were associated with efficacy, while expression levels of hormone receptors had no significant effect. In this study, patients were divided into "high" and "low" groups according to the proportion of cells staining positive for ER and PR, and the cut-off value was taken as 66% for this distinction. Patients in which both ER and PR were expressed over 66% were termed the "high" group. However, while separate risk groups were defined for ER and PR, risk groups formed by combining the two were not defined (47). In our study, besides defining separate risk groups for ER and PR according to hormone receptor expression levels, combined risk groups were formed by combining these two. Thus, the prognostic risk groups were better defined and it was determined that the group with ER $^{\text{strong positive}}$/PR $^{\text{moderate or strong positive}}$ was the best prognostic group. This was followed by the ER$^{\text{moderate positive}}$/PR$^{\text{moderate or strongly positive}}$ group, followed by the ER$^{\text{moderate positive}}$/PR$^{\text{negative}}$ group in a decreasing fashion. In our study, the positive effect on the prognosis, especially of being ER-strong positive, was more remarkable. In this sense, it was observed that both the response and survival were better in the ER-positive group, even if it was PR-negative. Therefore, our results, although not statistically significant, pointed out the prognostic importance of a high ER positivity. In this respect, it can be thought that our work has a different originality. In the above-mentioned Monaleesa-2 study, the fact that the strong positivity of the ER in both groups was not fully known may also have a role in the lower benefit observed in the PR+ group compared to the PR- group.

In line with all these studies, cyclin D1 is a direct transcriptional target of estrogen and therefore it is known that patients with high hormone receptor expression may obtain more benefit from CDK4/6i when combined with ET.

Our study has some limitations, such as its relatively small sample size, reflecting a single center experience, and retrospective design.

In conclusion, the results of this study suggest that the percentage of HR positivity may have a predictive and prognostic role in patients with HR+ HER-2- MBC who received CDK 4/6i with ET. As far as we know, our study is one of the few studies in the literature conducted with CDK 4/6i in breast cancer patients. We believe that the percentage of hormone receptor positivity and especially the strong positive ER should be taken into account in defining the patient group who will benefit more from the treatment in patients treated with ET plus CDK 4/6i. Our results are hypothesis generating and more comprehensive studies may be needed to further elucidate our findings.

## 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 humans were approved by Non-Invasive Research Ethics Committee of Dokuz Eylul University Faculty of Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

MK: Formal analysis, Methodology, Writing – original draft, Writing – review & editing. HSS: Writing – original draft, Writing – review & editing. TY: Supervision, Writing – original draft, Writing – review & editing. IO: Supervision, Writing – original draft, Writing – review & editing.

## Funding

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

## 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. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. (2020) 70:7–30. doi: 10.3322/caac.21590

2. Emens LA. Breast cancer immunotherapy: facts and hopes. *Clin Cancer Res*. (2018) 24:511–20. doi: 10.1158/1078-0432.CCR-16-3001

3. Nagini S. Breast cancer: current molecular therapeutic targets and new players. *Anticancer Agents Med Chem*. (2017) 17:152–63. doi: 10.2174/1871520616666160502122724

4. Clarke R, Tyson JJ, Dixon JM. Endocrine resistance in breast cancer—an overview and update. *Mol Cell Endocrinol*. (2015) 418:220–34. doi: 10.1016/j.mce.2015.09.035

5. Deluche E, Antoine A, Bachelot T, Lardy-Cleaud A, Dieras V, Brain E, et al. Contemporary outcomes of metastatic breast cancer among 22,000 women from the multicentre ESME cohort 2008–2016. *Eur J Cancer*. (2020) 129:60–70. doi: 10.1016/j.ejca.2020.01.016

6. Anderson WF, Chatterjee N, Ershler WB, Brawley OW. Estrogen receptor breast cancer phenotypes in the Surveillance, Epidemiology, and End Results database. *Breast Cancer Res Treat*. (2002) 76:27–36. doi: 10.1023/A:1020299707510

7. Yue W, Yager JD, Wang J-P, Jupe ER, Santen RJ. Estrogen receptor-dependent and independent mechanisms of breast cancer carcinogenesis. *Ster- oids*. (2013) 78:161–70. doi: 10.1016/j.steroids.2012.11.001

8. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Breast Cancer v3.2020 (2020). Available online at: https://www.nccn.org/professionals/physician_gls/pdf/breast_blocks.pdf (Accessed 20 Apr 2020).

9. Files JA, Ko MG, Pruthi S. Managing aromatase inhibitors in breast cancer survivors: not just for oncologists. *Mayo Clin Proc*. (2010) 85:560–6. doi: 10.4065/mcp.2010.0137

10. Nagaraj G, Ma CX. Clinical challenges in the management of hormone receptor-positive, human epidermal growth factor receptor 2-negative metastatic breast cancer: A literature review. *Adv Ther*. (2021) 38:109–36. doi: 10.1007/s12325-020-01552-2

11. Finn RS, Aleshin A, Slamon DJ. Targeting the cyclindependent kinases (CDK) 4/6 in estrogen receptorpositive breast cancers. *Breast Cancer Res*. (2016) 18:17. doi: 10.1186/s13058-015-0661-5

12. US Food and Drug Administration. Palbociclib highlights of prescribing information (2018). Available online at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/207103s007lbl.pdf (Accessed 06 Sep 2018).

13. US Food and Drug Administration. Ribociclib highlights of prescribing information (2018). Available online at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/209092s001lbl.pdf (Accessed 30 Jan 2019).

14. US Food and Drug Administration. Abemaciclib highlights of prescribing information (2018). Available online at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/208855s000lbl.pdf (Accessed 06 Sep 2018).

15. Spring LM, Wander SA, Andre F, Moy B, Turner NC, Bardia A. Cyclin-dependent kinase 4 and 6 inhibitors for hormone receptor-positive breast cancer: past, present, and future. *Lancet*. (2020) 395:817–27. doi: 10.1016/S0140-6736(20)30165-3

16. Shah M, Nunes MR, Stearns V. CDK4/6 inhibitors: game changers in the management of hormone receptor–positive advanced breast cancer? *Oncol (willist Park*. (2018) 32:216–22.

17. Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, et al. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. (2020) 38(12):1346–66. doi: 10.1200/JCO.19.02309

18. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Relevance of breast cancer hormone receptors and other factors to the effi cacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet*. (2011) 378 (9793):771–84. doi: 10.1016/S0140-6736(11)60993-8

19. Hill DA, Barry M, Wiggins C, Nibbe A, Royce M, Prossnitz E, et al. Estrogen receptor quantitative measures and breast cancer survival. *Breast Cancer Res Treat*. (2017) 166:855–64. doi: 10.1007/s10549-017-4439-6

20. Yoon KH, Park Y, Kang E, Kim EK, Kim JH, Kim SH, et al. Effect of estrogen receptor expression level and hormonal therapy on prognosis of early breast cancer. *Cancer Res Treat*. (2022) 54:1081–90. doi: 10.4143/crt.2021.890

21. Van den Eynden GG, Colpaert CG, Vermeulen PB, Weyler JJ, Goovaerts G, van Dam P, et al. Comparative analysis of the biochemical and immunohistochemical determination of hormone receptors in invasive breast carcinoma influence of the tumor-stroma ratio. *Pathol Res Pract*. (2002) 198:517–24. doi: 10.1078/0344-0338-00295

22. Sparano JA, Paik S. Development of the 21-gene assay and its application in clinical practice and clinical trials. *J Clin Oncol*. (2008) 26:721–8. doi: 10.1200/JCO.2007.15.1068

23. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet*. (2010) 376:687–97. doi: 10.1016/S0140-6736(10)61121

24. Hizal M, Bilgin B, Paksoy N, Atcı MM, Kahraman S, Kılıçkap S, et al. The percentage of ALK-positive cells and the efficacy of first-line alectinib in advanced non-small cell lung cancer: is it a novel factor for stratification? (Turkish Oncology Group Study). *J Cancer Res Clin Oncol*. (2022) 149(8):4141–8. doi: 10.1007/s00432-022-04252-2

25. Abdel-Rahman O. Validation of the 8th AJCC prognostic staging system for breast cancer in a population-based setting. *Breast*. (2018). doi: 10.1007/s10549-017-4577-x

26. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. (2009) 45:228–47. doi: 10.1016/j.ejca.2008.10.026

27. Available online at: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf (Accessed March 09, 2018).

28. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013

29. Murphy CG, Dickler MN. The role of CDK4/6 inhibition in breast cancer. *Oncologist*. (2015) 20:483–90. doi: 10.1634/theoncologist.2014-0443

30. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature*. (2012) 490:61–70. doi: 10.1038/nature11412

31. Finn RS, Martin M, Rugo HS, Jones S, Im SA, Gelmon K, et al. Palbociclib and letrozole in advanced breast cancer. *N Engl J Med*. (2016) 375:1925–36. doi: 10.1056/NEJMoa1607303

32. Hortobagyi GN, Stemmer SM, Burris HA, et al. Ribociclib as first-line therapy for HR-positive, advanced breast cancer. *N Engl J Med*. (2016) 375:1738–48. doi: 10.1056/NEJMoa1609709

33. Goetz MP, Toi M, Campone M, et al. MONARCH 3: abemaciclib as initial therapy for advanced breast cancer. *J Clin Oncol*. (2017) 35:3638–46. doi: 10.1200/JCO.2017.75.6155

34. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, dou- ble-blind, phas. *Lancet Oncol*. (2016) 17:425–39. doi: 10.1016/S1470-2045(15)00613-0

35. Rugo HS, Cristofanilli M, Loibl S, et al. Prognostic factors for overall survival in patients with hormone receptor-positive advanced breast cancer: analyses from PALOMA-3. *Oncologist*. (2021) 26(8):e1339–46. doi: 10.1002/onco.13833

36. Slamon DJ, Neven P, Chia S, et al. Phase III randomized study of ribociclib and fulvestrant in hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer: MONALEESA-3. *J Clin Oncol*. (2018) 36:2465–72. doi: 10.1200/JCO.2018.78.9909

37. Sledge GW Jr, Toi M, Neven P, et al. MONARCH 2: abemaciclib in combination with fulvestrant in women with HR+/HER2- advanced breast cancer who had progressed while receiving endocrine therapy. *J Clin Oncol*. (2017) 35:2875–84. doi: 10.1200/JCO.2017.73.7585

38. Im SA, Lu YS, Bardia A, Harbeck N, Colleoni M, Franke F, et al. Overall survival with ribociclib plus endocrine therapy in breast cancer. *N Engl J Med*. (2019) 381:307. doi: 10.1056/NEJMoa1903765

39. Johnston SRD, Harbeck N, Hegg R, Toi M, Martin M, Shao ZM, et al. Abemaciclib combined with endocrine therapy for the adjuvant treatment of HR+, HER2-, node-positive, high-risk, early breast cancer (monarchE). *J Clin Oncol*. (2020) 38:3987–98. doi: 10.1200/JCO.20.02514

40. Rossi V, Berchialla P, Giannarelli D, Nisticò C, Ferretti G, Gasparro S, et al. Should all patients with HR-positive HER2-negative metastatic breast cancer receive CDK 4/6 inhibitor as first-line based therapy? A network meta-analysis of data from the PALOMA 2, MONALEESA 2, MONALEESA 7, MONARCH 3, FALCON, SWOG and FACT trials. *Cancers*. (2019) 11:1661. doi: 10.3390/cancers11111661

41. Cancello G, Maisonneuve P, Rotmensz N, Viale G, Mastropasqua MG, Pruneri G, et al. Progesterone receptor loss identifies Luminal B breast cancer subgroups at higher risk of relapse. *Ann Oncol*. (2013) 24:661–8. doi: 10.1093/annonc/mds430

42. Gharib KE, Macaron W, Kattan J, Salloum MA, Farhat F, Smith M, et al. Palbociclib and letrozole in hormone-receptor positive advanced breast cancer: Predictive response and prognostic factors. *Curr Problems Cancer*. (2022) 46:100859. doi: 10.1016/j.currproblcancer.2022.100859

43. Canino F, Piacentini F, Omarini C, et al. Role of intrinsic subtype analysis with PAM50 in hormone receptors positive HER2 negative metastatic breast cancer: A systematic review. *Int J Mol Sci*. (2022) 23:7079. doi: 10.3390/ijms23137079

44. Prat A, Chaudhury A, Solovieff N, et al. Correlative biomarker analysis of intrinsic subtypes and efficacy across the MONALEESA phase III studies. *J Clin Oncol*. (2021) 39:1458–67. doi: 10.1200/JCO.20.02977

45. Bae SY, Kim S, Lee JH, Lee HC, Lee SK, Kil WH, et al. Poor prognosis of single hormone receptor- positive breast cancer: similar outcome as triple-negative breast cancer. *BMC Cancer*. (2015) 15:138. doi: 10.1186/s12885-015-1121-4

46. Ellis MJ, Miller WR, Tao Y, Evans DB, Chaudri Ross HA, Miki Y, et al. Aromatase expression and outcomes in the P024 neoadjuvant endocrine therapy trial. *Breast Cancer Res Treat*. (2009) 116:371–8. doi: 10.1007/s10549-008-0161-8

47. Shikanai A, Horimoto Y, Ishizuka Y, Uomori T, Nakai K, Arakawa A, et al. Clinicopathological features related to the efficacy of CDK4/6 inhibitor-based treatments in metastatic breast cancer. *Breast Cancer: Basic Clin Res*. (2022) 16:1–9. doi: 10.1177/11782234211065148

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 27 June 2024
DOI 10.3389/fonc.2024.1388087



OPEN ACCESS

EDITED BY
Zili Zhang,
Nanjing University of Chinese Medicine, China

REVIEWED BY
Ida Paris,
Fondazione Policlinico Agostino Gemelli IRCSS, Italy
Yukinori Ozaki,
Cancer Institute Hospital of Japanese Foundation for Cancer Research, Japan

*CORRESPONDENCE
Rachel Wuerstlein
rachel.wuerstlein@med.uni-muenchen.de

RECEIVED 19 February 2024
ACCEPTED 29 April 2024
PUBLISHED 27 June 2024

CITATION
Hester A, Henze F, Debes AM, Schubert CL, Koenig A, Harbeck N and Wuerstlein R (2024) What are the needs in oral antitumor therapy? An analysis of patients' and practitioners' preferences.
*Front. Oncol.* 14:1388087.
doi: 10.3389/fonc.2024.1388087



# What are the needs in oral antitumor therapy? An analysis of patients' and practitioners' preferences

Anna Hester, Franziska Henze, Anna Marie Debes, Charlotte Leonie Schubert, Alexander Koenig, Nadia Harbeck and Rachel Wuerstlein*

Department of Obstetrics and Gynecology, Breast Center and Comprehensive Cancer Center (CCC) Munich, University Hospital, LMU Munich, Munich, Germany

**Background:** Since the European approval of CDK4/6 inhibitors in 2016, the treatment of patients with hormone-receptor-positive, HER2-negative metastatic breast cancer has changed significantly. Compared with chemotherapy, endocrine-based therapy has different treatment regimens and is associated with new side effects. Oral therapy aims for optimal drug efficacy and long treatment times while maintaining maximum independence and quality of life resulting in the conservation of medical staff resources.

**Methods:** A monocentric analysis of therapy preferences of practitioners (25 nurses and physicians) and patients (11 on endocrine monotherapy, 17 on endocrine-based therapy, and 14 on intravenous chemotherapy) was performed using specific questionnaires. Preferences were assessed using a four-point Likert scale or bidirectional response options.

**Results:** All patients were highly supportive of oral therapy (mean agreement score on the Likert scale 1.3, $p < 0.001$ *vs*. all other options) and a consultation interval of 4 weeks (2.0, $p = 0.015$ *vs*. 3 weeks). Practitioners also preferred oral therapy (1.4) and visits every 4 weeks (1.6). In general, patients on oral therapies reported higher compatibility of their therapy with daily life than patients on chemotherapy (1.6 and 1.7 *vs*. 2.6, $p = 0.006$). Outpatient oncology is the main source of information for all patients, mainly in case of side effects (2.0) and open questions (1.8). Regarding oral antitumor therapy regimens, patients do not show a significant preference for a specific regimen, while practitioners prefer a continuous regimen (1.6) over a 21/7 regimen (21 days on and 7 days off therapy, 2.5). Patients are likely to accept mild side effects (e.g., neutropenia, diarrhea, polyneuropathy, fatigue) and would still adhere to their initial choice of regimen (continuous or 21/7). Only when side effects occur with a severity of CTCAE grade 3 do patients prefer the regimen in which the side effects occur for a shorter period of time.

**Conclusion:** Patients and practitioners prefer oral antitumor therapy—both continuous and 21/7 regimens—over other application forms. Patient education and proper therapy management, supported by additional tools, contribute to the specific management of side effects and high adherence. This allows quality of life to be maintained during long-term therapy with CDK4/6 inhibitors in patients with metastatic breast cancer.

KEYWORDS

CDK4/6 inhibitor, oral therapy, patient education, patient preference, e-health, metastatic breast cancer, continuos regimen, 21/7 regimen

## 1 Introduction

Breast cancer is the most common cancer in women worldwide with a lifetime risk of approximately 10% for women in Western countries. 4%–10% of all breast cancer patients present with primary metastatic disease, and approximately 20%–40% of the remaining patients develop metastases during the course of their disease (1). For clinical purposes and treatment decisions, breast cancer is biologically classified according to the presence or absence of expression of the hormone receptors (HRs) for estrogen and progesterone and a possible amplification of the human epidermal growth factor receptor 2 (HER2). HR-expressing (+) HER2 not amplificated (−) breast cancer is the most common subtype. Although it is often considered less aggressive than the other subtypes, recurrent disease or metastases can still occur a long time after primary diagnosis (2). The most common sites of metastasis in HR+ HER2− breast cancer are the bone, lung, liver, brain, and skin (3). With the development of new therapeutic options and the individualization of treatment regimens in recent decades, the therapy of primary and metastatic breast cancer has improved significantly. Treatment options for metastatic HR+ HER2− breast cancer have classically included oral or intramuscular endocrine therapies and oral or intravenous chemotherapies. However, due to recent therapeutic improvements, HR+ HER2− breast cancer can also be treated with oral, targeted therapies, such as the revolutionary cyclin-dependent kinase (CDK) 4/6 inhibitors (palbociclib, ribociclib, abemaciclib) (4). In addition, mammalian target of rapamycin (mTOR) inhibitors (everolimus), phosphatidylinositol 3-kinase (PI3K) inhibitors (alpelisib), and poly (ADP-ribose) polymerase (PARP) inhibitors (olaparib, talazoparib) are used in oral targeted therapy (5–8).

As of 2017, the standard first-line therapy regimen for patients with metastatic HR+ HER2− breast cancer is oral endocrine-based therapy with a classical endocrine therapy (aromatase inhibitor or fulvestrant) in combination with a CDK4/6 inhibitor and—in case of bone metastases—a bone-modifying drug (bisphosphonate or denosumab) (2). Chemotherapy, on the other hand, is only indicated in cases of visceral crisis (9, 10). Today, three different CDK4/6 inhibitors—palbociclib, ribociclib, and abemaciclib—are available for metastatic breast cancer.

All phase III trials of CDK4/6 inhibitors showed a significant increase in median progression-free survival (PFS) with CDK4/6 inhibitor therapy compared with placebo, but not all showed an increase in overall survival (OS) (4). Palbociclib (Ibrance®) became the first CDK4/6 inhibitor to be approved in Europe on 9 November 2016, based on data from the PALOMA trials: in the PALOMA-2 trial, postmenopausal patients with primary metastatic breast cancer received a combination of palbociclib and letrozole. PFS in the palbociclib/letrozole group increased significantly to 24.8 months compared with 14.5 months in the placebo/letrozole group (11). However, no statistically significant improvement in OS was observed after long-term follow-up (12). Ribociclib was evaluated in the MONALEESA study program: patients in the MONALEESA-2 trial benefited from endocrine-based therapy with ribociclib and letrozole with a PFS of 25.3 months compared with placebo/letrozole with a PFS of 16.0 months. OS was also significantly prolonged, with 63.9 months in the ribociclib arm compared with 51.4 months in the placebo arm (13, 14). Ribociclib (Kisqali®) was approved in Europe on 22 August 2017. The third CDK4/6 inhibitor, abemaciclib, also showed a significant and comparable improvement in PFS in combination with letrozole or fulvestrant in the MONARCH trials (15, 16). The final analysis of the MONARCH3 trial showed an increase in OS of 13.1 months compared with the control arm, which did not reach statistical significance (17). Abemaciclib was approved in Europe on 27 September 2018, under the trade name Verzenios®.

The various CDK4/6 inhibitors differ in their dosages, regimens, and potential side effects (Table 1). Palbociclib (standard dose: 125 mg) and ribociclib (standard dose: 600 mg) must be taken once daily for 21 consecutive days, followed by a 7-day rest before starting a new cycle (so-called “21/7 regimen”). Abemaciclib, on the other hand, is taken twice daily (at the standard dose of 150 mg) without a break (so-called “continuous regimen”). If side effects occur, the daily dose of all three CDK4/6 inhibitors can be reduced in two steps (see Table 1) according to the prescribing information (18–20).

The most common severe side effects of palbociclib [Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or higher] as described in the prescribing information are neutropenia,

TABLE 1 Dosing, treatment regimen, and most common side effects (≥ 30%, all grades) under therapy with palbociclib ($n$ = 768), ribociclib ($n$ = 1,065), and abemaciclib ($n$ = 768) in combination with endocrine therapy with letrozole or fulvestrant according to the prescribing information.

| | Palbociclib | | Ribociclib | | Abemaciclib |
|---|---|---|---|---|---|
| **Dosing** | 125 mg, 100 mg, 75 mg | **Dosing** | 600 mg, 400 mg, 200 mg | **Dosing** | 150 mg, 100 mg, 50 mg |
| **Treatment regimen** | 21/7 regimen, 1×/day | **Treatment regimen** | 21/7 regimen, 1×/day | **Treatment regimen** | Continuous regimen, 2×/day |
| AE | % of all patients | AE | % of all patients | AE | % of all patients |
| Neutropenia | 82.1 | Neutropenia | 74.3 | Diarrhea | 84.6 |
| Infections | 49.2 | Nausea | 51.5 | Neutropenia | 45.1 |
| Leucopenia | 48.6 | Infections | 50.3 | Infections | 43.6 |
| Fatigue | 41.5 | Fatigue | 36.5 | Nausea | 43.5 |
| Nausea | 36.0 | Diarrhea | 35.0 | Fatigue | 40.5 |
| Stomatitis | 30.3 | Alopecia | 33.2 | Anemia | 30.1 |
| | | Leucopenia | 32.9 | | |

Adapted after (18–20).
AE, adverse event.

leukopenia, and fatigue. In randomized clinical trials, 38.4% of patients receiving palbociclib required dose reductions or therapy adjustments due to adverse reactions, regardless of the endocrine combination partner (19). The most common grade 3 or grade 4 adverse events with ribociclib are neutropenia, leukopenia, abnormal liver function tests, QT time prolongation, nausea and vomiting, infections, and fatigue. Dose reductions due to adverse events were required in 37.3% of patients treated with ribociclib in phase III clinical trials, while 7% had to discontinue treatment permanently (20). Patients treated with abemaciclib mainly reported severe (CTCAE grade 3 or higher) side effects such as diarrhea, neutropenia, infections, leukopenia, abnormal liver function tests, nausea and vomiting, and fatigue. In contrast to the other two CDK4/6 inhibitors, diarrhea was significantly more common with abemaciclib, which may be pathophysiologically explained by the higher selectivity for CDK4 compared with CDK6 (18).

Regarding side effects, the prescribing information provides detailed instructions on therapy management including the required monitoring intervals. For palbociclib, a complete blood count is required before starting therapy, at the beginning of each cycle, on day 15 of the first two cycles, and as clinically indicated (19). For ribociclib, electrocardiograms (ECGs) and electrolytes should be monitored prior to initiation of treatment. ECGs should be repeated on approximately day 14 of the first cycle, at the beginning of the second cycle, and as clinically indicated. Electrolytes should be monitored at the beginning of each cycle for six cycles and as clinically indicated. Liver function tests (LFTs) and a complete blood count must be performed before starting treatment, every 2 weeks for the first two cycles, at the beginning of each of the subsequent four cycles, and as clinically indicated (20). For abemaciclib, complete blood counts and LFTs should be monitored prior to initiation of therapy, every 2 weeks for the first 2 months, monthly for the next 2 months, and as clinically indicated. Patients should be instructed to initiate antidiarrheal therapy, increase oral fluid intake, and notify their healthcare provider at the first sign of loose stools (18).

The fundamental change in the treatment of HR+ HER2− metastatic breast cancer from intravenous to oral tumor therapy and the long treatment periods with the new oral therapies pose new challenges for both practitioners and patients.

Since oral antitumor therapy with CDK4/6 inhibitors is taken independently by the patients in their home environment, extensive information and education of both patients and their families and caregivers is necessary prior to initiation of therapy (21): individual schedules—e.g., when to take the drug regularly or when to stop in case of a 21/7 regimen, or specifying the individual dose—should be discussed with both patients and their families. Important drug-specific features, such as interactions with over-the-counter medication, dietary supplements, and foods, also play an important role in targeted therapy and must be emphasized in discussions with patients. This information should be updated throughout the course of therapy. Detailed information about possible side effects of oral therapy should be explained to the patients and their families. To ensure adherence and safety, patients need a clear plan and detailed information about the regular check intervals (as described above) according to the prescribing information, and they need to know when to involve relatives or doctors, when to take additional medication, or even when to stop therapy in case of side effects. In addition to detailed pretreatment education, patients need to be followed closely during the treatment. They need regular and scheduled face-to-face visits to monitor adherence and discuss therapy details in person, but they also need emergency contact numbers and information options available at all times of the day (21).

New logistical challenges for some patients include the compatibility and flexibility of therapy-related appointments when returning to work. Early and seamless vacation planning can also be a challenge: if patients are planning to go on vacation, the most common side effects and their management in an emergency should be reviewed in detail, all relevant information and contact details should be available in writing, and prescriptions for therapy and concomitant medications should be available for the duration of the vacation. In addition to the new demands on the

patient in terms of self-responsibility, the treatment team is also faced with new challenges. Often, logistical and personnel restructuring is necessary to ensure continuous and high-quality patient care. Patients can generally be accompanied by either nurses or physicians. In addition, several digital tools have recently been introduced to monitor and manage therapy. Whereas in the past patients had to use calendars or written notes to document their therapy and possible side effects, digital tools are now available for this purpose (22). Similarly, various websites and apps support patients with detailed information and coaching modules for therapy accompaniment To our knowledge, there has been no detailed analysis of patients' preferences for personal therapy accompaniment and management.

The aim of the study was to evaluate preferences regarding oral antitumor therapy and therapy accompaniment in a real-world setting. The study was conducted at the Breast Cancer of the LMU University Hospital in Munich, Germany. Both patients and practitioners (nurses and physicians) were interviewed to assess what they expect from oral antitumor therapy, what kind of therapy accompaniment they prefer, and which therapy regimen is the most preferred in which clinical situation. We even assigned two different treatment schedules with equal therapy efficacy (an on/off schedule and a continuous schedule) with side effects of increasing severity and analyzed whether these side effects influence the preference for a specific treatment schedule. To our knowledge, no such analysis has been performed so far. Our results may help in counseling patients when choosing a CDK4/6 inhibitor for therapy—as they have all shown comparable efficacy, other factors need to be taken into account when making therapy decisions. In addition, the results on therapy accompaniment preferences may help practitioners caring for these patients in their daily clinical practice.

# 2 Materials and methods

## 2.1 Survey

Patients with metastatic breast cancer treated with either endocrine monotherapy, endocrine-based therapy, or chemotherapy at the Breast Center of the Department of Gynecology and Obstetrics of the LMU University Hospital in Munich, Germany, and practitioners/healthcare professionals (physicians and nurses) working at the same center were eligible for this project. The voluntary survey was conducted between December 2020 and March 2021 by distributing questionnaires to the study population, after the project was approved by the LMU Ethics Committee (ethical approval number 21-0848). The return date for the questionnaires was May 2021. The respective questionnaires were developed specifically for this study and are available in the Supplementary Files. The original questionnaires were in German and were translated for submission.

The "endocrine monotherapy" group included all patients receiving either letrozole, anastrozole, exemestane, or fulvestrant (in combination with a gonadotropin-releasing hormone analog if premenopausal). The "endocrine-based therapy" group included patients who received a CDK4/6 inhibitor (either palbociclib, ribociclib, or abemaciclib) in combination with one of the endocrine therapies above. Patients in the "chemotherapy" group received a classic intravenous or oral chemotherapy, such as paclitaxel, epirubicin/cyclophosphamide, or capecitabine.

The patient questionnaires covered the following areas in the first section: demographics, information about the disease, information about current therapy, and questions about therapy accompaniment and treatment regimen preferences. Multiple response options were available for the accompaniment and treatment regimen preference questions. Questions had predefined response options or were open-ended. Questions on treatment preferences were scored on a Likert scale from 1 to 4 (1 = strongly agree, 2 = somewhat agree, 3 = somewhat disagree, 4 = strongly disagree).

The second section was designed to assess patients' preferences for different CDK4/6 inhibitor regimens (21/7 regimen *vs*. continuous regimen). However, instead of specifically asking patients which CDK4/6 inhibitor and which intake regimen they preferred, two hypothetical but identically acting oral drugs should be compared by the patients. We did this to avoid confounding by possibly already known drug names. Patients were clearly told that both drugs were expected to have the same oncologic efficacy, regardless of their intake regimen. These hypothetical drugs therefore stood as substitutes for CDK4/6 inhibitors. One of these hypothetical drugs was to be taken continuously, the other in a 21/7 regimen. In 21 different questions, each drug was supposed to have a specific side effect. These side effects were neutropenia (and subsequent increased risk of infection), diarrhea, fatigue, and paresthesia in the fingers and toes (polyneuropathy). Each side effect could hypothetically occur with a severity of CTCAE grade 1, 2, or 3. Each side effect could occur for 2 days in the continuous scheme and for 7 days in the 21/7 regimen in a 28-day period (referred to in the analysis as "shorter duration of side effect in the continuous regimen") or vice versa (referred to in the analysis as "shorter duration of side effect in the 21/7 regimen"). In each question, patients were asked to decide if they would rather take drug A or drug B under the given condition. In this manuscript, the choice of a drug is shown in case of a grade 1 or grade 3 side effect (grade 2 is omitted for clarity).

The practitioner questionnaires included questions about demographics, the scope of practice at the breast center, and professional experience in oncology. Practitioners were also asked about their preferences for different treatment regimens, using a Likert scale with the same coding as above. As part of this survey, the practitioners were asked additional questions, the results of which are not presented in this publication. Therefore, the attached questionnaire for practitioners has been shortened to the questions relevant to this publication.

## 2.2 Statistics

Questionnaires were analyzed using Microsoft Excel and SPSS software. For patient and practitioner characteristics, data from questions with predefined responses were summarized, and median and range were presented when applicable (e.g., age, years of

therapy, years of experience). Data from the Likert-scale questions in the questionnaire (results in 3.2.1, 3.2.2, and 3.3) were analyzed as follows: the mean and standard deviation of the value on the Likert scale were calculated for each question, both for the overall cohorts of patients and practitioners and for the predefined subgroups (endocrine monotherapy, endocrine-based therapy, and chemotherapy and physicians and nurses). These results are described in this manuscript as "mean approval score." Differences between the subgroups regarding the mean approval score for each question were analyzed using the Kruskal–Wallis test (for more than two subgroups) and the Mann–Whitney *U* test (for two subgroups). Differences in the overall cohort on multiple variables (e.g., weekly *vs*. 3 weekly *vs*. 4 weekly) were analyzed using ANOVA.

Data from the questions in the questionnaire with only two response options (analysis of the second section regarding treatment regimen: 21/7 or continuous, results in 3.2.3) were analyzed for differences between the subgroups using chi-squared tests. *p*-values ≤0.05 were considered statistically significant.

# 3 Results

## 3.1 Characteristics of the patients and practitioners

The basic characteristics of the patients interviewed are shown in Table 2. Eleven patients on endocrine monotherapy were interviewed. They were generally the oldest patients (median age 71 years), more often already retired (81.8%), and had the lowest metastatic burden with mainly bone metastases. The 17 patients on endocrine-based therapy were generally younger (median age 66 years), more often still working (23.5% retired), and also had predominantly bone metastases. The 14 patients receiving chemotherapy were the youngest (median age 54.6 years) and most likely to be working part-time or full-time (only 21.4% were retired). They had the highest metastatic load, including major organ metastases (brain, liver).

The basic characteristics of the surveyed practitioners (both physicians and nurses) are shown in Table 3. Most were experienced practitioners, working in the field of oncology for at least 5 years.

We describe the preferences of the patients and practitioners interviewed in this study as their mean agreement score on a Likert scale to different options as explained in the *Methods* section (1 = strongly agree, 2 = partially agree, 3 = partially disagree, 4 = strongly disagree).

## 3.2 Patient preferences

### 3.2.1 Patient preferences regarding therapy accompaniment and management

#### 3.2.1.1 Therapy management tools

The overall level of agreement for the different therapy management tools (diaries, calendars, smartphone apps, or "other") was low among all patients surveyed in this study, and the respective mean agreement scores for each subgroup are shown in Table 4 and Figure 1. Diaries were not used at all by the patients in the study.

Patients on endocrine therapy were most likely to choose "other" therapy management tools (mean agreement score 2.7) to accompany their therapy, such as taking notes, counting blisters, or calling it just a "daily routine" to take their pills. Patients on endocrine-based therapy had the highest support for the use of calendars among all tools (mean agreement score 2.9), with a difference of borderline significance ($p$ = 0.059) compared with the other subgroups. Patients receiving chemotherapy were unlikely to use any of the tools, with no relevant differences between the different tools (Table 4, Figure 1).

#### 3.2.1.2 Compatibility of therapy

The overall cohort reported a high level of compatibility of the therapy with daily life (mean agreement score 1.6) and with leisure plans (2.0). Compatibility with vacation (2.4) or work (2.6) was lower (Table 4, Figure 1).

Patients on endocrine therapy showed even higher compatibility of the therapy with daily life (1.2) and leisure plans (1.6) than the overall cohort and showed the lowest compatibility with work (2.5, limited data, question answered by

TABLE 2 Basic characteristics of the patients surveyed in this study.

| | Endocrine monotherapy ($n$ = 11) | Endocrine-based therapy ($n$ = 17) | Chemotherapy ($n$ = 14) |
|---|---|---|---|
| **Age, years (median, range)** | 71 (44, 84) | 62.3 (35, 80) | 54.6 (32, 71) |
| **Time between primary diagnosis and metastases, years (median, range)** | 9.9 (0, 17) | 6.8 (0, 22) | 3.7 (0, 25) |
| **Localization of metastases, percentage ($n$)** | 81.8% (9) bone<br>18.2% (2) lung/pleural<br>18.2% (2) lymph nodes<br>9.1% (1) skin | 82.4% (14) bone<br>29.4% (5) lung/pleural<br>17.6% (3) liver<br>5.9% (1) lymph nodes | 64.3% (9) bone<br>28.6% (4) lungs/pleural<br>42.9% (6) liver<br>14.3% (2) peritoneum<br>14.3% (2) lymph nodes<br>14.3% (2) brain<br>7.1% (1) pericardium |

TABLE 3 Basic characteristics of the practitioners surveyed in this study.

| | *n* | % | | | |
|---|---|---|---|---|---|
| Number of practitioners | 25 | 100 | Age, years (median, range) | Years working in oncology | |
| **Physicians** | 11 | 44.0 | | n | |
| Specialized oncologist | 5 | 20.0 | 43.6 (33, 57) | 3 | >10 years |
| | | | | 2 | 5–10 years |
| Resident | 6 | 24.0 | 31.0 (28, 36) | 2 | 5–10 years |
| | | | | 4 | < 10 years |
| **Nurses** | 14 | 56.0 | 46.9 (31, 64) | | |
| Nurse specialized in oncology | 2 | 8.0 | | 5 | >10 years |
| Breast care nurse | 2 | 8.0 | | 5 | 5–10 years |
| General nurse | 5 | 20.0 | | 4 | < 5 years |
| Physician's assistant | 5 | 20.0 | | | |

TABLE 4 Patient characteristics and preferences regarding therapy accompaniment

| | Overall cohort (*n* = 42) | Endocrine monotherapy (*n* = 11) | Endocrine-based therapy (*n* = 17) | Chemotherapy (*n* = 14) | *p*-value comparing subgroups |
|---|---|---|---|---|---|
| **Agreement with various therapy management tools** | | | | | |
| Diary | 4 (0.0)[40] | 4 (0.0)[11] | 4 (0.0)[15] | 4 (0.0)[14] | 1.0 |
| Calendar | 3.3 (1.2)[42] | 4 (0.0)[11] | 2.9 (1.4)[17] | 3.2 (1.3)[14] | 0.059 |
| Smartphone app | 3.1 (1.4)[41] | 3.1 (1.3)[11] | 3.1 (1.4)[16] | 3.1 (1.4)[14] | 0.986 |
| Other: | 3.2 (1.3)[38] | 2.7 (1.5)[11] | 3.4 (1.2)[15] | 3.3 (1.4)[12] | 0.421 |
| Namely: | | Daily notes (*n* = 2), blisters (*n* = 2), routine (*n* = 1) | Daily notes (*n* = 1), routine (*n* = 2) | Blisters (*n* = 3) | |
| **Therapy is compatible with…** | | | | | |
| Daily life | 1.6 (0.6)[42] | 1.2 (0.4)[11] | 1.5 (0.5)[17] | 1.9 (0.7)[14] | **0.016*** |
| Leisure plans | 2.0 (1.0)[42] | 1.6 (0.9)[11] | 1.7 (0.8)[17] | 2.6 (1.0)[14] | **0.006*** |
| Vacations | 2.6 (1.1)[41] | 2.1 (0.8)[11] | 2.3 (1.0)[16] | 3.2 (1.1)[14] | **0.021*** |
| Work | 2.4 (1.2)[26] | 2.5 (2.1)[2] | 2.1 (1.2)[13] | 2.7 (1.1)[11] | 0.419 |
| **Agreement with various sources of information (for general questions)** | | | | | |
| Outpatient oncology unit | 1.8 (1.0)[41] | 2.4 (1.0)[11] | 1.5 (0.8)[16] | 1.6 (1.1)[14] | **0.035*** |
| Internet | 2.3 (1.2)[40] | 2.4 (1.1)[11] | 2.2 (1.2)[15] | 2.3 (1.2)[14] | 0.870 |
| Patient advocacy groups | 3.6 (0.9)[39] | 4 (0.0)[11] | 3.7 (0.6)[14] | 3.2 (1.3)[14] | 0.150 |
| Group chats | 3.7 (0.8)[39] | 4 (0.0)[11] | 3.8 (0.4)[14] | 3.3 (1.3)[14] | 0.155 |
| Other patients | 2.9 (1.0)[39] | 2.8 (0.9)[11] | 2.7 (1.0)[14] | 3.1 (1.2)[14] | 0.588 |
| Emergency department | 3.9 (0.5)[40] | 4 (0.0)[11] | 3.7 (0.8)[15] | 3.9 (0.4)[14] | 0.440 |

*(Continued)*

TABLE 4 Continued

| | Overall cohort ($n$ = 42) | Endocrine monotherapy ($n$ = 11) | Endocrine-based therapy ($n$ = 17) | Chemotherapy ($n$ = 14) | $p$-value comparing subgroups |
|---|---|---|---|---|---|
| Agreement with various sources of information (for general questions) | | | | | |
| **Wish to have a constant contact person** | 1.4 (0.5)[42] | 1.6 (0.5)[11] | 1.4 (0.6)[17] | 1.3 (0.5)[14] | 0.440 |
| His/her qualification should | | | | | |
| Physician | 1.1 (0.3)[42] | 1.3 (0.5)[11] | 1.1 (0.3)[17] | 1 (0.0)[14] | 0.119 |
| Nurse specialized in oncology | 1.3 (0.7)[42] | 1 (0.0)[11] | 1.3 (0.8)[17] | 1.4 (0.9)[14] | 0.280 |

For each option, the mean rating on a four-point Likert scale (1 = strongly agree, 2 = partially agree, 3 = partially disagree, 4 = strongly disagree) and the standard deviation (in parentheses) are shown in the overall cohort (gray background) and in the three different patient subgroups (patients on endocrine monotherapy, endocrine-based therapy, or chemotherapy).
Asterisks and bold type indicate significant p-values (from the Kruskal–Wallis test) when comparing the results between the three different patient subgroups. Superscript numbers indicate the number of patients ($n$) who answered the respective question (missing responses were common). The mean agreement scores are visualized in the corresponding Figure 1.

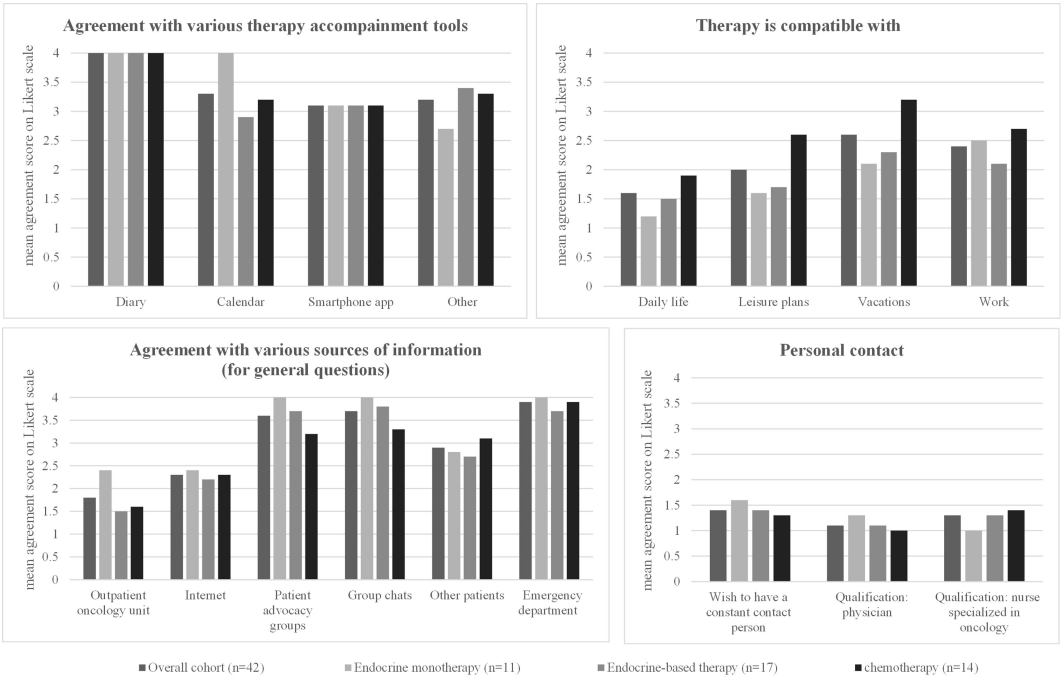



FIGURE 1
Patient preferences regarding side effects. Left: The number of pills (mean) already taken and accepted to reduce side effects is shown. Right: For each option, the mean rating on a four-point Likert scale (1 = strongly agree, 2 = partially agree, 3 = partially disagree, 4 = strongly disagree) in the overall cohort and in the three different patient subgroups (patients receiving endocrine monotherapy, endocrine-based therapy, or chemotherapy) is shown. The detailed values for each subgroup, standard deviations, and significant $p$-values when comparing the results between the three different patient subgroups are shown in the corresponding Table 4.

only two patients). Patients on endocrine-based therapy reported slightly lower but still high compatibility of their therapy with daily life (1.5) and leisure plans (1.7) and sufficient compatibility with work (2.1). Patients receiving chemotherapy reported different results: they reported significantly lower compatibility with daily life (1.9, $p$ = 0.016) and leisure plans (2.6, $p$ = 0.006). They also reported low compatibility with vacations (3.2) or work (2.7) (Table 4, Figure 1).

#### 3.2.1.3 Sources of information

Regarding sources of information for general questions (Table 4, Figure 1), direct contact with the oncologist received the highest agreement score in the general cohort (mean agreement score 1.8), followed by searching the Internet (mean agreement score 2.3). Contacting the emergency department, patient advocacy groups, or group chats were unlikely to be used in all three subgroups (Table 4, Figure 1).

Patients on endocrine therapy reported only the outpatient oncology unit (mean agreement score 2.4), the Internet (2.4), or other patients (2.8) as sources of information for general questions—all with comparable agreement scores (Table 4). Patients receiving endocrine-based therapy, on the other hand, strongly preferred contacting the outpatient oncology unit (1.5, $p = 0.035$), followed by the Internet (2.2), and then other patients (2.7). Similarly, patients receiving chemotherapy primarily contacted the outpatient oncology unit (1.6) or the Internet (2.3) as a source of information (Table 4, Figure 1).

Almost all patients surveyed in this study (regardless of the treatment regimen) stated that it was important for the person responsible for their oncological therapy to remain the same (mean agreement score 1.4). The qualification of this contact person seems to be less important: both the qualification as a physician (mean agreement score 1.1) and as a specialized nurse (mean agreement score 1.3) received high scores without significant differences between the patient subgroups (Table 4, Figure 1).

### 3.2.2 Patient preferences regarding treatment regimen

Regardless of the form of therapy currently being administered, all patients preferred oral tumor therapy over other forms of application [mean agreement score 1.3, $p < 0.001$ *vs.* each of the other options (intravenous, subcutaneous, and intramuscular)]. Oral therapy was followed by intravenous and subcutaneous therapy (mean agreement scores of 2.4 and 2.5, respectively). These mean agreement scores in the overall cohort were comparable to the agreement scores in each therapy subgroup (endocrine therapy *vs.* endocrine-based *vs.* chemotherapy). There were no significant differences in the preferred therapy form between the three patient subgroups (Table 5, Figure 2).

A 4-week interval between therapy visits received the highest agreement score (2.0, $p = 0.015$ *vs.* 3 weeks) in the overall cohort, and weekly visit intervals were the least approved of the options given (mean agreement score 3.4, $p < 0.001$ *vs.* 3 weeks) (Table 5, Figure 2).

In the subgroup analysis, patients on endocrine monotherapy showed very high support for 3-monthly visit intervals (mean agreement score 1.5, $p = 0.026$ *vs.* the other subgroups). Weekly (3.7) or 3-weekly (3.6) consultations were least supported by the patients (Table 5, Figure 2). Patients receiving endocrine-based therapy preferred a 4-week interval between their therapy visits (mean agreement score 1.5, $p = 0.005$ compared with the other subgroups), with the least approval for weekly (3.9) consultations. Patients receiving chemotherapy had similar approval scores for 3-weekly (2.1), 4-weekly (2.2), or 3-monthly (2.3) intervals. They had the lowest agreement score for weekly consultations of all options (2.7), but this score for weekly consultations was still significantly higher than in the other two subgroups ($p = 0.001$; Table 5, Figure 2).

### 3.2.3 Patient preferences regarding side effects

#### 3.2.3.1 Accompaniment in the event of side effects

All patients showed a high willingness to take additional medication to treat side effects even though they were already taking an average of 4.5 pills/day as co-medication (Table 6, Figure 3). Patients in the overall cohort would accept an average of 3.2 additional pills to reduce side effects. The number of additional pills accepted in the three subgroups was comparable to the overall cohort, with no significant differences between the subgroups (Table 6, Figure 3).

In the event of side effects, most patients in the overall cohort consult the outpatient oncology clinic (mean agreement score 2.0) or the Internet (2.4). Other sources (patient groups, group chats, other patients, emergency department) were very unlikely to be contacted for side effects (Table 6, Figure 3).

In the subgroup analysis, patients on endocrine therapy were equally likely to contact the outpatient oncology clinic (2.6) and the Internet (2.6). Patients on endocrine-based therapy showed the

TABLE 5 Patient preferences regarding treatment regimen.

| | Overall cohort ($n = 42$) | Endocrine monotherapy ($n = 11$) | Endocrine-based therapy ($n = 17$) | Chemotherapy ($n = 14$) | $p$-value comparing subgroups |
|---|---|---|---|---|---|
| **Agreement with different application forms** | | | | | |
| Oral therapy | 1.3 (0.5)$^{42}$ | 1.4 (0.5)$^{11}$ | 1.4 (0.5)$^{17}$ | 1.2 (0.4)$^{14}$ | 0.503 |
| Intravenous | 2.4 (0.9)$^{40}$ | 2.0 (0.9)$^{11}$ | 2.6 (0.7)$^{15}$ | 2.6 (1.0)$^{14}$ | 0.135 |
| Subcutaneous | 2.5 (0.6)$^{39}$ | 2.6 (0.7)$^{11}$ | 2.4 (0.6)$^{14}$ | 2.6 (0.6)$^{14}$ | 0.536 |
| Intramuscular | 3.1 (0.7)$^{39}$ | 3.4 (0.7)$^{11}$ | 2.9 (0.9)$^{14}$ | 3.1 (0.6)$^{14}$ | 0.263 |
| **Agreement with different consultation intervals** | | | | | |
| Weekly | 3.4 (0.9)$^{38}$ | 3.7 (0.5)$^{11}$ | 3.9 (0.4)$^{13}$ | 2.7 (1.1)$^{14}$ | **0.001*** |
| Every 3 weeks | 2.8 (0.9)$^{38}$ | 3.6 (0.5)$^{11}$ | 3 (0.8)$^{13}$ | 2.1 (0.9)$^{14}$ | **<0.001*** |
| Every 4 weeks | 2.0 (0.9)$^{41}$ | 2.5 (0.9)$^{11}$ | 1.5 (0.8)$^{16}$ | 2.2 (0.8)$^{14}$ | **0.005*** |
| Every 3 months | 2.2 (1.2)$^{39}$ | 1.5 (0.9)$^{11}$ | 2.7 (1.1)$^{14}$ | 2.3 (1.1)$^{14}$ | **0.026*** |

For each option, the mean rating average on a four-point Likert scale (1 = strongly agree, 2 = partially agree, 3 = partially disagree, 4 = strongly disagree) and the standard deviation (in parentheses) are shown in the overall cohort (gray background) and in the three different patient subgroups (patients receiving endocrine monotherapy, endocrine-based therapy, or chemotherapy).

Asterisks and bold type indicate significant $p$-values (from the Kruskal–Wallis test) when comparing the results between the three different patient subgroups. Superscript numbers indicate the number of patients ($n$) who answered the respective question (missing responses were common). The mean agreement scores are visualized in the corresponding Figure 2.

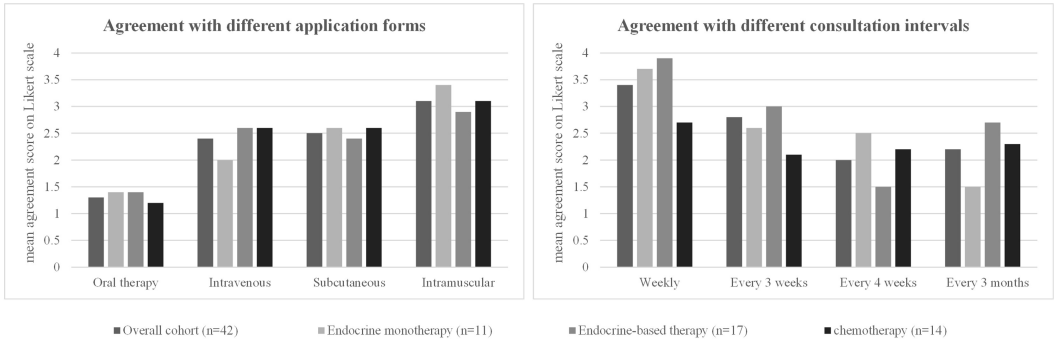



FIGURE 2
Patient preferences regarding treatment regimen. For each option, the mean rating on a four-point Likert scale (1 = strongly agree, 2 = partially agree, 3 = partially disagree, 4 = strongly disagree) in the overall cohort and in the three different patient subgroups (patients receiving endocrine monotherapy, endocrine-based therapy, or chemotherapy) is shown. The detailed values for each subgroup, standard deviations, and significant *p*-values when comparing the results between the three different patient subgroups are shown in the corresponding Table 5.

TABLE 6 Patient preferences regarding side effects.

| | Overall cohort (*n* = 42) | Endocrine monotherapy (*n* = 11) | Endocrine-based therapy (*n* = 17) | Chemotherapy (*n* = 14) | *p*-value comparing subgroups |
|---|---|---|---|---|---|
| **A) Pills taken daily**[a] | 4.5 (2.5)[42] | 5.6 (1.4)[11] | 4.3 (2.7)[17] | 3.7 (2.7)[14] | 0.071 |
| **B) Accepted additional daily pills to treat side effects**[b] | 3.2 (1.7)[42] | 3.5 (1.4)[11] | 3.1 (2.0)[17] | 3.1 (1.8)[14] | 0.659 |
| Agreement with different sources of information (for side effects) | | | | | |
| Outpatient oncology unit | 2.0 (1.2)[41] | 2.6 (1.2)[11] | 1.9 (1.1)[16] | 1.6 (1.1)[14] | 0.082 |
| Internet | 2.4 (1.2)[40] | 2.6 (1.2)[11] | 2.4 (1.2)[15] | 2.4 (1.4)[14] | 0.865 |
| Patient groups | 3.7 (0.8)[39] | 4 (0.0)[11] | 3.9 (0.0)[14] | 3.1 (1.2)[14] | **0.008*** |
| Group chats | 3.8 (0.5)[39] | 4 (0.0)[11] | 3.9 (0.5)[14] | 3.6 (0.6)[14] | 0.096 |
| Other patients | 3.5 (0.8)[39] | 3.5 (1.0)[11] | 3.6 (0.8)[14] | 3.6 (0.7)[14] | 0.971 |
| Emergency department | 3.8 (0.7)[40] | 4 (0.0)[11] | 3.7 (0.8)[15] | 3.6 (0.9)[14] | 0.283 |

A) and B): The number of pills (mean, standard deviation) is shown. C): For each option, the mean rating on a four-point Likert scale (1 = strongly agree, 2 = partially agree, 3 = partially disagree, 4 = strongly disagree) and the standard deviation (in parentheses) are shown. Results are shown for the overall cohort (grey background) and in the three different patient subgroups (patients receiving endocrine monotherapy, endocrine-based therapy, or chemotherapy). Asterisks and bold type indicate significant *p*-values (from the Kruskal–Wallis test) when comparing the results between the three different patient subgroups. Superscript numbers indicate the number of patients (*n*) who answered the respective question (missing responses were common). The mean number and mean agreement scores are visualized in the corresponding Figure 3.
[a]Patients were asked the question: "How many pills do you take in total per day?"
[b]Patients were asked the question: "If you could reduce the side effects of anti-tumor therapy by taking additional pills, how many would you be willing to take each day?"

highest agreement scores for the outpatient oncology unit (1.9) and the Internet (2.4) as a contact for side effects. Similar results were obtained for patients receiving chemotherapy, with a score of 1.6 for outpatient oncology and 2.4 for the Internet (Table 6, Figure 3).

### 3.2.3.2 Preferred treatment regimen in case of side effects

Specifically for oral therapies, patients were asked whether they would generally prefer a continuous regimen to a "21 days on–7 days off" (21/7) regimen or vice versa. For the overall cohort, patients did not prefer one regimen over the other (47.6% *vs.* 52.4%). However, there was a significant difference when analyzing for correlations between patient subgroups and regimen choice ($p = 0.023$): patients on endocrine monotherapy preferred a continuous regimen (81.8% *vs.* 18.2%), while most patients on endocrine-based therapies preferred a 21/7 regimen (29.6% *vs.* 70.6%). Patients receiving chemotherapy did not show any clear preference for either therapy regimen (42.9% *vs.* 57.1%).

In addition, it was examined in detail which oral therapy regimen (21/7 *vs.* continuous) is preferred by patients when the therapy regimen is associated with different side effects of varying severity (Figure 4). To answer this question, patients were asked to choose between two hypothetical drugs. Both were described as having the same efficacy and differed only in the therapy regimen (21/7 *vs.* continuous) and the intensity of different side effects. The side effects such as neutropenia CTCAE grade 3 with subsequent increased risk of infection (a), polyneuropathy [grade 1 (b) or grade 3 (c)], diarrhea [grade 1 (d) or grade 3 (e)], and fatigue [grade 1 (f) or grade 3 (d)] could hypothetically occur 2 or 7 days in a 28-day period and with both therapy regimens.

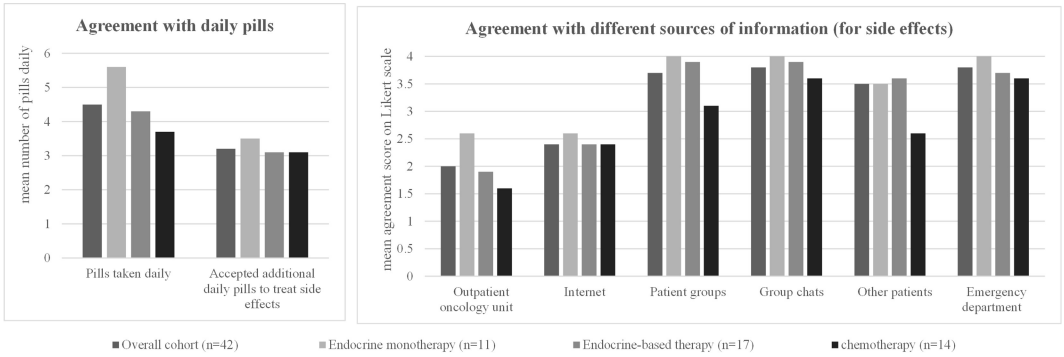



FIGURE 3
Patient characteristics and preferences regarding therapy accompaniment: for each option, the mean rating on a four-point Likert scale (1 = strongly agree, 2 = partially agree, 3 = partially disagree, 4 = strongly disagree) in the overall cohort and in the three different patient subgroups (patients receiving endocrine monotherapy, endocrine-based therapy, or chemotherapy) is shown. The detailed values for each subgroup, standard deviations, and significant *p*-values when comparing the results between the three different patient subgroups are shown in the corresponding Table 6.

The results of this evaluation regarding different side effects are summarized to provide an overview of the trends in treatment decisions. The choice of a treatment regimen for each side effect for each subgroup is shown in Figure 4.

Patients on endocrine monotherapy generally preferred the continuous regimen, despite the presence of side effects: 100% would choose a continuous regimen if it was the regimen with a shorter duration of side effects, regardless of their intensity. Only 35%–55% would choose a 21/7 regimen if it was the regimen with a shorter duration of CTCAE grade 1 side effects (neutropenia: grade 3). The remainder would still choose a continuous regimen even at the cost of a longer duration of side effects. However, if the intensity of side effects was CTCAE grade 3 and occurred for a shorter period with the 21/7 regimen, 100% would choose the 21/7 regimen (Figure 4).

Patients on endocrine-based therapy, on the other hand, have a high preference for 21/7: only approximately 55%–65% would choose the continuous regimen if it was the regimen with a shorter duration of CTCAE grade 1 side effects (neutropenia: grade 3). The remainder would still choose 21/7 even at the cost of a longer duration of side effects. If side effects occurred at an intensity of CTCAE grade 3 and for a shorter period of time with the continuous regimen, approximately 75% would choose the continuous regimen. However, if 21/7 was the regimen with fewer side effects, as many as 70%–100% would choose this regimen, regardless of side effect intensity. These values were comparable across all side effects compared (Figure 4).

Patients undergoing chemotherapy generally chose the therapy regimen where the side effects occurred for a shorter period, but they had a tendency to favor the continuous regimen. If the continuous regimen was the regimen with a shorter period of side effects, approximately 80% would choose it, regardless of the intensity of side effects. If the 21/7 regimen was the regimen with fewer side effects, approximately 55%–65% would choose it for side effects of CTCAE grade 1 (neutropenia: grade 3) and 80–90% for side effects of CTCAE grade 3 (Figure 4).

## 3.3 Practitioner preferences

Similar to the results obtained from patients, practitioners show a high preference for oral therapies (mean agreement score 1.4), while intramuscular injections were least preferred (2.8) (Table 7, Figure 5). Most practitioners prefer to see their patients every 3 weeks (mean agreement score 2.0) or 4 weeks (score 1.6) (Table 7, Figure 5). When asked specifically about the two different regimens for oral therapy, practitioners strongly prefer the continuous regimen (mean agreement score 1.6) over the 21/7 regimen (mean agreement score 2.5). Both physicians and nurses have similar preferences for treatment regimen and consultation intervals—there were no significant differences detectable between these two subgroups (Table 7, Figure 5).

# 4 Discussion

Oral targeted antitumor therapies with CDK4/6 inhibitors have revolutionized the treatment of HR+ HER2− metastatic breast cancer. In addition to their convincing oncological efficacy, these therapies offer numerous advantages in terms of treatment management for patients and practitioners. For example, frequent visits to the oncologist for long, time-consuming intravenous chemotherapy sessions can be avoided, and therapy can be more easily integrated into daily life. However, oral therapies require a high degree of patient responsibility, especially when it comes to managing side effects. This poses a challenge for pretherapeutic patient education. In addition, the oncology team must provide continuous oncologic care during therapy with various options for contact and information. Furthermore, interprofessional cooperation between physicians and nurses inside and outside the hospitals is necessary. As early as 1997, Liu G. et al. were able to show that patients with advanced cancer prefer oral antitumor therapy to intravenous chemotherapy if it does not compromise efficacy (23). The reasons for this choice included personal

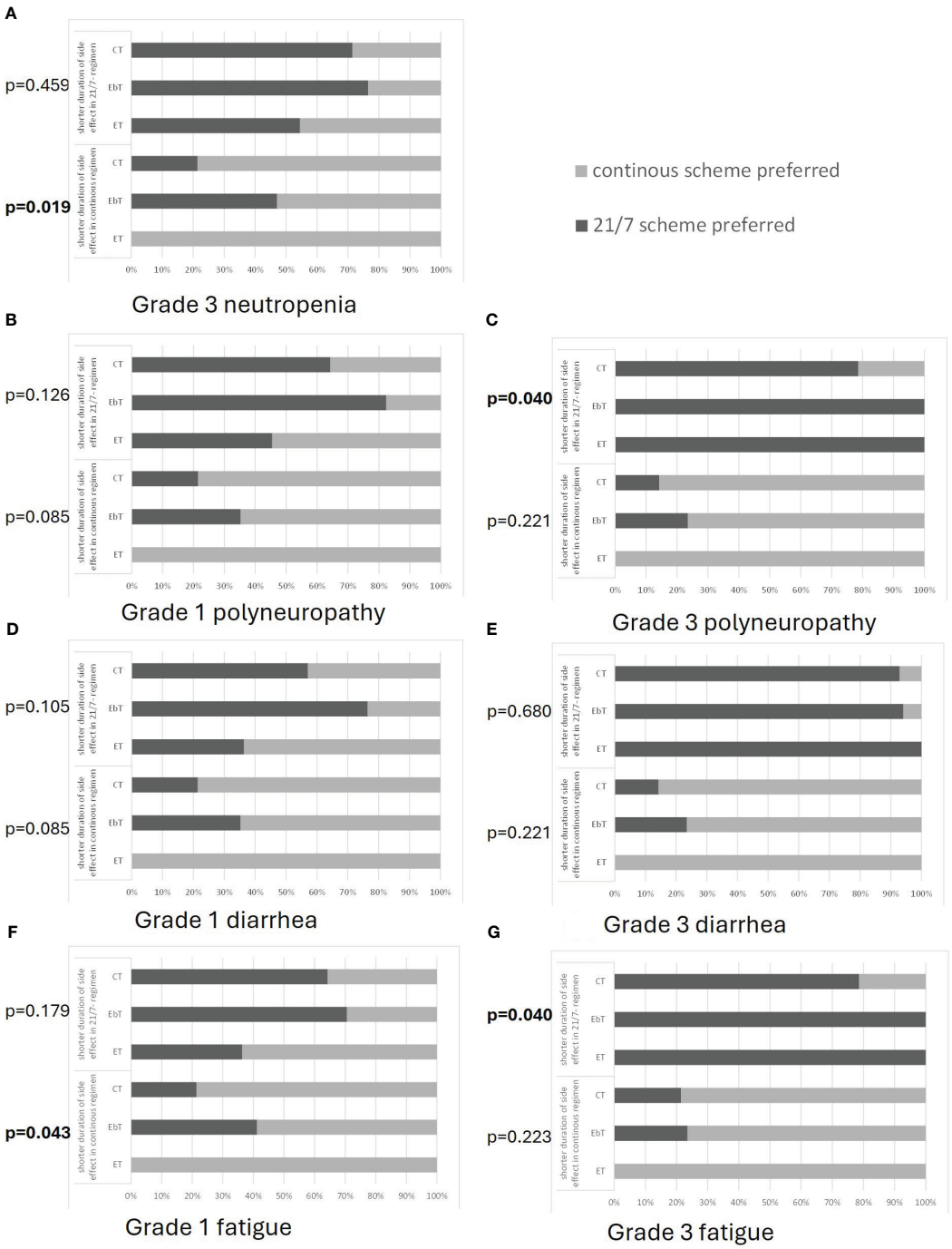



FIGURE 4
Patient preferences for either a 21-day on/7-day off regimen (21/7) or a continuous regimen with respect to the different patient groups of patients undergoing chemotherapy (CT), endocrine-based therapy (EbT), and endocrine monotherapy (ET). Each side effect [shown in **(A–G)**] could hypothetically occur for 2 days in the 21/7 regimen and for 7 days in the continuous regimen (upper part of each graph) or vice versa (lower part of each graph) Preferences for grade 3 neutropenia **(A)** (grade 1 not evaluated), in case of polyneuropathy **(B, C)**, diarrhea **(D, E)**, and fatigue **(F, G)** are shown. *p*-values indicate the result of a chi-squared test analyzing the statistical dependencies of the categorical variables.

problems with intravenous lines and better compatibility with daily life by administering oral therapy in the home environment. A similar conclusion was reached by Eek D. et al. in a literature search of a total of 14 publications on patient preference for oral *vs.* intravenous administration of antitumor therapies (24). The advantages of oral therapies were seen in terms of the concrete therapeutic regimen (e.g., daily intake at home vs. weekly visits to the oncologist) and specific side effects. At the virtual San Antonio Breast Cancer Symposium in December 2020, Jaisle et al. presented the results of their online survey on expectations and preferences for oral *vs.* intravenous chemotherapy in patients with metastatic breast cancer. Assuming equal efficacy of both treatments, most

10.3389/fonc.2024.1388087

TABLE 7 Practitioner preferences regarding treatment regimen.

| | All practitioners (*n* = 25) | Physicians (*n* = 11) | Nurses (*n* = 14) | *p*-value |
|---|---|---|---|---|
| **Agreement with different application forms** | | | | |
| Oral therapy | 1.4 (0.5) | 1.4 (0.5) | 1.4 (0.5) | 0.974 |
| Intravenous | 2.2 (0.8) | 2.2 (0.9) | 2.1 (0.8) | 0.861 |
| Subcutaneous | 1.9 (0.8) | 2.0 (0.8) | 1.9 (0.9) | 0.642 |
| Intramuscular | 2.8 (1.0) | 2.6 (0.9) | 2.9 (1.0) | 0.491 |
| **Agreement with different consultation intervals** | | | | |
| Weekly | 2.8 (0.9) | 2.6 (0.8) | 2.9 (1.0) | 0.301 |
| Every 3 weeks | 2.0 (0.8) | 1.8 (0.8) | 2.1 (0.8) | 0.431 |
| Every 4 weeks | 1.6 (0.6) | 1.6 (0.5) | 1.6 (0.6) | 0.756 |
| Every 3 months | 2.3 (1.0) | 1.9 (0.7) | 2.6 (1.1) | 0.103 |
| **Agreement with different therapy regimens** | | | | |
| …Continuous regimen | 1.6 (0.5) | 1.6 (0.5) | 1.6 (0.5) | 0.899 |
| …21/7 regimen | 2.5 (0.7) | 2.5 (0.7) | 2.5 (0.8) | 0.754 |

For each option, the mean rating on a four-point Likert scale (1 = strongly agree, 2 = partially agree, 3 = partially disagree, 4 = strongly disagree) and the standard deviation (in parentheses) are shown in the overall cohort (gray background) and in the subgroups of nurses and physicians. *p*-values (from the Kruskal–Wallis test) when comparing the results between the subgroups are shown. The mean agreement scores are visualized in the corresponding Figure 5.

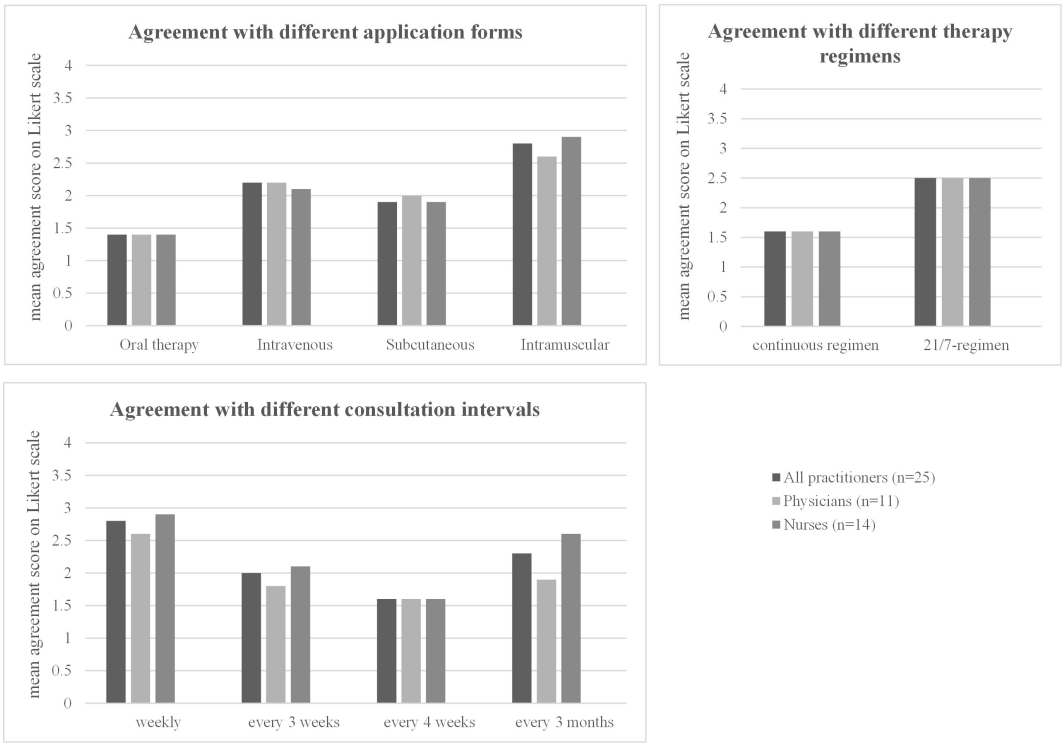



FIGURE 5
Practitioner preferences regarding the treatment regimen. For each option, the mean rating on a four-point Likert scale (1 = strongly agree, 2 = partially agree, 3 = partially disagree, 4 = strongly disagree) in the overall cohort and in the subgroups of nurses and physicians is shown. The detailed values for each subgroup, standard deviations, and *p*-values when comparing the results between the subgroups are shown in the corresponding Table 7.

respondents indicated a preference for oral (72%) over intravenous (11%) chemotherapy. The most frequently cited advantages of oral chemotherapy were ease of drug administration at home (76%), fewer appointments at the treatment center (81%), and better compatibility with work or leisure time (73%). The survey also assessed patients' tolerance of various adverse events of varying severity caused by oral chemotherapy. Respondents were least willing to tolerate adverse events such as hand-foot syndrome, diarrhea, neuropathy, and nausea with a severity of CTCAE grade III/IV (25).

Similar results regarding treatment regimen preferences were obtained in the real-world survey conducted as part of this work. All patients, whether they had previous experience with oral therapies (patients currently receiving endocrine therapy or endocrine-based

therapy) or not (patients currently receiving chemotherapy), strongly preferred oral therapy when asked for their approval of different therapy regimens. Patients currently receiving endocrine-based therapy reported that therapy was more compatible with daily life, leisure time, and vacations than patients receiving chemotherapy. This is encouraging, because in a study of patients with metastatic breast cancer that did not focus specifically on treatment regimens, more than half reported that their disease had "very much" affected their family's well-being, and one-fifth reported that it had strongly affected their responsibilities and social life (26). In our survey, endocrine-based therapy was also reported to be highly compatible with work. This is important because women with metastatic breast cancer who could continue to work had a better quality of life than those who could not (26). This is probably one of the greatest benefits of oral antitumor therapy that we were able to could confirm in our study: the drugs are taken at home, independent of a clinical setting, and can be flexibly integrated into a person's daily routine.

The different oral CDK4/6 inhibitors differ in terms of side effects and regimen between continuous intake and 21-day intake followed by a 7-day break. Our study does not provide a clear indication as to whether patients prefer a particular dosing regimen for oral antitumor therapy: 47.6% of the respondents chose the 21/7 regimen and 52.4% the continuous regimen. Looking at different patient groups, it becomes clear that patients are most likely to vote for the regimen they are already familiar with: patients on endocrine monotherapy voted predominantly for the continuous regimen. Patients on endocrine-based therapy (with a high proportion of palbo-/ribociclib-experienced patients) voted for the 21/7 regimen and patients on chemotherapy—unfamiliar with either regimen—voted approximately 50/50%. Practitioners, on the other hand, strongly favored the continuous regimen, probably because it appears to be easier to manage. When the therapy regimens were associated with specific side effects of varying severity, most of the respondents chose the regimen in which the adverse event occurred for a shorter period of time, e.g., only 2 days per month instead of 7 days per month. However, patients on endocrine monotherapy still tended to stick with the continuous regimen and patients on endocrine-based therapy tended to stick with the 21/7 regimen—even if this was the regimen in which the side effect lasted longer. This was true for mild CTCAE grade 1 side effects (diarrhea, fatigue, polyneuropathy) and also for CTCAE grade 3 neutropenia. This result again reflects the tendency of patients to prefer a treatment regimen with which they are already familiar. However, when the side effect was severe (CTCAE grade 3), the influence of "habit" became less important: patients generally chose the treatment regimen in which the side effect occurred for a shorter period of time, regardless of whether it was a continuous or a 21/7 regimen.

In general, the establishment of a continuous and intimate relationship between practitioners and patients, as well as regular appointments with the possibility of needs-based therapy support, can contribute significantly to increase adherence to oral antitumor therapy. Regarding therapy accompaniment, we might assume certain wishes of our patients, but we should analyze scientifically what is really important to our patients, to establish tools, personal contact, and visits preferred and to abandon those not much used.

In our study, most patients preferred to see their oncologist every 4 weeks. In general, patients receiving endocrine monotherapy and endocrine-based therapy were more comfortable with longer intervals between visits than those currently receiving chemotherapy. The general trend of highest support for a visit frequency of approximately once a month (every 3 or 4 weeks) is also reflected in the practitioners' survey. They also seem to have the best experience with these consultation intervals and do not want to see their patients less often, even though this would further reduce their workload—presumably, regular check-ups are still necessary for them to monitor the therapy. There were no differences between physicians and nurses. It also seems to be important for all patients, regardless of their treatment regimen, to have a constant contact person in the outpatient oncology setting, who may be either a physician or a specialized nurse. Specialized nurses are an adaptation that most oncology facilities have already implemented to improve therapy accompaniment. All breast centers in Germany already work with breast care nurses, and some also employ advanced practice nurses (APNs). APNs are academically qualified nurses, who offer specialized, nurse-led consultations, with extensive pretreatment discussions and close monitoring during therapy (27). However, in a recent UK study, only 56% of patients with metastatic breast cancer had access to a specialized nurse (26). A further development to provide patients with the best possible support throughout their treatment is the patient/onco-coach treatment model. Onco-coaches are the central link between the physician and the patient. They provide support and education to patients throughout the entire treatment process and act as a personal advisor. In addition, they collect feedback from patients and pass it on in a targeted manner (28). Onco-coaches are not yet common in Germany.

In-depth patient education is one of the central aspects of oral antitumor therapy. Regular personal training sessions on the individual drugs, interactions, side effects, prophylaxis, and behavior in everyday life can increase patients' knowledge, especially about oral tumor therapies, and help to promote motivation and self-management. A recent survey of metastatic breast cancer patients reported that information is very important to the patients: 71% of all patients in this study reported that they wished they had known more about metastatic breast cancer before their diagnosis and 47% reported that they still do not fully understand their disease (26). In addition to treatment management, breast cancer patients also have some very specific information needs, such as information about sexual health (29). Even 15 months after diagnosis, breast cancer patients report unmet needs (30).

It is likely that patient education should be more in-depth for patients undergoing endocrine-based therapies compared with traditional intravenous chemotherapy, where patients receive their medications and co-medications in a controlled medical setting. In our survey, outpatient oncology consultations received the highest agreement scores as sources of information, regardless of treatment regimen and for both general questions and side effects. However, the Internet is also used as a source of information (also reflected in high agreement scores). A recent study of newly diagnosed breast cancer patients found similar

results: in this study, physicians and nurses were the most important sources of information, closely followed by the Internet which was used by 81% of all patients in this study (31). Numerous other studies also report the use of the Internet as an important source of information (26, 32). These findings highlight the importance of developing evidence-based websites and online information tools for patients to obtain reliable, peer-reviewed information and to share these web-based information resources with patients during face-to-face consultations. This will contribute significantly to patient education and will prevent patients from receiving unfiltered and even false information from various not well-controlled websites. Various tools and websites already exist, e.g., from patient support groups or oncology societies. In Germany, new legislation allows for the prescription of validated eHealth tools: the digital coach "PINK!" contributes to patient education through coaching modules. Patients receive pseudo-individualized information, practical tips, instructions, and tutorials in the areas of exercise, nutrition, and mental health (33).

Additional tools can be helpful not only for patient education but also for treatment management (34). Traditional tools include patient diaries or calendars that record, for example, pill intake, side effects and complaints, and upcoming appointments. However, all these tools received rather low approval rates in our study. For some time now, eHealth-based therapy support has been available, such as the CANKADO app with artificial intelligence-based individualized support to reduce severe side effects and increase adherence. CANKADO reliably reminds patients of upcoming pills or other medication-specific tasks, such as blood glucose measurements, and offers the possibility to document daily symptoms. The recorded health status can serve as a basis for the next discussion with the attending physician. The goal is to conserve resources on the part of care providers (staff, time) and patients (independence, time) (22). It has been shown that the use of CANKADO in metastatic breast cancer patients can prolong the time to quality of life deterioration (35). The use of the app received the highest agreement scores of all therapy management tools in our survey but with a still rather low mean agreement score of 3.1 in the overall cohort. This score was also not significantly higher in patients on endocrine-based therapy, for whom it could be a helpful tool for self-management of their therapy. Regular support and assistance from a specially trained caregiver like an APN or an onco-coach may increase the number of users and thus improve adherence in patients with oral antitumor therapy.

Our study represents an important real-world experience of patients with breast cancer, but several limitations should still be considered. The total number of patients—with 42 patients divided into three subgroups—is quite small. However, our study still provides a representative sample of breast cancer patients treated in oncology centers today. Comparable studies, analyzing, e.g., the information-seeking behavior of breast cancer patients, achieve comparable patient numbers and come to comparable results (31). Therefore, we were able to present qualitative data on the important wishes and needs of these patients and can also provide important subgroup analyses.

As the questionnaires were designed specifically for this study, we cannot provide a validated survey. However, it is difficult to obtain such individual and subjective information in a validated and rigid survey. Often, such valuable information is provided in patient workshops, so that qualitative surveys are already an improvement over pure workshops (36). Another limitation may be that the different CDK4/6 inhibitors, which were grouped under the term "endocrine-based therapy" in our study, have different treatment regimens and side effects, which may also influence the needs reported by patients. However, the limited number of patients in this subgroup ($n$ = 17) would have resulted in very small patient numbers if each CDK4/6 inhibitor were considered in isolation. This analysis addresses the overall difference between CDK4/6 inhibitors and chemotherapy or endocrine monotherapy. Even if the CDK4/6 inhibitors have different treatment regimens, the differences with completely different forms of therapy, such as intravenous chemotherapy, are likely to be much greater. The aim of this study was to identify the general differences in treatment preferences among patients undergoing endocrine-based therapy. Furthermore, our study is monocentric and center-specific effects cannot be excluded. In addition, the patient population may be somewhat biased due to a selection for more complex cases at a university hospital. Patients in smaller outpatient oncology centers with, e.g., fewer additional diagnoses, might have slightly different wishes.

In conclusion, our study showed a high desire for oral antitumor therapies among both patients and practitioners without a clear preference for a specific therapy regimen. Since oral therapies are taken by patients on their own, thorough patient education—about the individual therapy regimen, relevant side effects, and their management and potential interactions—is necessary to ensure therapy safety. Patients need to be provided with written booklets with detailed information about their therapy, which they can use to look up information at home, but which they can also take with them in case of an emergency to inform the medical team in the emergency department. Of course, they need to know all emergency contacts and phone numbers. For outpatient oncology care, patients should be seen regularly every 4 weeks, either in person or by phone or video call. Specialized nurses or additional tools such as eHealth modules can help to improve treatment management.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving humans were approved by LMU Munich ethics comittee, LMU Munich, Munich, Germany. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/ next of kin because the only way of gathering information on patients was by asking for them in questionnaires. Patients were

informed about the survey when they received the questionnaires. When patients returned the questionnaires, this was regarded as consent to participate and therefore no separate informed consent was required according to the ethical approval.

## Author contributions

AH: Formal analysis, Validation, Visualization, Writing – original draft. FH: Data curation, Formal analysis, Investigation, Project administration, Visualization, Writing – review & editing. AD: Writing – review & editing. CS: Writing – review & editing. AK: Writing – review & editing. NH: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. RW: Conceptualization, Funding acquisition, Resources, Supervision, Validation, Writing – review & editing.

## Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by an educational grant by Lilly. The financial sponsor had no influence on planning and execution of the project, data analysis or on the manuscript.

## Acknowledgments

We thank all the patients and practitioners who were willing to contribute to this analysis. We thank Verena Semmlinger for the statistical advice.

## Conflict of interest

AH received honoraria for advisory boards and lectures and support for training programs/congress participation from Roche, Pfizer, Gilead and Sandoz/Hexal. RW reports potential COIs with Agendia, Amgen, Aristo, Astra Zeneca, Boeringer Ingelheim, Carl Zeiss, Celgene, Clovis Oncology, Daiichi-Sankyo, Eisai, Exact Sciences, Genomic Health, Gilead, Glaxo Smith Kline, Hexal, Lilly, Medstrom Medical, MSD, Mundipharma, Mylan, Nanostring, Novartis, Odonate, Paxman, Palleos, Pfizer, Pierre Fabre, PINK, PumaBiotechnolgogy, Riemser, Roche, Sandoz/Hexal, Sanofi Genzyme, Seattle Genetics/Seagen, Stemline, Tesaro Bio, Teva, Veracyte, Viatris, FOMF, Aurikamed, Clinsol, Pomme Med, medconcept, MCI. NH reports honoraria for lectures and/or consulting from: Amgen, AstraZeneca, Daiichi-Sankyo, Gilead, Lilly, MSD, Novartis, Pierre-Fabre, Pfizer, Roche, Sandoz, and Seagen.

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

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1388087/full#supplementary-material

SUPPLEMENTARY FILE 1
Questionnaire for patients (in German).

SUPPLEMENTARY FILE 2
Questionnaire for practitioners (in German).

SUPPLEMENTARY FILE 3
Questionnaire for patients (translated to English for submission).

SUPPLEMENTARY FILE 4
Questionnaire for practitioners (translated to English for submission, shortened to the questions relevant for this publication).

## References

1. Manders K, van de Poll-Franse LV, Creemers GJ, Vreugdenhil G, van der Sangen MJ, Nieuwenhuijzen GA, et al. Clinical management of women with metastatic breast cancer: a descriptive study according to age group. *BMC cancer*. (2006) 6:179. doi: 10.1186/1471-2407-6-179

2. Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, et al. Breast cancer. *Nat Rev Dis primers*. (2019) 5:66. doi: 10.1038/s41572-019-0111-2

3. Kimbung S, Loman N, Hedenfalk I. Clinical and molecular complexity of breast cancer metastases. *Semin Cancer Biol*. (2015) 35:85–95. doi: 10.1016/j.semcancer.2015.08.009

4. Eggersmann TK, Degenhardt T, Gluz O, Wuerstlein R, Harbeck N. CDK4/6 inhibitors expand the therapeutic options in breast cancer: palbociclib, ribociclib and abemaciclib. *BioDrugs: Clin immunotherapeutics biopharmaceuticals Gene Ther*. (2019) 33:125–35. doi: 10.1007/s40259-019-00337-6

5. Baselga J, Campone M, Piccart M, Burris HA, Rugo HS, Sahmoud T, et al. Everolimus in postmenopausal hormone-receptor–positive advanced breast cancer. *New Engl J Med*. (2011) 366:520–9. doi: 10.1056/NEJMoa1109653

6. André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for PIK3CA-mutated, hormone receptor–positive advanced breast cancer. *New Engl J Med*. (2019) 380:1929–40. doi: 10.1056/NEJMoa1813904

7. Robson M, Im S-A, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. *New Engl J Med*. (2017) 377:523–33. doi: 10.1056/NEJMoa1706450

8. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee K-H, et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. *New Engl J Med*. (2018) 379:753–63. doi: 10.1056/NEJMoa1802905

9. Cardoso F, Senkus E, Costa A, Papadopoulos E, Aapro M, André F, et al. 4th ESO–ESMO international consensus guidelines for advanced breast cancer (ABC 4)†. *Ann Oncol*. (2018) 29:1634–57. doi: 10.1093/annonc/mdy192

10. Lüftner D, Fasching PA, Haidinger R, Harbeck N, Jackisch C, Müller V, et al. ABC6 consensus: assessment by a group of german experts. *Breast Care*. (2022) 17:90–100. doi: 10.1159/000522068

11. Finn RS, Martin M, Rugo HS, Jones S, Im SA, Gelmon K, et al. Palbociclib and letrozole in advanced breast cancer. *New Engl J Med*. (2016) 375:1925–36. doi: 10.1056/NEJMoa1607303

12. Finn RS, Rugo HS, Dieras VC, Harbeck N, Im S-A, Gelmon KA, et al. Overall survival (OS) with first-line palbociclib plus letrozole (PAL+LET) versus placebo plus letrozole (PBO+LET) in women with estrogen receptor–positive/human epidermal growth factor receptor 2–negative advanced breast cancer (ER+/HER2– ABC): Analyses from PALOMA-2. *J Clin Oncol*. (2022) 40:LBA1003–LBA. doi: 10.1200/JCO.2022.40.17_suppl.LBA1003

13. Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Paluch-Shimon S, et al. Ribociclib as first-line therapy for HR-positive, advanced breast cancer. *New Engl J Med*. (2016) 375:1738–48. doi: 10.1056/NEJMoa1609709

14. Hortobagyi GN, Stemmer SM, Burris HA, Yap Y-S, Sonke GS, Hart L, et al. Overall survival with ribociclib plus letrozole in advanced breast cancer. *New Engl J Med*. (2022) 386:942–50. doi: 10.1056/NEJMoa2114663

15. Johnston S, Martin M, Di Leo A, Im S-A, Awada A, Forrester T, et al. MONARCH 3 final PFS: a randomized study of abemaciclib as initial therapy for advanced breast cancer. *NPJ Breast Cancer*. (2019) 5:5. doi: 10.1038/s41523-018-0097-z

16. Sledge GW Jr., Toi M, Neven P, Sohn J, Inoue K, Pivot X, et al. The effect of abemaciclib plus fulvestrant on overall survival in hormone receptor–positive, ERBB2-negative breast cancer that progressed on endocrine therapy—MONARCH 2: A randomized clinical trial. *JAMA Oncol*. (2020) 6:116–24. doi: 10.1001/jamaoncol.2019.4782

17. Goetz MP, Toi M, Huober J, Sohn J, Tredan O, Park IH, et al. LBA15 MONARCH 3: Interim overall survival (OS) results of abemaciclib plus a nonsteroidal aromatase inhibitor (NSAI) in patients (pts) with HR+, HER2-advanced breast cancer (ABC). *Ann Oncol*. (2022) 33:S1384. doi: 10.1016/j.annonc.2022.08.009

18. Prescribing information Verzenios® 50/100/150mg. Available online at: https://www.ema.europa.eu/en/documents/product-information/verzenios-epar-product-information_en.pdf (Accessed 01-09-2023).

19. Prescribing information Ibrance® 75/100/125mg. Available online at: https://www.ema.europa.eu/en/documents/product-information/ibrance-epar-product-information_en.pdf (Accessed 01-09-2023).

20. Prescribing information Kisqali® 200mg. Available online at: https://www.ema.europa.eu/en/documents/product-information/kisqali-epar-product-information_en.pdf (Accessed 01-09-2023).

21. Thill M, Schmidt M. Management of adverse events during cyclin-dependent kinase 4/6 (CDK4/6) inhibitor-based treatment in breast cancer. *Ther Adv Med Oncol*. (2018) 10:1758835918793326. doi: 10.1177/1758835918793326

22. CANKADO. PRO-React-Onco. Available online at: www.cankado.com (Accessed 01-09-2023).

23. Liu G, Franssen E, Fitch MI, Warner E. Patient preferences for oral versus intravenous palliative chemotherapy. *J Clin Oncol*. (1997) 15:110–5. doi: 10.1200/JCO.1997.15.1.110

24. Eek D, Krohe M, Mazar I, Horsfield A, Pompilus F, Friebe R, et al. Patient-reported preferences for oral versus intravenous administration for the treatment of cancer: a review of the literature. *Patient preference adherence*. (2016) 10:1609–21. doi: 10.2147/PPA

25. Jaisle A, Fortune EE, Jacobs J, Saxton C, Ackourey J, Zaleta AK. (2020). Abstract PS9-07: Metastatic breast cancer patients' preferences and expectations for oral chemotherapy, presented at the conference: *San Antonio Breast Cancer Virtual Symposium; December 8-11, 2020*, San Antonio, Texas. doi: 10.1158/1538-7445.SABCS20-PS9-07

26. Fallowfield L, Starkings R, Palmieri C, Tait A, Stephen L, May S, et al. Living with metastatic breast cancer (LIMBER): experiences, quality of life, gaps in information, care and support of patients in the UK. *Support Care Cancer*. (2023) 31:459. doi: 10.1007/s00520-023-07928-8

27. Tracy MF, O'Grady E. *Hamric and Hanson's Advanced Practice Nursing: An integrative approach. 6th ed*. St. Louis: Elsevier (2018).

28. Zamora P, Riese C, Borges U, et al. Patientenkompetenz in der oralen Krebstherapie (PACOCT). *Forum*. (2015) 29:42–5. doi: 10.1007/s12312-013-1085-8

29. Aupomerol M, Chaltiel D, Pautier P, Wehrer D, Véron L, Degousée L, et al. Breast cancer patients' Experience and wishes regarding communication on sexual health: the BEROSE study. *Cancer Invest*. (2022) 40:483–93. doi: 10.1080/07357907.2022.2066112

30. Lo-Fo-Wong DN, de Haes HC, Aaronson NK, van Abbema DL, Admiraal JM, den Boer MD, et al. Health care use and remaining needs for support among women with breast cancer in the first 15 months after diagnosis: the role of the GP. *Fam Pract*. (2020) 37:103–9. doi: 10.1093/fampra/cmz043

31. Ludwigson A, Huynh V, Vemuru S, Romandetti K, Fisher C, Coons HL, et al. Characterizing informational needs and information seeking behavior of patients with breast cancer. *Am J Surg*. (2024) 227:100–5. doi: 10.1016/j.amjsurg.2023.09.047

32. van Eenbergen M, Vromans RD, Boll D, Kil PJM, Vos CM, Krahmer EJ, et al. Changes in internet use and wishes of cancer survivors: A comparison between 2005 and 2017. *Cancer*. (2020) 126:408–15. doi: 10.1002/cncr.32524

33. Bundesinstitut für Arzneimittel und Medizinprodukte. DiGa-Verzeichnis. PINK!-Coach. Available online at: https://diga.bfarm.de/de/verzeichnis/1464 (Accessed 01-09-2023).

34. Fenerty SD, West C, Davis SA, Kaplan SG, Feldman SR. The effect of reminder systems on patients' adherence to treatment. *Patient preference adherence*. (2012) 6:127–35. doi: 10.2147/ppa.S26314

35. Harbeck N, Fasching PA, Wuerstlein R, Degenhardt T, Lüftner D, Kates RE, et al. CANKADO PRO-React eHealth support in patients with HR+ HER2- metastatic breast cancer receiving palbociclib and endocrine therapy and the affect on time to deterioration of quality of life: Primary outcome analysis of the multicenter randomized PreCycle trial. *J Clin Oncol*. (2023) 41:1008. doi: 10.1200/JCO.2023.41.16_suppl.1008

36. Fallowfield L, Boyle FM, Travado L, Kiely BE, Jewell P, Aubel D, et al. Gaps in care and support for patients with advanced breast cancer: A report from the advanced breast cancer global alliance. *JCO Glob Oncol*. (2021) 7:976–84. doi: 10.1200/GO.21.00045

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 10 July 2024
DOI 10.3389/fendo.2024.1433707



OPEN ACCESS

EDITED BY
Barbara Altieri,
University Hospital of Wuerzburg, Germany

REVIEWED BY
Claudia Pivonello,
University of Naples Federico II, Italy
Piero Ferolla,
Umbria Regional Cancer Network, Italy

*CORRESPONDENCE
Giovanni Vitale
giovanni.vitale@unimi.it

†These authors have contributed equally to this work

RECEIVED 16 May 2024
ACCEPTED 25 June 2024
PUBLISHED 10 July 2024

CITATION
Oldani M, Cantone MC, Gaudenzi G, Carra S, Dicitore A, Saronni D, Borghi MO, Lombardi A, Caraglia M, Persani L and Vitale G (2024) Exploring the multifaceted antitumor activity of axitinib in lung carcinoids. *Front. Endocrinol.* 15:1433707. doi: 10.3389/fendo.2024.1433707



# Exploring the multifaceted antitumor activity of axitinib in lung carcinoids

Monica Oldani[1†], Maria Celeste Cantone[1†], Germano Gaudenzi[1], Silvia Carra[2], Alessandra Dicitore[3], Davide Saronni[3,4], Maria Orietta Borghi[5,6], Angela Lombardi[7], Michele Caraglia[7,8], Luca Persani[2,3] and Giovanni Vitale[1,3*]

[1]Laboratory of Geriatric and Oncologic Neuroendocrinology Research, IRCCS, Istituto Auxologico Italiano, Milan, Italy, [2]Laboratory of Endocrine and Metabolic Research, IRCCS, Istituto Auxologico Italiano, Milan, Italy, [3]Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy, [4]PhD Program in Experimental Medicine, University of Milan, Milan, Italy, [5]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy, [6]Experimental Laboratory of Immuno-Rheumatology, Istituto Auxologico Italiano, IRCCS, Milan, Italy, [7]Department of Precision Medicine, University of Campania "L. Vanvitelli", Naples, Italy, [8]Laboratory of Molecular and Precision Oncology, Biogem Scarl, Ariano Irpino, Italy

**Introduction:** Lung carcinoids (LCs) are a type of neuroendocrine tumor (NET) that originate in the bronchopulmonary tract. LCs account for 20–25% of all NETs and approximately 1–2% of lung cancers. Given the highly vascularized nature of NETs and their tendency to overexpress vascular growth factor receptors (VEGFR), inhibiting angiogenesis appears as a potential therapeutic target in slowing down tumor growth and spread. This study evaluated the long-term antitumor activity and related mechanisms of axitinib (AXI), a VEGFR-targeting drug, in LC cell lines.

**Methods:** Three LC cell lines (NCI-H727, UMC-11 and NCI-H835) were incubated with their respective $EC_{50}$ AXI concentrations for 6 days. At the end of the incubation, FACS experiments and Western blot analyses were performed to examine changes in the cell cycle and the activation of apoptosis. Microscopy analyses were added to describe the mechanisms of senescence and mitotic catastrophe when present.

**Results:** The primary effect of AXI on LC cell lines is to arrest tumor growth through an indirect DNA damage. Notably, AXI triggers this response in diverse manners among the cell lines, such as inducing senescence or mitotic catastrophe. The drug seems to lose its efficacy when the DNA damage is mitigated, as observed in NCI-H835 cells.

**Conclusion:** The ability of AXI to affect cell viability and proliferation in LC tumor cells highlights its potential as a therapeutic agent. The role of DNA damage and the consequent activation of senescence seem to be a prerequisite for AXI to exert its function.

KEYWORDS

**lung carcinoid, tyrosine kinase inhibitors, axitinib, cell cycle, senescence, mitotic catastrophe, reactive oxygen species**

# 1 Introduction

Lung carcinoids (LCs) are a type of neuroendocrine tumor (NET) that originate in the bronchopulmonary tract. LCs account for 20–25% of all NETs and approximately 1–2% of lung cancers. More than 80% of LCs are diagnosed at TNM stage I or II (1). In advanced tumors, the goals of therapeutic management are to control tumor proliferation and manage functioning syndromes through a multidisciplinary approach (2–4). However, LCs can be highly heterogeneous, responding differently to treatments. This variability can make it challenging to define the therapeutic approach and to find new effective therapies (5).

NETs are highly vascularized tumors, with 64–80% of cases exhibiting an overexpression of endothelial vascular growth factor (VEGF) and Vascular Endothelial Growth Factor receptors 1, 2 and 3 (VEGFR-1, -2, and -3) (6–8). Based on this, the inhibition of angiogenesis could have a key role in reducing the metastatic potential of these neoplasms. Several investigations have reported an important involvement of angiogenesis in the progression of lung NETs. Angiogenic factors, such as VEGF, Angiopoietin 2 (ANG2) and prokineticin 2 (PROK2) correlate with tumor aggressiveness (9–12). Uncontrolled activity of angiogenic factors in lung NETs can contribute to invasive tumor behavior, endothelial cell growth, and occurrence of metastasis (13). The significant roles played by VEGF in the growth and spread of lung NETs are supported by higher serum VEGF levels detected in patients with larger primary tumor sizes, nodal involvement, and distant metastases (13). Notably, LCs exhibit higher expression levels of VEGFR-2 and -3 compared to the other lung NETs (8). Moreover, a significant increase in VEGF expression is strongly associated with reduced survival in patients with LCs (14). All these findings have prompted to consider monoclonal antibodies against VEGF and VEGFR tyrosine kinase inhibitors (TKIs) as a possible treatment for lung NETs (9, 10, 15–21). Among these drugs, axitinib (AXI), a selective tyrosine kinase inhibitor targeting VEGF receptors (22–24), has shown to improve outcomes in patients with NETs (17).

In our previous research (25), we evaluated the antitumor activity of AXI on different human LC cell lines. We demonstrated that AXI reduced *in vitro* the viability rate of LC cell lines and induced a cell cycle arrest in the $G_2$/M phase after 3 days of treatment. AXI inhibited tumor-induced angiogenesis and reduced the invasiveness of LC cells in zebrafish *Tg(fli1a: EGFP)*$^{y1}$ embryos. All these findings supported the potential of AXI as a therapeutic agent in LCs. However, observing the effects on cells after a short-term treatment period, we cannot exclude the possibility that LC cells may develop drug resistance with prolonged treatment.

In the present study, we evaluated the long-term antitumor activity of AXI on human LC cell lines in inducing programmed cell death programs (senescence, apoptosis and mitotic catastrophe) and/or cell cycle arrest. Recognizing these cellular outcomes is of paramount importance to understand the cell behavior changes induced by AXI and to design new therapeutic approaches.

# 2 Materials and methods

## 2.1 Cells and reagents

Human LC cell lines NCI-H727, UMC-11 and NCI-H835 were purchased from ATCC and standard protocols were followed for their maintenance. These three different cell lines are representative of well-differentiated pulmonary NETs, classified as typical LCs. Among these three cell lines, the responses to pharmacological treatments can vary significantly, reflecting the heterogeneity reported in this tumor and making them useful for comparative studies (26–28). In brief, cells were routinely seeded in T75 flasks containing RPMI medium (EuroClone™, Milan, Italy) and supplemented with 10% heat-activated fetal bovine serum (FBS) (EuroClone™, Milan, Italy) and $10^5$ U·L−1 penicillin/streptomycin (EuroClone™, Milan, Italy). Prior to experiments, LC cell lines were counted using a standard hemocytometer. Cells utilized in all experiments were below 5 passages. Axitinib (AXI) (MedChemExpress, Monmouth Junction, NJ, USA) was diluted in dimethyl sulfoxide (DMSO) at the concentration of $10^{-2}$ M and stored at -80 C°.

## 2.2 Cell cycle and apoptosis evaluation

Cell cycle and apoptosis were investigated after 6 days of incubation with AXI. 1 x $10^5$ cells/well were seeded in 6-well plates in duplicate for both NCI-H727 and UMC-11, while 3 x $10^5$ cells/well were counted for NCI-H835. Twenty-four hours after seeding, the cell medium was replaced with RPMI supplemented with 0.1% DMSO (referred to as CTR) or with the specific $EC_{50}$ dose of AXI for each cell line. In detail, 2 x $10^{-6}$ M of AXI was used to treat NCI-H727 cell line, while a concentration of 4 x $10^{-7}$ M and 2.4 x $10^{-7}$ M were tested on UMC-11 and NCI-H835 cell lines, respectively. After 3 days, the RPMI medium was replaced again preserving the above-mentioned conditions for the CTR and AXI treatment. The sixth day, cells were harvested by trypsinization, washed with PBS, and collected with centrifugation. For cell cycle, a propidium iodide (PI) solution (50 µg/ml PI, 0.05% Triton X-100 and 0.6 µg/ml RNase A in 0.1% sodium citrate, all from Sigma-Aldrich® Merck KGaA, Darmstadt, Germany) was added to stain the pellets at 4°C for 30 minutes in the dark. For apoptosis, each sample was resuspended in 100 µl of 1X binding buffer (BB: 1.4M NaCl, 0.1M HEPES/NaOH, pH 7.4, 25 mM CaCl2) and incubated with 5 µl Annexin-V-fluorescein isothiocyanate (FITC) (BD Pharmingen, San Diego, CA, USA) and 10 µl PI (50 µg/ml in PBS) for 15 minutes at room temperature in the dark. Additional 400 µl of 1X BB have been added to each sample before the acquisition. Both Samples stained for cell cycle and apoptosis were analyzed through BD FACSLyric™ (BD Pharmingen, San Diego, CA, USA) flow cytometer using BD FACSuite™ Software on 10,000 events (BD Pharmingen, San Diego, CA, USA) (29).

## 2.3 Cell lysis and western blot analysis

LC cells were plated as described in the previous paragraph and incubated without (CTR) and with AXI for 6 days. Thereafter, the seeded cells were scraped in 50 µl of radio-immuno-precipitation assay lysis buffer (RIPA: 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) added with phosphatase (Roche, Basel, Switzerland) or protease inhibitors cocktail tablets (Sigma-Aldrich® Merck KGaA, Darmstadt, Germany). The cellular lysates were harvested by centrifuging at 15,000g for 30 minutes at 4°C. PierceTM BCA Protein Assay Kit (Thermo ScientificTM, Pierce Biotechnology, Illinois, USA) was used following manufacturer's instructions to detect the protein content in each supernatant. Ten micrograms of proteins per lane were separated on Mini-PROTEAN TGX 4–20% precast polyacrylamide gels (Bio-Rad, Bio-Rad Laboratories, Inc, USA) and transferred through iBlot Gel Transfer Stacks Nitrocellulose (Invitrogen by Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, nitrocellulose membranes were incubated with specific first antibody at 4°C overnight. Antibodies, all diluted 1:1000 and provided by Cell Signaling Technology (Danvers, MA, USA), were as follow: Caspase-3 (D3R6Y) Rabbit Ab; Poly(ADP-ribose)polymerase (PARP) (46D11) Rabbit Ab; Phospho-Chk1 (Ser345) (133D3) Rabbit mAb; Chk1 (2G1D5) Mouse mAb; p21 WAF1/Cip1 (12D1) Rabbit mAb; Phospho-p53 (Ser15) (16G8) Mouse mAb; Cyclin-B1 Rabbit Ab; Phospho-Histone H2A.X (Ser139) (D7T2V) Mouse mAb (γ-H2AX); Nuclear factor erythroid 2-related factor 2 (Nrf2) (D1Z9C) XP Rabbit mAb; Kelch-like ECH-associated protein 1 (Keap1) (D6B12) Rabbit mAb; and βActin (8H10D10) mouse mAb. After incubation with anti-mouse or anti-rabbit IgG, HRP-linked Ab (dilution 1:5000) (Cell Signaling Technology, Danvers, MA, USA), signals were detected through ECL Star Enhanced Chemiluminescent Substrate (Euroclone, Milan, Italy), and band exposition were revealed by Azure Imaging Systems (Azure Biosystems, Dublin, CA, USA). Band intensities were expressed as absolute unit, normalized by the level of β-actin expression in each sample and, at the end, compared with the untreated cell band.

## 2.4 Analysis of reactive oxygen species

The general production of intracellular reactive oxygen species (ROS) and nitric oxide (•NO) was revealed by the oxidation of 2′,7′-dichlorofluorescin diacetate (H2DCFDA) (Sigma Chemical Co., St. Louis, MO). Human LC cell lines were seeded in 96-well plates at density of 6 x $10^3$ cells/well for NCI-H727 and UMC-11 and 5 x $10^4$ cells/well for NCI-H835. The day after, cell medium was replaced without or with different concentration of AXI, as reported above. At the end of the third or sixth day, cells were incubated in the dark with 5 µM H2DCFDA diluted in PBS. At the end of 20 minutes incubation at 37°C, the probe fluorescence intensity was measured by the FL-1 channel (excitation = 485 nm; emission = 528 nm) using a microtiter plate reader (VICTOR X3, PerkinElmer) and analyzed by the PerkinElmer 2030 Manager software for Windows. H2DCFDA fluorescence was normalized by the total protein content (PierceTM BCA Protein Assay Kit, see above) in each sample.

## 2.5 β-gal senescence assay

Cells were seeded in 6-well plates in duplicate and incubated without and with AXI for 6 days, as previously described. Thereafter, we performed the β-galactosidase staining (pH 6.0) for measuring cell senescence, according to manufacturer's instructions (Cell Signaling Technology, Danvers, MA, USA). All images of stained cells were acquired by the inverted and epifluorescent Leica DMIRE2 microscope (Leica Microsystems, Illinois, USA) using the same parameters of magnification and light exposure. The blue pixel percentage in the total area of each image were performed using (Fiji Is Just) ImageJ software. At least 3 images were analyzed for each condition.

## 2.6 Morphological analysis

After 6 days of AXI treatment, LC cells were stained with two fluorescent dyes to quantify the crucial morphological parameters related to the mitotic catastrophe phenomenon. Hoechst Stain (Invitrogen$^{TM}$) diluted 1:1000 in PBS was used to identify cell nuclei, whereas Cell Tracker Green CMFDA (5-chloromethylfluorescein diacetate) staining (Invitrogen$^{TM}$) was used to monitoring the whole cell body. Human LC cell lines were seeded in duplicate in 6-well plates and incubated without and with AXI for 6 days, as described so far. At this point, two similar procedures have been applied for adherent (NCI-H727 and UMC-11) or in suspension (NCI-H835) cell lines. The NCI-H727 and UMC-11 cells were first incubated with Hoechst stain and then with Cell Tracker Green (0.125 mM). For both passages, an incubation time of 10 minutes at 37°C in the dark and an intermediate and gentle wash with PBS was required. On the other hand, for enhancing the adherence of cells to the plate, NCI-H835 cell line was primarily transferred into a new 6-well plate previously poly-lysinated and, after 3h of incubation, cells were stained with both Hoechst, as previously described. Detection of cell fluorescence for Hoechst blue images (excitation = 350 nm; emission = 450 nm filter) was performed through the inverted and epifluorescent Leica DMIRE2 microscope (Leica Microsystems, Illinois, USA). Thereafter, blue fluorescent signals for each cell in the images were quantified using (Fiji) Imagej software for analyzing the morphological parameters such as Area, Perimeter, and Circularity. All the quantified images have maintained the same pixel size and at least 3 images were analyzed for each condition.

## 2.7 Statistical analysis

All experiments were performed at least in triplicate. Statistical differences among groups were first calculated applying a Normality test (Shapiro-Wilk test), after we have carried out either an unpaired t test or a Two-ways ANOVA test followed by a *post hoc* test (Sidak's multiple comparison test). A p value $<0.05$ was considered significant. The values reported in the figures represent the mean ± Standard Error of the Mean (SEM). For statistical analysis, GraphPad Prism 8.0.1 (GraphPad Software, San Diego, CA, USA) was used.

10.3389/fendo.2024.1433707

# 3 Results

## 3.1 Human LC cell lines do not activate apoptosis after 6 days of AXI treatment

We have previously reported that the three LC cell lines showed different responsiveness to AXI in terms of inhibition of cell viability (25). UMC-11 cell line was the most sensitive to AXI treatment, with a maximal growth inhibitory effect of approximately 93%. The maximal growth inhibition induced by AXI on NCI-H727 was about 65%. Interestingly, NCI-H835 cells were sensitive to lower concentrations of AXI if compared to NCI-H727 cell line. However, the growth inhibitory effects of AXI on NCI-H727 were more pronounced at higher concentrations (Table 1). The AXI maximal growth inhibitory effect generally increased over the time (after 3 and 6 days of treatment) for all LC cell lines (Table 1). Based on these observations, we analyzed the possible activation of apoptosis after 6 days of treatment, which could be due to the decrease in viability observed in all tumor cell lines by either MTT or MTS assays (25). Therefore, each cell line was treated with its own $EC_{50}$ at 6 days of incubation (Table 1). Western blot analyses revealed that apoptotic mechanisms were activated in both UMC-11 and NCI-H835 cells, as demonstrated by a significant increase in cleaved caspase-3 (UMC-11 cells) and PARP (both cell lines) after treatment with AXI (Figure 1). However, cytofluorimetric analyses after Annexin-V and PI labelling showed that the percentage of dead LC cells were extremely low and did not increase after treatment with AXI (Figure 2). Moreover, a slight accumulation of cells in early and late apoptosis (EA and LA) or necrosis (N) was observed in UMC-11 and NCI-H835 cell lines, respectively (Figure 2).

## 3.2 Human LC cell lines undergo cell cycle arrest after 6 days of AXI treatment

Flow cytometric analysis after labelling of methanol-fixed cells with PI showed that the cell cycle was not altered before or after AXI in NCI-H835 (Figure 3). However, NCI-H727 and UMC-11 cell lines underwent to cell cycle arrest in $G_2$/M phase after treatment with AXI (Figure 3). Specifically, a significant reduction of the percentage of both cell lines in the $G_0/G_1$ phase and an increase in the $G_2$/M phase were observed after AXI (Figure 3). Cell cycle arrest at this stage could be indicative of not repairable DNA damage. Moreover, the observed increase of the cell population in the sub-$G_1$ phase, typically indicative of DNA fragmentation without detectable cell death, could be another indication of a possible DNA damage. AXI significantly induced an increase of the percentage of polyploid (>4N) cells only in NCI-H727 cells (Figure 3).

TABLE 1 Summary of MTT or MTS data.

| Cell lines | $EC_{50}$ 3D (M) | $EC_{50}$ 6D (M) | Maximal inhibition effect 3D | Maximal inhibition effect 6D |
|---|---|---|---|---|
| NCI-H727 | $8{,}6*10^{-6}$ | $1{,}9*10^{-6}$ | -27% | -65% |
| UMC-11 | $5*10^{-6}$ | $4*10^{-7}$ | -63% | -93% |
| NCI-H835 | $2.8*10^{-7}$ | $2*10^{-7}$ | -20% | -47% |

The table presents EC50 absolute values and the maximal inhibition effect obtained from three independent experiments assessing the effect of AXI on cell viability after 3 (3D) or 6 days (6D) of AXI treatment [data derived from 25]. These values are calculated by generating dose-response curves following MTT assays (for NCI-H727 and UMC-11) or MTS assays (for NCI-H835).

## 3.3 AXI induces DNA damage in human LC cell lines

The next step was to determine whether AXI could actually induce DNA damage in LC cells. In this view, γ-H2AX, a variant of the H2A histone family, was analyzed by Western blot, as it is rapidly phosphorylated at sites of DNA double-strand breaks and serves as a marker for these lesions. γ-H2AX levels were significantly enhanced after 6 days of AXI treatment in NCI-H727 and UMC-11, while no changes were recorded in NCI-H835 cells (Figures 4A, D). As suggested by Morelli et al. (30), AXI appeared to cause DNA damage through increased intracellular ROS. Indeed, we found a significant increase in ROS production after AXI treatment, measured at both short (3 days) and long times (6 days) of exposure to AXI in both NCI-H727 and UMC-11 cells (Figures 4E, F). As ROS levels remained high over time in NCI-H727 and UMC-11cells (Figures 4E, F), it is possible that ROS induced DNA damage and this condition might be sufficient to maintain a cell cycle arrest in both cell lines. On the other hand, the NCI-H835 cell line maintained a stable level of ROS over time (Figures 4E, F), with a concurrent downregulation of Keap1 ($p<0.05$) and upregulation of Nrf2 ($p<0.001$) after incubation with AXI (Figures 4B–D).

## 3.4 Pathways activated to counteract DNA damage in NCI-H727 and UMC-11 cell lines

Two critical pathways related to DNA damage and cell cycle arrest in the $G_2$/M phase were analyzed by Western blot: Chk1 and p53. In particular, during DNA damage or replication stress, ChK1 is activated by phosphorylation at Ser 345 (P-ChK1). Once activated, ChK1 affects cell cycle progression leading to cell accumulation, mainly at the $G_2$/M phase, as previously shown. We recorded an activation of ChK1 with an increase of about 1.5-fold in both NCI-H727 and UMC-11 cells after treatment with AXI (Figures 5A, B). The phosphorylation of p53 at Ser15 (pp53) is another regulatory event in cell response to DNA damage, contributing to both p53 stabilization and activation. In fact, after activation, pp53 induces cell cycle arrest at $G_2$/M phase and/or apoptosis in case of irreparable DNA damage. The phosphorylation of p53 at Ser 15 was different in these two cell lines. In UMC-11 cells, pp53 was 1.5-fold increased if compared to untreated controls, whereas in NCI-H727 cells it was more 0.5-fold decreased after exposure to AXI (Figures 5C, D). Notably, the p21 (WAF1/Cip1) protein, one of p53 main downstream effectors and key regulator of the $G_2$/M transition, was also augmented in UMC-11 cells after treatment with AXI (Figures 5C, D). However, a significative

10.3389/fendo.2024.1433707

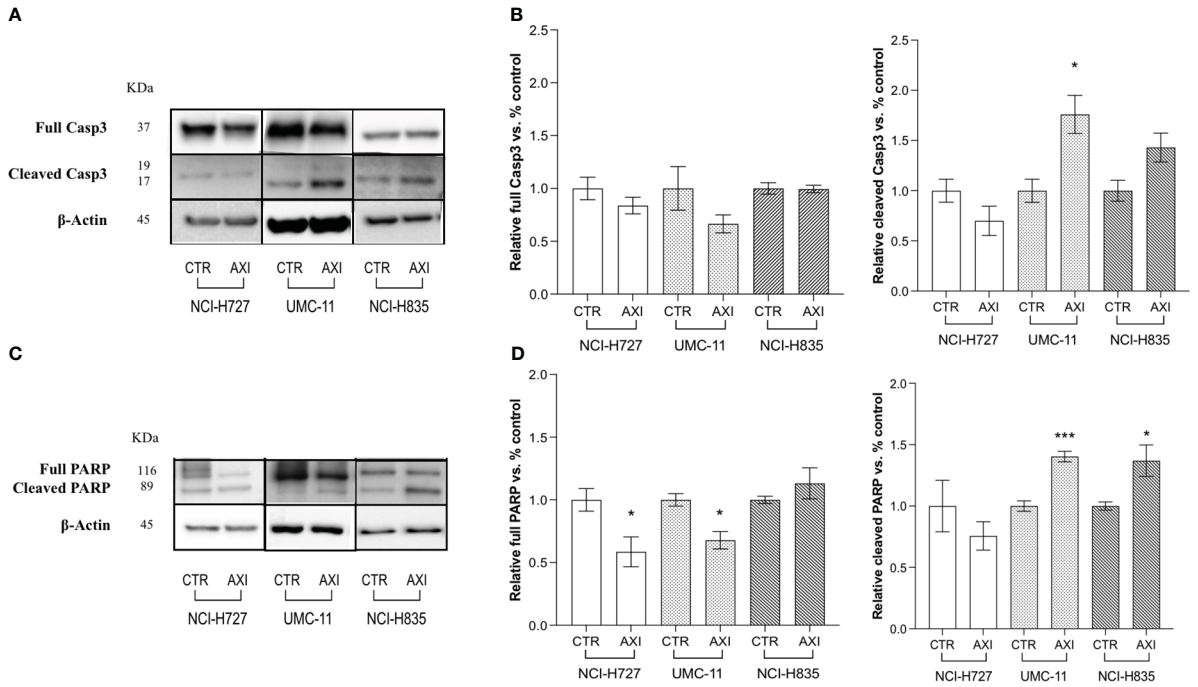



FIGURE 1
Examination of apoptosis activation by Western blot analysis after 6 days of incubation with or without AXI in each LC cell line. **(A, C)** are representative Western blot images for each target in LC cell lines. Histograms **(B, D)** summarize the relative expression change of each target. The targets analyzed are full and cleaved Caspase-3 **(A, B)** and PARP **(C, D)**. β-Actin is used as a loading control. Values represent the mean ± S.E.M. of a minimum of 3 independent experiments. The significance is calculated by performing an unpaired t-test between the control (CTR) and the treated group: *p<0.05; ***p<0.001.

increase in p21 expression was also observed in NCI-H727 cells, apparently independent from the p53 activation (Figures 5C, D).

## 3.5 NCI-H727 and UMC-11 cell lines show senescence features after AXI treatment

We hypothesized that p21 high expression can also serve as a biomarker for the activation of senescence. Therefore, we evaluated the activity of senescence-associated β-galactosidase (SA-β-gal), a conventional hallmark of senescence. A significant ($p<0.001$) increase in SA-β-gal activity was recorded in both NCI-H727 and UMC-11 cell lines treated with AXI confirming the senescence induction (Figures 6A, B). On the other hand, NCI-H835 cell line did not exhibit any feature of senescence after treatment with AXI (data not shown). However, the distinct morphological alterations seen exclusively in NCI-H727 cells treated with AXI, namely their increased size and flattened shape, could be not only attributed to senescence occurrence but also to mitotic catastrophe.

## 3.6 AXI induces mitotic catastrophe in NCI-H727 cells

Tetraploid tumor cells observed in NCI-H727 (see section 3.2, >4N), intrinsically susceptible to mitotic aberrations, could be relevantly sensitive to the induction of mitotic catastrophe. Since mitotic catastrophe is characterized by large cells with multiple micronuclei, the shape of tumor cells and their nuclei was evaluated in LC cells treated with AXI. After staining with Hoechst 33258, only NCI-H727 cells had a significant enlargement of the nucleus for both their area and circumference after AXI (Figures 6C, D). As a result of abnormal mitosis, the nuclei lose their circularity, showing a more complex shape, as indicated by an increase in the aspect ratio (AR) value (Figure 6D). NCI-H727 cells area and perimeter also increased (Figure 6E). Moreover, after closer inspection of the nuclear to cytoplasmic area ratio, it was observed that despite an overall increase in cell size after treatment with AXI, the nuclei of NCI-H727 cells did not enlarge with a similar extent to occupy a larger part of the cell (Figure 6F). In addition, Western blot analysis indicated that polyploid and senescent NCI-H727 cells showed lower levels of cyclin-B1, additionally suggesting the potential role of cell cycle arrest and senescence in this context (Figures 6G, H).

# 4 Discussion

LCs are complex tumors that demand a multidisciplinary approach and a sophisticated therapeutic strategy (2–4). Recently, several TKIs have been investigated in LCs (31–36). Among these, AXI, a potent and selective second-generation inhibitor of VEGFRs, has demonstrated effectiveness in treating metastatic NETs (37), advanced hepatocellular carcinoma (HCC) (38), non-small cell lung cancer (23, 39), advanced renal carcinoma (40), epithelial ovarian

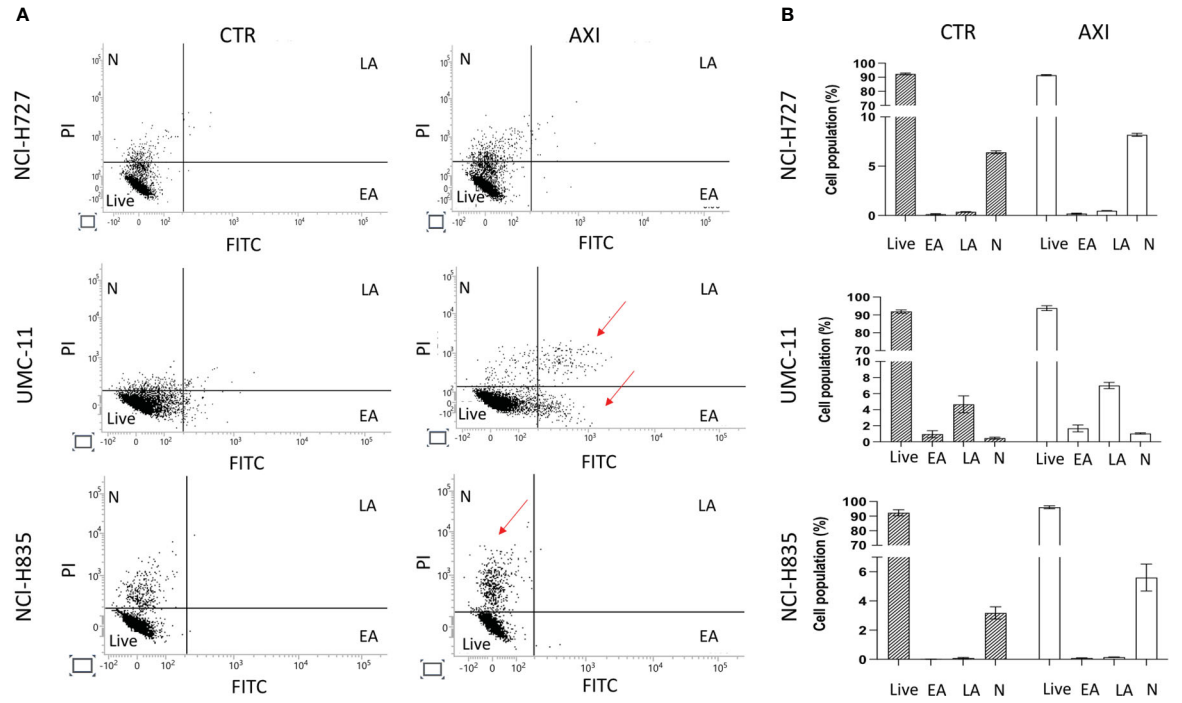



FIGURE 2
Apoptosis Analyses. **(A)** shows a representative distribution of the cell population in untreated cells (CTR) and AXI treated samples. A single event, represented as a dot, is correlated to FITC/PI fluorescence detection. The dials in each graph indicate the percentage of live cells (Live), cells in the early stages of apoptosis (EA), cells in the late stages of apoptosis (LA) or necrosis (N). The red arrows indicate where LC cells have been typically accumulated after treatment with AXI. Histograms in **(B)** summarize the average percentage ± S.E.M. of Live, EA, LA and N of three independent experiments. The results are analyzed by comparing the value in each dial between AXI and CTR samples (2way ANOVA test).

cancer (EOC) (41). In a recent study (25), we demonstrated that AXI reduced cell viability in preclinical models of human LC cell lines. Specifically, in NCI-H727 AXI induced high expression of cleaved PARP and caspase-3. At the same time, AXI showed a potent anti-proliferative effect in lung NCI-H727 and UMC-11 cell lines that was correlated to cell cycle arrest in $G_2$/M phase. Additionally, using *Tg(fli1a:EGFP)*$^{y1}$ zebrafish embryos implanted with the same LC cell lines, AXI was found to significantly inhibit tumor-induced angiogenesis and tumor cell invasiveness.

Our current research has unveiled a range of anti-tumor effects exerted by AXI after prolonged treatment, as depicted in Figure 7. AXI inhibited cell viability in human LC cell lines in a time-dependent manner. By comparing the MTT or MTS results acquired after 3 or 6 days of cell exposure to AXI, we observed that the anti-tumor effect of this drug increased over time (Table 1). Notably, for the NCI-H727 cells, extending the treatment from 3 to 6 days resulted in a 4-fold reduction in the $EC_{50}$ value and a 3-fold increase in the maximal growth inhibitory effect (Table 1). The UMC-11, the most sensitive cell line, exhibited a decrease in the $EC_{50}$ value from $5*10^{-6}$ M to $4*10^{-7}$ M, with only around 10% of cells surviving at the maximum AXI concentration (Table 1). Even the relatively resistant NCI-H835 cell line showed a decrease in cell viability with the drug. Although the change in $EC_{50}$ values between 3 and 6 days was not significant (from $2.8*10^{-7}$ M to $2*10^{-7}$ M), the drug reduced cell viability at the maximum inhibition dose by half (Table 1). The reduction in cell viability can be attributed either to the activation of cell death processes or to a cell cycle arrest. While our expectations were that prolonged exposure to AXI might increase cell mortality, the results revealed significant variability of these cells in the response to the drug (Figure 7).

NCI-H727 cells, which initiated apoptotic processes at a short incubation time (25), did not sustain the activation of the apoptotic pathways after 6 days. UMC-11 cell lines maintained an active apoptotic response, at least at molecular level. Finally, NCI-H835 cells, which did not display an early response to the drug during the short exposure (25), showed an increase in cleaved PARP that could be due to the potential activation of the apoptotic pathway. However, cytofluorimetry analyses indicated that the induction of cell death was not the primary mechanism behind the decrease in the cell viability after 6 days of treatment with AXI in all LC cell lines. Indeed, the number of cells that were annexin-V and/or PI positive after 6 days was generally low.

One potential drug tolerance strategy adopted by tumor cells is the modification of their own cell cycle (42). On this light, NCI-H835 cell line showed no cell cycle arrest, whereas both NCI-H727 and UMC-11 cell lines showed cell cycle arrest in the $G_2$/M phase.

The underlying causes of this cell cycle arrest could be linked to the occurrence of DNA damage in the treated cells, as indicated by the notable elevation in γ-H2AX expression compared to the control cells observed in the Western blot analysis (43). Since the production of ROS can result in permanent DNA damage (44), we also assessed whether AXI could induce ROS increase in the cytosol of treated cells. We observed an upregulation of activated γ-H2AX and an increase in ROS levels only in NCI-H727 and UMC-11 cells, suggesting that AXI

10.3389/fendo.2024.1433707

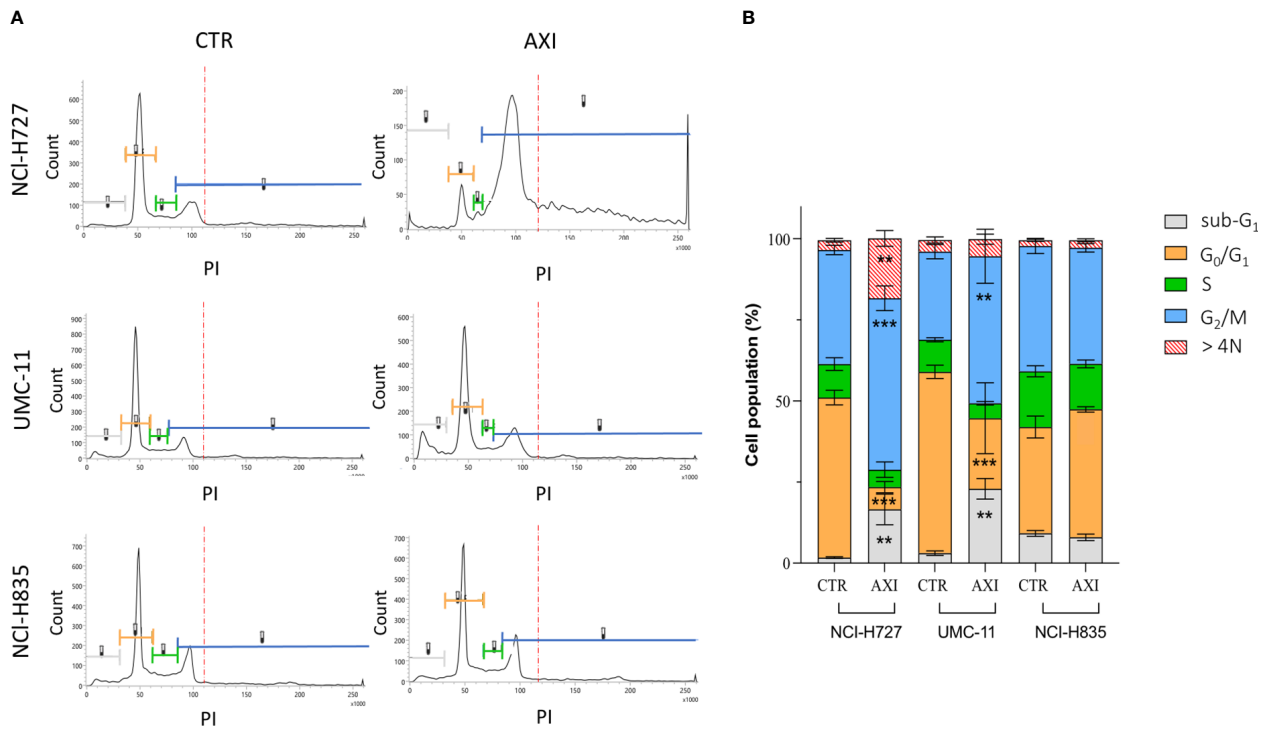



FIGURE 3
Modulation of cell cycle in NCI-H727, UMC-11 and NCI-H835 cell lines after 6 days of incubation with AXI. The different phases of the cell cycle are associated with the same reference colors in both images. **(A)** Compendium of representative profiles of the cell cycle distribution of the different cell lines without or with AXI. **(B)** Graph of the mean percentage of cells in the sub-$G_1$, $G_0/G_1$, S, $G_2$/M phases and >4N for control (CTR) and treated samples. The results of three independent experiments are analyzed by comparing the cell percentage in each phase between AXI and CTR samples (2way ANOVA test): ** p<0.01; ***p<0.001.

utilizes this mechanism to induce a block of cell cycle progression in the $G_2$/M phase (Figure 7). Supporting this hypothesis, similar findings were reported in other *in vitro* studies on renal cell carcinoma (30, 45) and glioma cell lines (46) treated with AXI. DNA damage normally leads to apoptosis, but efficient DNA repair mechanisms prevent cell death, allowing cells to survive despite the initial damage. Furthermore, the rise in cell population in the sub-$G_1$ phase in both NCI-H727 and UMC-11 cell lines, typically indicative of DNA fragmentation, without detectable cell death, suggests effective repair mechanisms or potential involvement of alternative cellular processes, such as senescence. These processes may represent protective responses to oxidative stress. Briefly, we hypothesize that all these results can indicate the presence of a potential reciprocal relationship between DNA damage response and ROS generation, which is adequate to sustain cell cycle arrest and, potentially, to trigger senescence in both cell lines (47). These data also suggest that the arrest of cell proliferation and cellular senescence induced by DNA damage could play a crucial role in the response of tumors to AXI, similar to what occurs in chemotherapy (48–50). Indeed, we evaluated the expression of pp53, Chk1, activated by the presence of DNA damage, and of p21 (WAF1/Cip1), playing a major role in cell cycle arrest and senescence, respectively (51–54). Both UMC-11 and NCI-H727 cell lines showed high levels of p21 and activation of Chk1 after AXI. However, the activation of p53 was observed only in UMC-11 cells. Moreover, accordingly to the role of the activation of both p53 and p21 in the growth arrest of senescent cells, UMC-11 cells showed positive staining for senescence-specific SA-β-gal (55, 56). This may help to explain why, after 6 days, a substantial number of apoptotic cells were not detected, but the activation of cell death pathways remained evident. In UMC-11 cells, pp53 seems to regulate the cell cycle by activating p21. This protein stops cell proliferation to allow DNA repair by entering the cells in a state of senescence. However, since the DNA repair seems to be ineffective or impossible due to severe damage, pathways that lead to cell death are activated. In contrast, NCI-H727 cell line, which did not show p53 phosphorylation, underwent to senescence and mitotic catastrophe occurring through a p53-independent pathway (57) (Figure 7). In NCI-H727 cells, where p53 is reported to be defective, the cell cycle arrest is ineffective, leading to the accumulation of DNA damage and the inability to undergo complete mitosis. This scenario results in the increase of tetraploid cells, as shown after cytofluorimetric analyses. Interestingly, there are three different pathways associated to mitotic catastrophe, and only two lead to cell death (58–60). The first pathway, referred to as 'mitotic death' is characterized by increased levels of cyclin B1, which our cells do not appear to show. In the second pathway, cells exit mitosis through a process known as 'slippage' and undergo cell death in the subsequent $G_1$ phase of the cell cycle (61, 62). This mechanism appears unlikely to explain the effects of AXI. The third pathway does not lead to cell death but instead induces senescence characterized by decreased levels of cyclin B1 (63, 64), as observed in our experimental model. Thus, in our study, the decline in cyclin B1 levels seems to play a role in

10.3389/fendo.2024.1433707

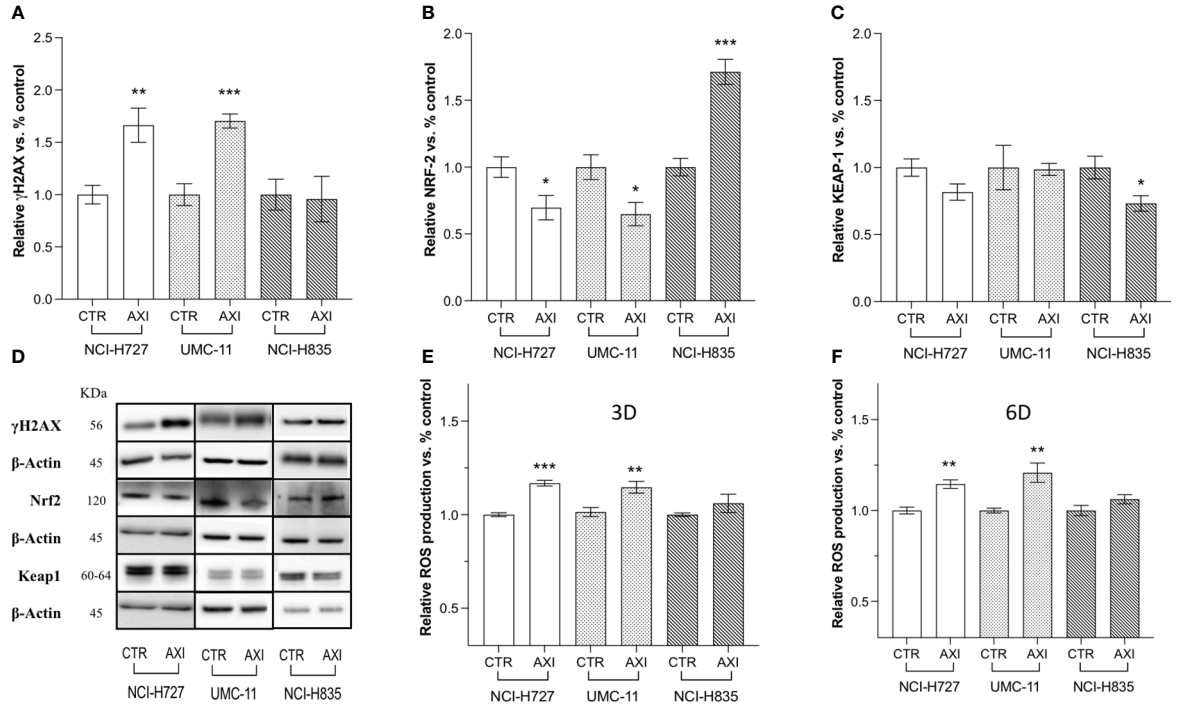



FIGURE 4
Western blot analysis of DNA damage and quantification of ROS in untreated (CTR) and AXI-treated cells. Histograms **(A–C)** summarize the relative expression change of one target after 6 days of treatment with AXI in each LC cell line. **(D)** Representative Western blot images for each target in LC cell lines. The analyzed targets are phospho-Histone H2A.X (Ser139) (γH2AX), Nrf2, Keap1. βActin is used as loading control. Panels **(E, F)** show the relative quantification of intracellular ROS production after 3 and 6 days of incubation, respectively. Values represent the mean ± S.E.M. of a minimum of three independent experiments. Significance is calculated by performing an unpaired t-test between the control and treated groups: *p<0.05; ** p<0.01; ***p<0.001.

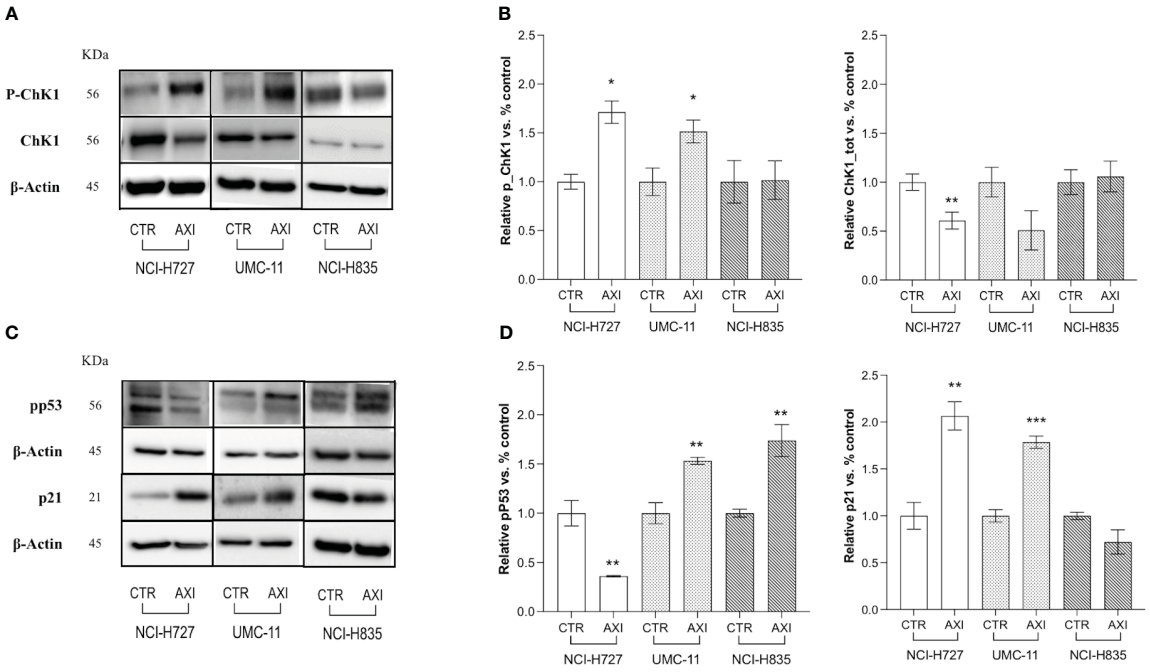



FIGURE 5
Examination of cell cycle arrest by Western blot analysis. **(A, C)** Representative Western blot images for each target in LC cell lines. Histograms **(B, D)** summarize the change in expression of one target after 6 days of treatment with AXI in each LC cell line. The analyzed targets are phospho-Chk1 (Ser345) (P-ChK1), total Chk1 (Chk1) **(A, B)**, phospho-p53 (Ser15) (pp53) and p21 Waf1/Cip1 **(C, D)**. βActin is used as a loading control. Values represent the mean ± S.E.M. of a minimum of three independent experiments. The significance is calculated by performing an unpaired t-test between the control and the treated group: *p<0.05; ** p<0.01; ***p<0.001.

10.3389/fendo.2024.1433707

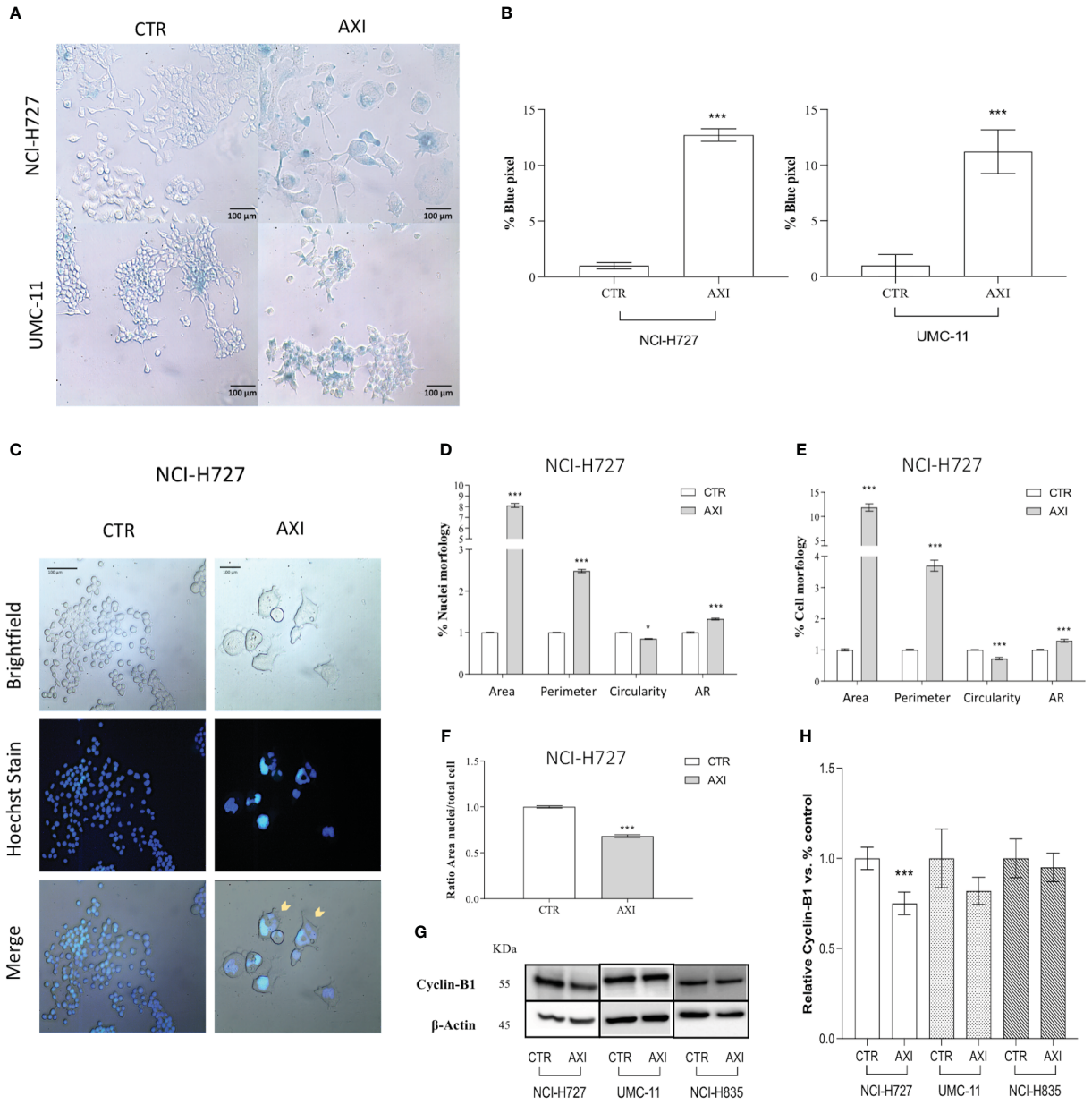



FIGURE 6
Evaluation of cell senescence and morphological changes of LC cells after treatment with AXI. **(A)** Representative bright field images of AXI-treated human LC and control cells (CTR) after staining with SA-β-gal. All quantified images have the same pixel size. **(B)** Histograms show the quantification of the blue pixel percentage present in the total image area of treated and control cells after staining with SA-β-gal. Values show the mean ± S.E.M. of a minimum of 3 independent experiments. In **(C)** LC cells are stained with a fluorescent dyes to quantify the main morphological parameters related to the mitotic catastrophe phenomenon. Hoechst dye is used to identify nuclei. **(D–F)** are representative of NCI-H727 cell line. Morphological parameters related to the nucleus or cell shape and their changes can be visualized in **(D, E)**, respectively. The analysis on the ratio between the cell area and nucleus are shown in **(F)**. Representative Western blot images and relative quantifications for the expression of Cyclin-B1 after 6 days of treatment with AXI in each LC cell line are shown in **(G, H)**. βActin is used as loading control. Values represent the mean ± S.E.M. of a minimum of three independent experiments. Significance was calculated by performing an unpaired t-test between the control and treated groups: *p<0.05; ***p<0.001.

polyploidization by sustaining an irreversible stop in the cell cycle. This prevents the proliferation of genomically unstable cells and potentially enables DNA replication in NCI-H727 cells undergoing AXI-induced senescence (49, 60, 65).

A separate consideration is necessary for the NCI-H835 cell line. The NCI-H835 cell line, which demonstrated no evidence of DNA damage and maintained a stable level of ROS over time, was able to activate a ROS resistance mechanism, probably through the Keap1/Nrf2 signaling. This mechanism, previously reported in renal cell carcinoma treated with AXI (45, 66), involves reduced Keap1 expression, increased Nrf2 expression, and overall decreased susceptibility to AXI. The Keap1/Nrf2 pathway is critical for antioxidant responses and cellular defense mechanisms, contributing significantly to tumor progression and resistance to chemotherapy and

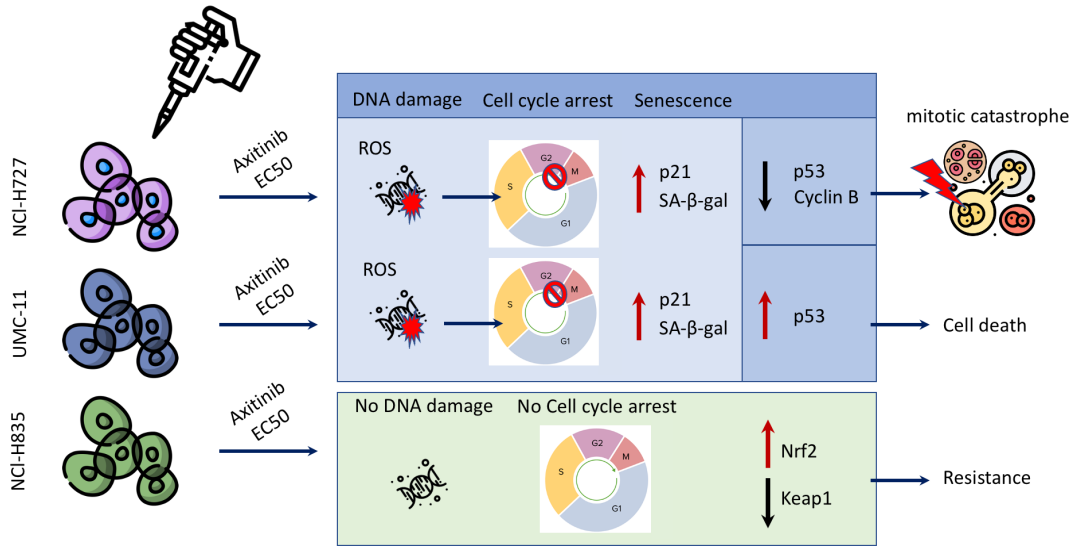



FIGURE 7
Scheme summarizing the different cell fates following treatment with AXI in LC cell lines.

radiotherapy in different cancer types (67, 68). This mechanism occurring through increased Nrf2 activity was initially described in non-small cell lung cancer cells (69, 70), but was quickly also associated to high-grade pulmonary NETs (71). Moreover, Keap1 promoter hypermethylation was identified in about 50% of the tissues from patients with LCs (69). Looking at a possible correlation between methylation, mutations, and loss of heterozygosity (LOH) in the KEAP1 gene and the disease course, it was observed that the degree of KEAP1 inhibition showed a trend of association with a higher risk of tumor progression. By activating the Keap1/Nrf2 signaling pathway, NCI-H835 cells effectively mitigated the production of ROS and the consequent DNA damage that AXI could potentially induce, as observed in other tumor cell lines (45, 72). However, the specific reasons why only this particular tumor cell line activated this defense mechanism remain unclear, requiring further investigation.

A limitation of this study is the exclusive use of cell lines from LCs, due to the challenges of obtaining primary cultures from this tumor type, given the limited tissue availability and low mitotic activity.

In conclusion, AXI exhibits a time-dependent enhancement of efficacy in LC cell lines and, notwithstanding the diverse responses of cells, the role of DNA damage and the consequent activation of senescence following treatment seem to be a prerequisite for AXI to exert its antitumor activity. When cells manage to repair DNA damage, the effectiveness of the drug is reduced. However, it is evident that the variability of AXI in mechanisms of action could potentially represent an advantage in light of the heterogeneity reported in LCs. Nevertheless, AXI capacity to induce a range of anti-tumor effects, from apoptosis to senescence, and its significant impact on cell viability and proliferation suggest its role as a possible therapeutic agent. The inclusion of AXI in a poly-therapeutic approach could also improve the overall treatment efficacy, especially when considering the drug ability to induce cell cycle arrest. Given the complexity of tumor biology, additional studies are warranted to define the potential role of AXI in patients with advanced LCs.

## Data availability statement

The datasets presented in this study can be found in online repositories. The datasets for this study can be found in Zenodo (https://doi.org/10.5281/zenodo.11105090).

## Ethics statement

Ethical approval was not required in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

## Author contributions

MO: Data curation, Formal analysis, Investigation, Validation, Visualization, Writing – original draft. MCC: Conceptualization, Data curation, Investigation, Validation, Visualization, Writing – review & editing. GG: Conceptualization, Writing – review & editing. SC: Conceptualization, Writing – review & editing. AD: Conceptualization, Data curation, Investigation, Writing – review & editing. DS: Data curation, Investigation, Writing – review & editing. MB: Data curation, Investigation, Validation, Writing – review & editing. AL: Writing – review & editing. MC: Writing – review & editing. LP: Writing – review & editing. GV: Resources, Supervision, Writing – review & editing.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was partially funded by the Neuroendocrine Tumor Research Foundation (NETRF) (Pilot Study 2019) and the Italian Ministry of Health (Ricerca Corrente). The publication fee has been supported by Ricerca Corrente from Italian Ministry of Health.

## Conflict of interest

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

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

## Publisher's note



## References

1. Baudin E, Caplin M, Garcia-Carbonero R, Fazio N, Ferolla P, Filosso PL, et al. Corrigendum to 'Lung and thymic carcinoids: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up': [Annals of Oncology 32 (2021) 439-451]. *Ann Oncol Off J Eur Soc Med Oncol.* (2021) 32:1453–5. doi: 10.1016/j.annonc.2021.08.2150

2. Malandrino P, Feola T, Mikovic N, Cannavale G, Molfetta SD, Altieri B, et al. Radioligand therapy in patients with lung neuroendocrine tumors: A systematic review on efficacy and safety. *Semin Nucl Med.* (2024), S0001–2998(24)00043-6. doi: 10.1053/j.semnuclmed.2024.05.001

3. Ferolla P, Berruti A, Spada F, Brizzi MP, Ibrahim T, Marconcini R, et al. Efficacy and safety of lanreotide autogel and temozolomide combination therapy in progressive thoracic neuroendocrine tumors (Carcinoid): results from the phase 2 ATLANT study. *Neuroendocrinology.* (2023) 113:332–42. doi: 10.1159/000526811

4. Caplin ME, Baudin E, Ferolla P, Filosso P, Garcia-Yuste M, Lim E, et al. Pulmonary neuroendocrine (carcinoid) tumors: European Neuroendocrine Tumor Society expert consensus and recommendations for best practice for typical and atypical pulmonary carcinoids. *Ann Oncol Off J Eur Soc Med Oncol.* (2015) 26:1604–20. doi: 10.1093/annonc/mdv041

5. Uprety D, Halfdanarson TR, Molina JR, Leventakos K. Pulmonary neuroendocrine tumors: adjuvant and systemic treatments. *Curr Treat Options Oncol.* (2020) 21:86. doi: 10.1007/s11864-020-00786-0

6. La Rosa S, Uccella S, Finzi G, Albarello L, Sessa F, Capella C. Localization of vascular endothelial growth factor and its receptors in digestive endocrine tumors: correlation with microvessel density and clinicopathologic features. *Hum Pathol.* (2003) 34:18–27. doi: 10.1053/hupa.2003.56

7. Cortez E, Gladh H, Braun S, Bocci M, Cordero E, Björkström NK, et al. Functional Malignant cell heterogeneity in pancreatic neuroendocrine tumors revealed by targeting of PDGF-DD. *Proc Natl Acad Sci.* (2016) 113:E864–73. doi: 10.1073/pnas.1509384113

8. Mairinger FD, Walter RFH, Werner R, Christoph DC, Ting S, Vollbrecht C, et al. Activation of angiogenesis differs strongly between pulmonary carcinoids and neuroendocrine carinomas and is crucial for carcinoid tumourgenesis. *J Cancer.* (2014) 5:465–71. doi: 10.7150/jca.9235

9. La Salvia A, Carletti R, Verrico M, Feola T, Puliani G, Bassi M, et al. Angioside: the role of angiogenesis and hypoxia in lung neuroendocrine tumours according to primary tumour location in left or right parenchyma. *J Clin Med.* (2022) 11:5958. doi: 10.3390/jcm11195958

10. Puliani G, Sesti F, Anastasi E, Verrico M, Tarsitano MG, Feola T, et al. Angiogenic factors as prognostic markers in neuroendocrine neoplasms. *Endocrine.* (2022) 76:208–17. doi: 10.1007/s12020-021-02942-4

11. Melen-Mucha G, Niedziela A, Mucha S, Motylewska E, Lawnicka H, Komorowski J, et al. Elevated peripheral blood plasma concentrations of tie-2 and angiopoietin 2 in patients with neuroendocrine tumors. *Int J Mol Sci.* (2012) 13:1444–60. doi: 10.3390/ijms13021444

12. Srirajaskanthan R, Dancey G, Hackshaw A, Luong T, Caplin ME, Meyer T. Circulating angiopoietin-2 is elevated in patients with neuroendocrine tumours and correlates with disease burden and prognosis. *Endocr Relat Cancer.* (2009) 16:967–76. doi: 10.1677/ERC-09-0089

13. Telega A, Kos-Kudła B, Foltyn W, Blicharz-Dorniak J, Rosiek V. Selected neuroendocrine tumour markers, growth factors and their receptors in typical and atypical bronchopulmonary carcinoids. *Endokrynol Pol.* (2012) 63:477–82.

14. Zhang J, Jia Z, Li Q, Wang L, Rashid A, Zhu Z, et al. Elevated expression of vascular endothelial growth factor correlates with increased angiogenesis and decreased progression-free survival among patients with low-grade neuroendocrine tumors. *Cancer.* (2007) 109:1478–86. doi: 10.1002/cncr.22554

15. Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, et al. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. *N Engl J Med.* (2011) 364:501–13. doi: 10.1056/NEJMoa1003825

16. Xu J, Shen L, Zhou Z, Li J, Bai C, Chi Y, et al. Surufatinib in advanced extrapancreatic neuroendocrine tumours (SANET-ep): a randomised, double-blind, placebo-controlled, phase 3 study. *Lancet Oncol.* (2020) 21:1500–12. doi: 10.1016/S1470-2045(20)30496-4

17. Garcia-Carbonero R, Benavent M, Jiménez Fonseca P, Castellano D, Alonso T, Teule A, et al. A phase II/III randomized double-blind study of octreotide acetate LAR with axitinib versus octreotide acetate LAR with placebo in patients with advanced G1-G2 NETs of non-pancreatic origin (AXINET trial-GETNE-1107). *J Clin Oncol.* (2021) 39:360–0. doi: 10.1200/JCO.2021.39.3_suppl.360

18. Kulke MH, Lenz HJ, Meropol NJ, Posey J, Ryan DP, Picus J, et al. Activity of sunitinib in patients with advanced neuroendocrine tumors. *J Clin Oncol Off J Am Soc Clin Oncol.* (2008) 26:3403–10. doi: 10.1200/JCO.2007.15.9020

19. Choueiri TK, Powles T, Burotto M, Escudier B, Bourlon MT, Zurawski B, et al. Nivolumab plus Cabozantinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *N Engl J Med.* (2021) 384:829–41. doi: 10.1056/NEJMoa2026982

20. Cella CA, Cazzoli R, Fazio N, De Petro G, Gaudenzi G, Carra S, et al. Cabozantinib in neuroendocrine tumors: tackling drug activity and resistance mechanisms. *Endocr Relat Cancer.* (2023) 30:e230232. doi: 10.1530/ERC-23-0232

21. Carra S, Gaudenzi G, Dicitore A, Cantone MC, Plebani A, Saronni D, et al. Modeling lung carcinoids with zebrafish tumor xenograft. *Int J Mol Sci.* (2022) 23:8126. doi: 10.3390/ijms23158126

22. Cella D, Escudier B, Rini B, Chen C, Bhattacharyya H, Tarazi J, et al. Patient-reported outcomes for axitinib vs sorafenib in metastatic renal cell carcinoma: phase III (AXIS) trial. *Br J Cancer.* (2013) 108:1571–8. doi: 10.1038/bjc.2013.145

23. Bondarenko IM, Ingrosso A, Bycott P, Kim S, Cebotaru CL. Phase II study of axitinib with doublet chemotherapy in patients with advanced squamous non–small-cell lung cancer. *BMC Cancer.* (2015) 15:339. doi: 10.1186/s12885-015-1350-6

24. Hu-Lowe DD, Zou HY, Grazzini ML, Hallin ME, Wickman GR, Amundson K, et al. Nonclinical antiangiogenesis and antitumor activities of axitinib (AG-013736), an oral, potent, and selective inhibitor of vascular endothelial growth factor receptor tyrosine kinases 1, 2, 3. *Clin Cancer Res Off J Am Assoc Cancer Res.* (2008) 14:7272–83. doi: 10.1158/1078-0432.CCR-08-0652

25. Dicitore A, Gaudenzi G, Carra S, Cantone MC, Oldani M, Saronni D, et al. Antitumor activity of axitinib in lung carcinoids: A preclinical study. *Cancers.* (2023) 15:5375. doi: 10.3390/cancers15225375

26. Olszewski U, Zeillinger R, Geissler K, Hamilton G. Genome-wide gene expression analysis of chemoresistant pulmonary carcinoid cells. *Lung Cancer Targets Ther.* (2010) 1:107–17. doi: 10.2147/LCTT.S12874

27. *In vitro* cytotoxicity of novel platinum-based drugs and dichloroacetate against lung carcinoid cell lines | DCA Guide (2021). Available online at: https://www.dcaguide.org/dca-information/dca-papers-and-clinical-trials/in-vitro-cytotoxicity-of-novel-platinum-based-drugs-and-dichloroacetate-against-lung-carcinoid-cell-lines/.

28. Boora GK, Kanwar R, Kulkarni AA, Pleticha J, Ames M, Schroth G, et al. Exome-level comparison of primary well-differentiated neuroendocrine tumors and their cell lines. *Cancer Genet.* (2015) 208:374–81. doi: 10.1016/j.cancergen.2015.04.002

29. Dicitore A, Castiglioni S, Saronni D, Gentilini D, Borghi MO, Stabile S, et al. Effects of human recombinant type I IFNs (IFN-α2b and IFN-β1a) on growth and migration of primary endometrial stromal cells from women with deeply infiltrating endometriosis: A preliminary study. *Eur J Obstet Gynecol Reprod Biol.* (2018) 230:192–8. doi: 10.1016/j.ejogrb.2018.10.004

30. Morelli MB, Amantini C, Santoni M, Soriani A, Nabissi M, Cardinali C, et al. Axitinib induces DNA damage response leading to senescence, mitotic catastrophe, and increased NK cell recognition in human renal carcinoma cells. *Oncotarget.* (2015) 6:36245–59. doi: 10.18632/oncotarget.v6i34

31. Granberg D, Eriksson B, Wilander E, Grimfjärd P, Fjällskog ML, Öberg K, et al. Experience in treatment of metastatic pulmonary carcinoid tumors. *Ann Oncol.* (2001) 12:1383–91. doi: 10.1023/A:1012569909313

32. Granberg D, Wilander E, Oberg K. Expression of tyrosine kinase receptors in lung carcinoids. *Tumour Biol J Int Soc Oncodevelopmental Biol Med.* (2006) 27:153–7. doi: 10.1159/000092718

33. Chan JA, Mayer RJ, Jackson N, Malinowski P, Regan E, Kulke MH. Phase I study of sorafenib in combination with everolimus (RAD001) in patients with advanced neuroendocrine tumors. *Cancer Chemother Pharmacol*. (2013) 71:1241–6. doi: 10.1007/s00280-013-2118-9

34. Torniai M, Scortichini L, Tronconi F, Rubini C, Morgese F, Rinaldi S, et al. Systemic treatment for lung carcinoids: from bench to bedside. *Clin Transl Med*. (2019) 8:22. doi: 10.1186/s40169-019-0238-5

35. Grillo F, Florio T, Ferraù F, Kara E, Fanciulli G, Faggiano A, et al. Emerging multitarget tyrosine kinase inhibitors in the treatment of neuroendocrine neoplasms. *Endocr Relat Cancer*. (2018) 25:R453–66. doi: 10.1530/ERC-17-0531

36. Dicitore A, Cantone MC. Targeting receptor tyrosine kinases in neuroendocrine neoplasm: what's going on with lung carcinoids? *Minerva Endocrinol*. (2022) 47:261–3. doi: 10.23736/S2724-6507.22.03879-9

37. Strosberg JR, Cives M, Hwang J, Weber T, Nickerson M, Atreya CE, et al. A phase II study of axitinib in advanced neuroendocrine tumors. *Endocr Relat Cancer*. (2016) 23:411–8. doi: 10.1530/ERC-16-0008

38. Jiang H, Liao J, Wang L, Jin C, Mo J, Xiang S. The multikinase inhibitor axitinib in the treatment of advanced hepatocellular carcinoma: the current clinical applications and the molecular mechanisms. *Front Immunol*. (2023) 14:1163967. doi: 10.3389/fimmu.2023.1163967

39. King JW, Lee SM. Axitinib for the treatment of advanced non-small-cell lung cancer. *Expert Opin Investig Drugs*. (2013) 22:765–73. doi: 10.1517/13543784.2013.775243

40. Gross-Goupil M, Françlois L, Quivy A, Ravaud A. Axitinib: A review of its safety and efficacy in the treatment of adults with advanced renal cell carcinoma. *Clin Med Insights Oncol*. (2013) 7:CMO.S10594. doi: 10.4137/CMO.S10594

41. Paik ES, Kim TH, Cho YJ, Ryu J, Choi JJ, Lee YY, et al. Preclinical assessment of the VEGFR inhibitor axitinib as a therapeutic agent for epithelial ovarian cancer. *Sci Rep*. (2020) 10:4904. doi: 10.1038/s41598-020-61871-w

42. Liang XW, Liu B, Chen JC, Cao Z, Chu Fr, Lin X, et al. Characteristics and molecular mechanism of drug-tolerant cells in cancer: a review. *Front Oncol*. (2023) 13:1177466. doi: 10.3389/fonc.2023.1177466

43. Tanaka T, Huang X, Halicka HD, Zhao H, Traganos F, Albino AP, et al. Cytometry of ATM activation and histone H2AX phosphorylation to estimate extent of DNA damage induced by exogenous agents. *Cytomet A*. (2007) 71A:648–61. doi: 10.1002/cyto.a.20426

44. Gruosso T, Mieulet V, Cardon M, Bourachot B, Kieffer Y, Devun F, et al. Chronic oxidative stress promotes H2AX protein degradation and enhances chemosensitivity in breast cancer patients. *EMBO Mol Med*. (2016) 8:527–49. doi: 10.15252/emmm.201505891

45. Huang H, Wu Y, Fu W, Wang X, Zhou L, Xu X, et al. Downregulation of Keap1 contributes to poor prognosis and Axitinib resistance of renal cell carcinoma via upregulation of Nrf2 expression. *Int J Mol Med*. (2019) 43:2044–54. doi: 10.3892/ijmm

46. Morelli MB, Amantini C, Nabissi M, Cardinali C, Santoni M, Bernardini G, et al. Axitinib induces senescence-associated cell death and necrosis in glioma cell lines: The proteasome inhibitor, bortezomib, potentiates axitinib-induced cytotoxicity in a p21(Waf/Cip1) dependent manner. *Oncotarget*. (2016) 8:3380–95. doi: 10.18632/oncotarget.v8i2

47. Passos JF, Nelson G, Wang C, Richter T, Simillion C, Proctor CJ, et al. Feedback between p21 and reactive oxygen production is necessary for cell senescence. *Mol Syst Biol*. (2010) 6:347. doi: 10.1038/msb.2010.5

48. Kahlem P, Dörken B, Schmitt CA. Cellular senescence in cancer treatment: friend or foe? *J Clin Invest*. (2004) 113:169–74. doi: 10.1172/JCI20784

49. Park SS, Choi YW, Kim JH, Kim HS, Park TJ. Senescent tumor cells: an overlooked adversary in the battle against cancer. *Exp Mol Med*. (2021) 53:1834–41. doi: 10.1038/s12276-021-00717-5

50. Roninson IB. Tumor cell senescence in cancer treatment. *Cancer Res*. (2003) 63:2705–15.

51. Senturk E, Manfredi JJ. p53 and cell cycle effects after DNA damage. *Methods Mol Biol Clifton NJ*. (2013) 962:49–61. doi: 10.1007/978-1-62703-236-0_9

52. Müllers E, Cascales HS, Macurek L, Lindqvist A. Cdk activity drives senescence from G2 phase. *bioRxiv*. (2016), 041723. doi: 10.1101/041723v1

53. Di Micco R, Krizhanovsky V, Baker D, d'Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Nat Rev Mol Cell Biol*. (2021) 22:75–95. doi: 10.1038/s41580-020-00314-w

54. Lai SL, Perng RP, Hwang J. p53 gene status modulates the chemosensitivity of non-small cell lung cancer cells. *J BioMed Sci*. (2000) 7:64–70. doi: 10.1007/BF02255920

55. Tian H, Faje AT, Lee SL, Jorgensen TJ. Radiation-induced Phosphorylation of Chk1 at S345 is Associated with p53-dependent Cell Cycle Arrest Pathways. *Neoplasia N Y N*. (2002) 4:171–80. doi: 10.1038/sj.neo.7900219

56. Mijit M, Caracciolo V, Melillo A, Amicarelli F, Giordano A. Role of p53 in the regulation of cellular senescence. *Biomolecules*. (2020) 10:420. doi: 10.3390/biom10030420

57. Phalke S, Mzoughi S, Bezzi M, Jennifer N, Mok WC, Low DHP, et al. p53-Independent regulation of p21Waf1/Cip1 expression and senescence by PRMT6. *Nucleic Acids Res*. (2012) 40:9534–42. doi: 10.1093/nar/gks858

58. Castedo M, Perfettini JL, Roumier T, Andreau K, Medema R, Kroemer G. Cell death by mitotic catastrophe: a molecular definition. *Oncogene*. (2004) 23:2825–37. doi: 10.1038/sj.onc.1207528

59. Sazonova EV, Petrichuk SV, Kopeina GS, Zhivotovsky B, link between mitotic defects A. and mitotic catastrophe: detection and cell fate. *Biol Direct*. (2021) 16:25. doi: 10.1186/s13062-021-00313-7

60. Vitale I, Galluzzi L, Castedo M, Kroemer G. Mitotic catastrophe: a mechanism for avoiding genomic instability. *Nat Rev Mol Cell Biol*. (2011) 12:385–92. doi: 10.1038/nrm3115

61. Mc Gee MM. Targeting the mitotic catastrophe signaling pathway in cancer. *Mediators Inflamm*. (2015) 2015:146282. doi: 10.1155/2015/146282

62. Sinha D, Duijf PHG, Khanna KK. Mitotic slippage: an old tale with a new twist. *Cell Cycle*. (2019) 18:7–15. doi: 10.1080/15384101.2018.1559557

63. Huang X, Tran T, Zhang L, Hatcher R, Zhang P. DNA damage-induced mitotic catastrophe is mediated by the Chk1-dependent mitotic exit DNA damage checkpoint. *Proc Natl Acad Sci USA*. (2005) 102:1065–70. doi: 10.1073/pnas.0409130102

64. Kikuchi I, Nakayama Y, Morinaga T, Fukumoto Y, Yamaguchi N. A decrease in cyclin B1 levels leads to polyploidization in DNA damage-induced senescence. *Cell Biol Int*. (2010) 34:645–53. doi: 10.1042/CBI20090398

65. Jackson JG, Pereira-Smith OM. p53 is preferentially recruited to the promoters of growth arrest genes p21 and GADD45 during replicative senescence of normal human fibroblasts. *Cancer Res*. (2006) 66:8356–60. doi: 10.1158/0008-5472.CAN-06-1752

66. Huang H, Zhang J, Jiang P, Xu X, Huang F, Zhao B, et al. FXR1 facilitates axitinib resistance in clear cell renal cell carcinoma via regulating KEAP1/Nrf2 signaling pathway. *Anticancer Drugs*. (2023) 34:248–56. doi: 10.1097/CAD.0000000000001416

67. Baird L, Yamamoto M. The molecular mechanisms regulating the KEAP1-NRF2 pathway. *Mol Cell Biol*. (2020) 40:e00099–20. doi: 10.1128/MCB.00099-20

68. Taguchi K, Yamamoto M. The KEAP1–NRF2 system in cancer. *Front Oncol*. (2017) 7:85. doi: 10.3389/fonc.2017.00085

69. Sparaneo A, Fabrizio FP, la Torre A, Graziano P, Di Maio M, Fontana A, et al. Effects of KEAP1 silencing on the regulation of NRF2 activity in neuroendocrine lung tumors. *Int J Mol Sci*. (2019) 20:2531. doi: 10.3390/ijms20102531

70. Rossi G, Bertero L, Marchiò C, Papotti M. Molecular alterations of neuroendocrine tumours of the lung. *Histopathology*. (2018) 72:142–52. doi: 10.1111/his.13394

71. Tanca A, Addis MF, Pagnozzi D, Cossu-Rocca P, Tonelli R, Falchi G, et al. Proteomic analysis of formalin-fixed, paraffin-embedded lung neuroendocrine tumor samples from hospital archives. *J Proteomics*. (2011) 74:359–70. doi: 10.1016/j.jprot.2010.12.001

72. Deshmukh P, Unni S, Krishnappa G, Padmanabhan B. The Keap1–Nrf2 pathway: promising therapeutic target to counteract ROS-mediated damage in cancers and neurodegenerative diseases. *Biophys Rev*. (2017) 9:41–56. doi: 10.1007/s12551-016-0244-4

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 07 August 2024
DOI 10.3389/fendo.2024.1388861



OPEN ACCESS

EDITED BY
Min Tu,
Nanjing Medical University, China

REVIEWED BY
Xiaoqiang Wang,
City of Hope, United States
Chun Zhang,
Nanjing University of Chinese Medicine, China

*CORRESPONDENCE
Taijun Huang
202320337@sr.gxmu.edu.cn
Miaofeng Liu
liumiaofeng2024@126.com

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

RECEIVED 20 February 2024
ACCEPTED 24 July 2024
PUBLISHED 07 August 2024

CITATION
Wei C, Ai H, Mo D, Wang P, Wei L, Liu Z, Li P, Huang T and Liu M (2024) A nomogram based on inflammation and nutritional biomarkers for predicting the survival of breast cancer patients.
*Front. Endocrinol.* 15:1388861.
doi: 10.3389/fendo.2024.1388861



# A nomogram based on inflammation and nutritional biomarkers for predicting the survival of breast cancer patients

Caibiao Wei[1†], Huaying Ai[2†], Dan Mo[3†], Peidong Wang[1†], Liling Wei[4], Zhimin Liu[1], Peizhang Li[1], Taijun Huang[1*] and Miaofeng Liu[1*]

[1]Department of Clinical Laboratory, Guangxi Medical University Cancer Hospital, Nanning, China, [2]Department of Injection Room, The People's Hospital of Yingtan, Yingtan, Jiangxi, China, [3]Department of Breast, Guangxi Zhuang Autonomous Region Maternal and Child Health Care Hospital, Nanning, China, [4]Department of Anesthesiology, First Affiliated Hospital of Guangxi Medical University, Nanning, China

**Background:** We aim to develop a new prognostic model that incorporates inflammation, nutritional parameters and clinical-pathological features to predict overall survival (OS) and disease free survival (DFS) of breast cancer (BC) patients.

**Methods:** The study included clinicopathological and follow-up data from a total of 2857 BC patients between 2013 and 2021. Data were randomly divided into two cohorts: training (n=2001) and validation (n=856) cohorts. A nomogram was established based on the results of a multivariate Cox regression analysis from the training cohorts. The predictive accuracy and discriminative ability of the nomogram were evaluated by the concordance index (C-index) and calibration curve. Furthermore, decision curve analysis (DCA) was performed to assess the clinical value of the nomogram.

**Results:** A nomogram was developed for BC, incorporating lymphocyte, platelet count, hemoglobin levels, albumin-to-globulin ratio, prealbumin level and other key variables: subtype and TNM staging. In the prediction of OS and DFS, the concordance index (C-index) of the nomogram is statistically greater than the C-index values obtained using TNM staging alone. Moreover, the time-dependent AUC, exceeding the threshold of 0.7, demonstrated the nomogram's satisfactory discriminative performance over different periods. DCA revealed that the nomogram offered a greater overall net benefit than the TNM staging system.

**Abbreviations:** BC, Breast cancer; TNM, tumor-node-metastasis staging; HR, Hazard ratio; CI, Confidence interval; OS, Overall survival; DFS, Disease free survival, IDC, Invasive ductal carcinoma; ILC, Invasive lobular carcinoma; NLR, Neutrophil–lymphocyte ratio; PLR, Platelet–lymphocyte ratio; LMR, Lymphocyte-to-monocyte ratio; PLT, Platelet count; MON, Monocyte; NEU, Neutrophil; LYM, Lymphocyte; ALB, Albumin; HGB, Hemoglobin; TRF, Transferrin, TP, Total albumin; AGR, Albumin to globulin; PA, Prealbumin; SF, Serum ferritin

**Conclusion:** The nomogram incorporating inflammation, nutritional and clinicopathological variables exhibited excellent discrimination. This nomogram is a promising instrument for predicting outcomes and defining personalized treatment strategies for patients with BC.

KEYWORDS

breast cancer, inflammation, nutrition, nomogram, prognosis

# 1 Introduction

Breast cancer (BC) is the most frequently diagnosed cancer and is associated with one of the highest mortality rates among female malignant tumors (1). It is estimated that 287,000 new cases of BC will be diagnosed in 2023 (2). The current treatment modalities for BC encompass surgery, chemotherapy, hormonal therapy, and radiation therapy (3, 4). Despite remarkable advancements in early detection and therapeutic approaches, BC patients have poor prognoses (5, 6).

Although the traditional TNM staging system of the American Joint Committee on Cancer (AJCC) is extensively used for predicting prognosis and guiding clinical management, its ability to accurately identify patients at high risk of cancer-related mortality may be limited (7, 8). This limitation arises from the inherent heterogeneity observed in BC, where patients sharing the same stage can exhibit diverse clinical outcomes (9, 10). Patients with BC at the same stage may have different outcomes due to the heterogeneity of the disease (11). Consequently, there exists a critical need to identify reliable and easily applicable predictive models that can complement the TNM staging system and offer more precise predictions of individual patient outcomes.

Extensive Research consistently demonstrates the link between systemic inflammation and poor prognosis in cancer patients (12, 13). Chronic inflammation drives tumor progression, angiogenesis, and metastasis while suppressing the immune response (14, 15). In parallel, the significance of nutritional status in cancer patients' survival has been widely recognized (16, 17). Cancer-associated malnutrition weakens immune function and triggers inflammation, worsening treatment outcomes (18). These findings underscore the importance of considering both systemic inflammation and nutritional status in the management and prognosis of cancer patients.

Recent studies have shed light on the prevalence of cancer-associated systemic inflammation and malnutrition in the majority of patients with malignancy, including those with BC (14, 19, 20). These factors have been closely linked to tumor progression and have been shown to have a detrimental impact on patient's clinical outcomes (12, 21, 22). Various inflammation-based and nutritional markers, such as neutrophil (NEU), lymphocyte (LYM), platelet count (PLT), serum ferritin (SF) and lymphocyte-to-monocyte ratio (LMR), as well as prognostic nutritional index hemoglobin (HGB), albumin (ALB), transferrin (TRF), albumin to globulin (AGR), and prealbumin (PA), have been identified as promising clinical prognostic predictors for various cancers due to their simplicity and cost-effectiveness (23–29). However, it is worth noting that many nomogram studies in the literature often fail to consider the assessment of hematological markers encompassing both inflammation and nutritional biomarkers in conjunction with tumor characteristics (30–32). There are two studies of prognostic scoring systems for patients with BC, published by Hua X, et al. (33) and Jiang C, et al. (34). The scoring system developed from those studies provides useful tools for clinicians and researchers to predict the prognostic value of BC patients. However, two of the studies simply classified patients as early-stage or underwent neoadjuvant chemotherapy BC patients, their clinical application is restricted to a subset of patients, not all females with BC treated at their center. Meanwhile, both were developed using limited sample sizes. Furthermore, these models have not received any independent validation, most likely because of the small sample size. In addition, most current studies have primarily focused on the combination of one or a few inflammatory and nutritional parameters with clinicopathological factors, without incorporating more accurate variables such as TNM staging, subtype, and tumor size (35–37). These factors are of great importance in the treatment of BC. Therefore, the assessment of hematological markers including inflammation and nutritional biomarkers, could be of great importance in revealing the survival of patients with BC, which most nomogram studies did not mention.

In this study, our objective was to develop an inexpensive, trustworthy, and more accurate prognostic model by simultaneously combining inflammation and nutritional biomarkers collected from a substantial cohort of nearly 3000 patients diagnosed with BC. By incorporating these diverse factors, we aimed to enhance the accuracy and reliability of prognosis analysis in BC patients.

# 2 Materials and methods

## 2.1 Study population

This study included a total of 2857 patients who were diagnosed with BC at Guangxi Medical University Cancer Hospital between 2013 and 2021. Inclusion criteria for the study required: (1)

confirmation of the pathological diagnosis of BC; (2) no preoperative chemotherapy or other radiation therapy; (3) absence of acute infections or other inflammatory conditions in the two weeks preceding surgery; (4) availability of complete follow-up information and clinical data; (5) availability of peripheral blood hematological markers before treatment. Patients were removed from the study if any of the following conditions were met: (1) receipt of relevant antitumor therapy (e.g., chemotherapy, radiotherapy) (n=889); (2) lack of clear and definite pathological diagnosis and medical history information (n=96); (3) presence of other malignant tumors except for BC or distant metastasis(n=63); (4) diagnosis of autoimmune diseases or chronic inflammatory conditions (n=79); (5) relapse or *de novo* BC(n=35). All included patients were divided into two groups, with a ratio of 7:3, resulting in a training cohort of 2001 patients and a validation cohort of 856 patients. Figure 1 depicts the comprehensive workflow for patient selection.

## 2.2 Ethics approval and consent to participate

Our study was approved by the Guangxi Medical University Cancer Hospital Ethical Review Committee (Approve No.LW2023087), Guangxi Zhuang Autonomous Region Maternal and Child Health Care Hospital Ethical Review Committee (Approve No. 6–1, 2024) and conducted following the ethical principles outlined in the Helsinki Declaration of 1964 and its subsequent amendments or other ethical standards with equivalent requirements. To ensure patient confidentiality, the identities of the individuals included in this study were anonymized using computer-generated ID numbers. On admission, all patients provided written consent for their anonymized medical data to be analyzed and published for research purposes.

## 2.3 Data acquisition

In this study, we collected a comprehensive set of clinicopathological, demographic, and laboratory data from 2857 BC patients. Clinicopathological data included the patient's age, tumor size, histologic type, grade, subtype, and clinical TNM stage based on the most recent AJCC staging system (8th edition) (38), as well as outcomes such as mortality. Pre-treatment inflammation and nutritional biomarkers included the levels of NEU, LYM, MON, PLT, SF, HGB, ALB, TRF, TP, AGR and PA. To facilitate analysis, we also transformed certain clinicopathological characteristics into categorical variables. Furthermore, we

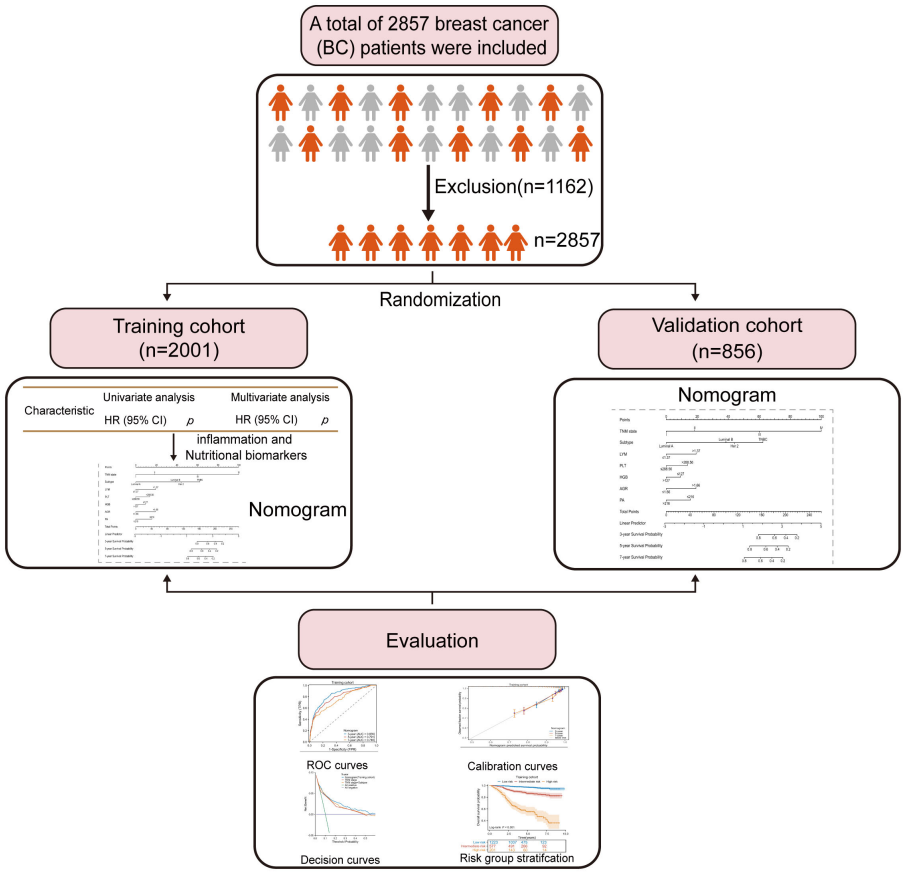



FIGURE 1
Flow diagram of enrolled participants and evaluation process.

calculated several inflammation-related ratios, such as NLR, PLR, and LMR, based on their known associations with the outcomes of interest.

## 2.4 Patient follow-up

We conducted follow-up assessments using a combination of phone interviews and an outpatient surveillance system. The median follow-up time was 54 months (range: 52–55 months). Our primary endpoint of interest was overall survival (OS), which was defined as the duration between the date of surgery and the occurrence of death from any cause or the date of the last follow-up, whichever came first. The period of disease-free survival (DFS) was measured from the date of diagnosis until the occurrence of any recurrence or death. The follow-up period for our study extended until December 2022, or until the date of a patient's death if it transpired earlier.

## 2.5 Nomogram development and validation

Receiver operating characteristic (ROC) curves were employed to determine the optimal cutoff points for plasma/serum biomarkers using MedCalc software. Statistical analysis was performed using R software version 4.2.1 and SPSS 23.0. The relevance of clinicopathologic characteristics between the training and validation cohorts was analyzed using the chi-square test or Fisher's exact test. Survival curves were plotted using the Kaplan-Meier method, and differences between groups were assessed using the log-rank test. Univariate and multivariate Cox regression analyses were conducted to identify factors influencing OS. Hazard ratios (HRs) and 95% confidence intervals (CIs) were used to assess the association between patients' indices and prognosis. In the multivariate Cox regression analysis, only variables with a significance level of $p < 0.05$ in the univariate analysis were included.

A nomogram was constructed using the training cohort of 2001 BC patients based on significant predictors identified through multivariable Cox regression analysis, utilizing R software with the survival and rms packages. The performance of the nomograms was evaluated using the concordance index (C-index), time-dependent receiver operating characteristic (ROC) curve, and the area under the curve (AUC). The accuracy of the model was assessed using calibration plots to compare the predicted and actual OS and DFS. Additionally, decision curve analysis (DCA) was conducted to determine the clinical usefulness of the nomograms by quantifying the net benefits at different threshold probabilities. Results with $p < 0.05$ were considered statistically significant.

## 2.6 External validation of the nomogram

We further validated the feasibility of our model by using BC patients from the Guangxi Zhuang Autonomous Region Maternal and Child Health Care Hospital as an external validation cohort. For external validation of the model, we utilized TNM stage, subtype, LYM, PLT, HGB, AGR, and PA indicators, along with their respective cutoff values, to construct the model in independent cohorts, aiming to assess the robustness and applicability of the model in this study.

# 3 Results

## 3.1 Clinicopathologic characteristics

A total of 2001 patients from the training cohort and 856 patients from the validation cohort were included in our analyses. The demographic and clinical characteristics of the patients are summarized in Table 1. No significant differences were observed between the primary and validation cohorts, except for age, LYM, LMR, ALB level, and TRF level. The median follow-up times for the primary and validation cohorts were 55.0 months (range: 53.0 to 57.0 months) and 54.0 months (range:52.0 to 56.0 months), respectively.

## 3.2 Factors correlated with plasma levels of LYM, PLT, AGR, PA and HGB in BC patients with their interrelationship

The interrelationships between plasma levels of LYM, PLT, AGR, PA, HGB and clinical factors in BC patients are presented in Figure 2. AGR levels showed a correlation with age and subtype, indicating that AGR levels were influenced by these factors. LYM levels, on the other hand, only showed a correlation with age. Interestingly, HGB and PA levels did not show significant correlations with age or subtype. Furthermore, PLT levels were found to be negatively correlated with age, suggesting that PLT levels decrease as age increases. PA levels, on the other hand, were negatively correlated with TNM stage, and tumor size, indicating that higher PA levels were associated with less advanced disease.

In terms of interrelationships among the biomarkers, PLT levels were not correlated with HGB levels. However, they were negatively correlated with LYM, PA, and AGR levels, indicating that higher PLT levels were associated with lower LYM, PA, and AGR levels. Additionally, AGR, PA, and HGB levels showed correlations with LMR, suggesting a potential relationship between these biomarkers and LMR.

These findings provide insights into the interrelationships between plasma biomarker levels and various clinical factors in BC patients, highlighting their potential as prognostic indicators and contributing to our understanding of the disease.

## 3.3 Univariate analyses and multivariate analyses

The univariate analysis of potential factors associated with BC revealed significant associations with the following factors: age

10.3389/fendo.2024.1388861

TABLE 1 Characteristics of training cohort and validation cohort.

| Characteristic | All patients | Training cohort | Validation cohort | *p* |
|---|---|---|---|---|
| | No. (%) | No. (%) | No. (%) | |
| **Total** | 2857 | 2001 | 856 | |
| **Age (years)** | | | | **0.024** |
| ≤52 | 1910(66.9%) | 1364(68.2%) | 546(63.8%) | |
| >52 | 947(33.1%) | 637(31.8%) | 310(36.2%) | |
| **Histologic type** | | | | 0.545 |
| IDC | 2121(74.2%) | 1490(74.5%) | 631(73.7%) | |
| ILC | 76(2.7%) | 49(2.4%) | 27(3.2%) | |
| Others | 660(23.1%) | 462(23.1%) | 198(23.1%) | |
| **Grade** | | | | 0.906 |
| I | 419(14.7%) | 290(14.5%) | 129(15.1%) | |
| II | 1265(44.3%) | 886(44.3%) | 379(44.3%) | |
| III | 1173(41.0%) | 825(41.2%) | 348(40.6%) | |
| **Subtype** | | | | 0.363 |
| Luminal A | 402(14.1%) | 292(14.6%) | 110(12.9%) | |
| Luminal B | 1760(61.6%) | 1217(60.8%) | 543(63.4%) | |
| Her 2 | 391(13.7%) | 283(14.1%) | 108(12.6%) | |
| TNBC | 304(10.6%) | 209(10.5%) | 95(11.1%) | |
| **TNM stage** | | | | 0.834 |
| I | 644(22.5%) | 451(22.5%) | 193(22.5%) | |
| II | 1525(53.4%) | 1076(53.8%) | 449(52.5%) | |
| III | 539(18.9%) | 369(18.4%) | 170(19.9%) | |
| IV | 149(5.2%) | 105(5.3%) | 44(5.1%) | |
| **Tumor size(cm)** | | | | 0.255 |
| ≤2 | 912(31.9%) | 652(32.6%) | 260(30.4%) | |
| >2 | 1945(68.1%) | 1349(67.4%) | 596(69.6%) | |
| **PLT ($10^9$/L)** | | | | 0.478 |
| ≤288.56 | 1734(60.7%) | 1223(61.2%) | 511(59.7%) | |
| >288.56 | 1123(39.3%) | 778(38.8%) | 345(40.3%) | |
| **MON ($10^9$/L)** | | | | 0.361 |
| ≤0.39 | 1690(59.2%) | 1195(59.7%) | 495(57.8%) | |
| >0.39 | 1167(40.8%) | 806(40.3%) | 361(42.2%) | |
| **NEU ($10^9$/L)** | | | | 0.484 |
| ≤4.84 | 2254(78.9%) | 1586(79.3%) | 668(78.0%) | |
| >4.84 | 603(21.1%) | 415(20.7%) | 188(22%) | |
| **LYM ($10^9$/L)** | | | | **<0.001** |
| ≤1.37 | 471(16.5%) | 295(14.7%) | 176(20.6%) | |
| >1.37 | 2386(83.5%) | 1706(85.3%) | 680(79.4%) | |

*(Continued)*

TABLE 1 Continued

| Characteristic | All patients | Training cohort | Validation cohort | *p* |
|---|---|---|---|---|
| | No. (%) | No. (%) | No. (%) | |
| **PLR ($10^9$/L)** | | | | 0.322 |
| ≤196.4 | 2316(81.1%) | 1632(81.6%) | 684(79.9%) | |
| >196.4 | 541(18.9%) | 369(18.4%) | 172(20.1%) | |
| **LMR ($10^9$/L)** | | | | **<0.001** |
| ≤4.92 | 1187(41.5%) | 907(45.3%) | 280(32.7%) | |
| >4.92 | 1670(58.8%) | 1094(54.7%) | 576(67.3%) | |
| **NLR ($10^9$/L)** | | | | 0.932 |
| ≤1.69 | 988(34.6%) | 691(34.5%) | 297(34.7%) | |
| >1.69 | 1869(65.4%) | 1310(65.5%) | 559(65.3%) | |
| **ALB (g/L)** | | | | **0.005** |
| ≤44.6 | 2382(83.4%) | 1643(82.1%) | 739(86.3%) | |
| >44.6 | 475(16.6%) | 358(17.9%) | 117(13.7%) | |
| **HGB (g/L)** | | | | 0.220 |
| ≤127 | 1375(48.1%) | 948(47.4%) | 427(49.9%) | |
| >127 | 1482(51.9%) | 1053(52.6%) | 429(50.1%) | |
| **TRF (g/L)** | | | | **0.024** |
| ≤2.25 | 684(23.9%) | 455(22.7%) | 229(26.8%) | |
| >2.25 | 2173(76.1%) | 1546(77.3%) | 627(73.2%) | |
| **TP (g/L)** | | | | 0.094 |
| ≤70.2 | 1406(49.2%) | 964(48.2%) | 442(51.6%) | |
| >70.2 | 1451(50.8%) | 1037(51.8%) | 414(48.4%) | |
| **AGR(g/L)** | | | | 0.091 |
| ≤1.66 | 2480(86.8%) | 1751(87.5%) | 729(85.2%) | |
| >1.66 | 377(13.2%) | 250(12.5%) | 127(14.8%) | |
| **PA (mg/L)** | | | | 0.067 |
| ≤216 | 924(32.3%) | 626(31.3%) | 298(34.8%) | |
| >216 | 1933(67.7%) | 1375(68.7%) | 558(65.2%) | |
| **SF (μg/L)** | | | | 1.000 |
| ≤240 | 2374(83.1%) | 1674(83.7%) | 700(81.8%) | |
| >240 | 483(16.9%) | 372(16.3%) | 156(18.2%) | |
| **LDH (U/L)** | | | | 0.741 |
| ≤212 | 2387(83.5%) | 1675(83.7%) | 712(83.2%) | |
| >212 | 470(16.5%) | 326(16.3%) | 144(16.8%) | |

Bold values indicate P-values < 0.05.

($p$=0.005), subtype ($p$ <0.001), TNM stage ($p$<0.001), tumor size ($p$<0.001), PLT ($p$<0.001), MON ($p$<0.001), NEU ($p$<0.001), LYM ($p$=0.048), LMR ($p$=0.001), NLR ($p$=0.002), HGB ($p$=0.007), TRF ($p$=0.001), AGR ($p$=0.002), PA ($p$<0.001), SF ($p$<0.001), and LDH ($p$<0.001). Based on these significant factors, a multivariate analysis was conducted. The results showed that the following factors were significantly associated with BC: subtype (HR=6.461; 95% CI=2.860~14.595; $p$<0.001), TNM stage (HR=9.603; 95% CI=4.080~22.602; $p$<0.001), PLT (HR=1.374; 95% CI=1.016~1.860; $p$ =0.039), LYM (HR=1.748; 95% CI=1.043~2.930; $p$ =0.034), HGB (HR= 0.690; 95% CI=0.510~0.935 $p$ =0.017), AGR (HR=1.951; 95% CI=1.389~2.740; $p$ <0.001), and PA (HR= 0.694;95%

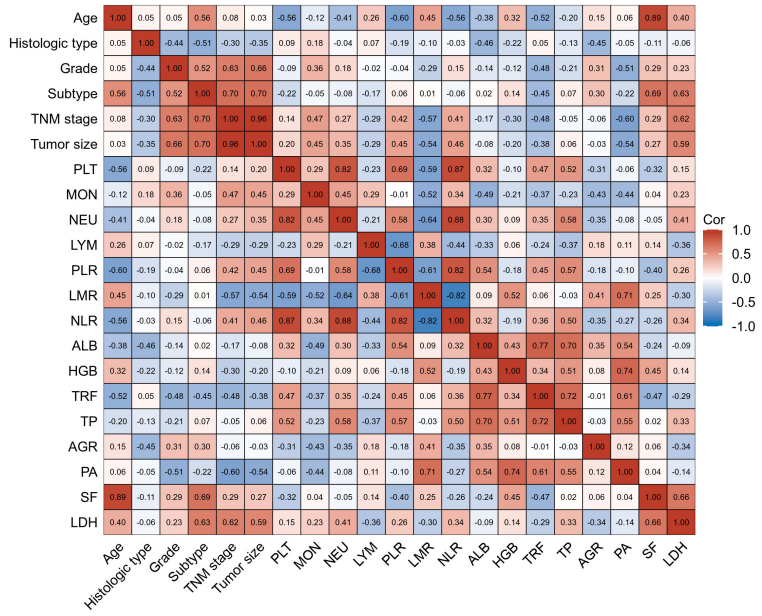



FIGURE 2
Factors correlated with plasma levels of LYM, PLT, AGR, PA, and HGB in training cohort BC patients with their interrelationship. The heat map showing a red background indicates a positive correlation, and the blue background indicates a negative correlation.

CI=0.507~0.949; $p$ =0.022). The detailed univariate and multivariate analysis results are presented in Table 2.

## 3.4 Construction and validation of the nomogram

In our study, TNM stage, subtype, LYM, PLT, HGB, AGR, and PA indicators, along with their respective cutoff values, performed well in the OS model, we have chosen to apply these indicators and cutoff values to the DFS model. The purpose of this is to ensure that our DFS model maintains reliable predictive performance and consistency with the OS model. The nomogram, based on the multivariate analysis results from the training cohort, was constructed to predict OS as well as DFS in BC patients. The nomogram incorporated all the independent prognostic factors identified in the multivariate analysis, including TNM stage, subtype, PLT, LYM, HGB, AGR, and PA. Figure 3 represents the nomogram for the training cohort. It is straightforward to estimate the 3-year, 5-year and 7-year OS and DFS probabilities by summing the scores associated with each variable and projecting the sums to the bottom scales. The model predicted the OS and DFS rates of BC with high accuracy in the training cohort, as indicated by a C-index of 0.820 (95% CI, 0.805–0.835) for OS and 0.760 (95% CI, 0.744–0.776) for DFS. In the training cohort, the calibration plot in Figures 4A, C shows a strong correlation between the predicted probabilities of 3-year, 5-year, and 7-year OS and DFS from the nomogram and the actual observed survival rates after surgery, indicating a high degree of concordance. Similarly, consistent results were observed in the validation cohort. The C-index of the nomogram for predicting OS and DFS in the validation cohort was 0.838 (95% CI, 0.818–0.858) and 0.755 (95% CI, 0.730–0.780), respectively. In the validation cohort, the calibration plot in Figures 4B, D also demonstrates good consistency between the predicted and actual OS and DFS. These findings imply that a nomogram is an accurate method for estimating survival in BC patients and offers useful data for clinical decision-making and patient counseling.

## 3.5 Risk stratification of OS and DFS

The X-tile program was used to determine total point thresholds, based on which patients in both the training and validation cohorts were divided into low-, intermediate-, and high-risk groups for both OS and DFS. In the training cohort, the OS rates for the low-risk, intermediate-risk, and high-risk groups were 97.5%, 87.5%, and 54.7%, respectively ($p$<0.001, Figure 5A), while the DFS rates for the training cohort were 94.3%, 85.2%, and 51.6%, respectively ($p$<0.001, Figure 5C). Similarly, in the validation cohort, the OS rates for these risk categories were 97.3%, 86.8%, and 53.8%, respectively ($p$<0.001, Figure 5B), and the DFS rates were 92.6%, 84.8%, and 55.4%, respectively ($p$<0.001, Figure 5D). This risk stratification accurately determined survival outcomes for the three different categories within the training and validation cohorts.

## 3.6 Comparison of predictive accuracy and clinical usability between nomogram and TMN staging systems

ROC analysis further confirmed the superiority of the nomogram over the TNM stage model, exhibiting higher AUC

10.3389/fendo.2024.1388861

TABLE 2 Univariate and multivariate analysis of OS in the training cohort.

| Characteristic | Univariate analysis HR (95% CI) | *p* | Multivariate analysis HR (95% CI) | *p* |
|---|---|---|---|---|
| Age (years) | | | | |
| ≤52 vs >52 | 1.513(1.135~2.018) | 0.005 | 1.349(0.967~1.882) | 0.078 |
| **Histologic type** | | 0.789 | | |
| IDC vs ILC | 1.295(0.573~2.927) | 0.535 | | |
| IDC vs Others | 0.955(0.660~1.380) | 0.805 | | |
| **Grade** | | 0.150 | | |
| I vs II | 1.100(0.693~1.744) | 0.687 | | |
| I vs III | 1.412(0.903~2.208) | 0.130 | | |
| **Subtype** | | <0.001 | | **<0.001** |
| Luminal A vs Luminal B | 3.663(1.707~7.862) | 0.001 | 3.291(1.527~7.091) | 0.002 |
| Luminal A vs Her-2 | 5.901(2.640~13.193) | <0.001 | 3.671(1.626~8.290) | 0.002 |
| Luminal A vs TNBC | 7.544(3.357~16.953) | <0.001 | 6.461(2.860~14.595) | <0.001 |
| **TNM stage** | | <0.001 | | **<0.001** |
| I vs II | 1.850(0.992~3.452) | 0.053 | 1.013(0.441~2.327) | 0.975 |
| I vs III | 7.555(4.110~13.889) | <0.001 | 3.501(1.540~7.959) | 0.003 |
| I vs IV | 24.867(13.236~46.717) | <0.001 | 9.603(4.080~22.602) | <0.001 |
| Tumor size (cm) | | | | |
| ≤2 vs >2 | 3.427(2.235~5.255) | <0.001 | 1.654(0.909~3.010) | 0.099 |
| PLT ($10^9$/L) | | | | |
| ≤288.56 vs >288.56 | 1.739(1.311~2.307) | <0.001 | 1.374(1.016~1.860) | **0.039** |
| MON ($10^9$/L) | | | | |
| ≤0.39 vs >0.39 | 1.687(1.272~2.236) | <0.001 | 1.292(0.912~1.831) | 0.149 |
| NEU ($10^9$/L) | | | | |
| ≤4.84 vs >4.84 | 1.976(1.466~2.662) | <0.001 | 1.385(0.966~1.984) | 0.076 |
| LYM ($10^9$/L) | | | | |
| ≤1.37 vs >1.37 | 1.631(1.004~2.650) | 0.048 | 1.748(1.043~2.930) | **0.034** |
| PLR ($10^9$/L) | | | | |
| ≤196.4 vs >196.4 | 1.340(0.960~1.871) | 0.085 | | |
| LMR ($10^9$/L) | | | | |
| ≤4.92 vs >4.92 | 0.611(0.460~0.811) | 0.001 | 0.976(0.676~1.411) | 0.899 |
| NLR ($10^9$/L) | | | | |
| ≤1.69 vs >1.69 | 1.680(1.212~2.328) | 0.002 | 1.166(0.795~1.710) | 0.433 |
| ALB (g/L) | | | | |
| ≤44.6 vs >44.6 | 1.230(0.884~1.712) | 0.219 | | |
| HGB (g/L) | | | | |
| ≤127 vs >127 | 0.676(0.509~0.898) | 0.007 | 0.690(0.510~0.935) | **0.017** |
| TRF (g/L) | | | | |

*(Continued)*

TABLE 2 Continued

| Characteristic | Univariate analysis HR (95% CI) | p | Multivariate analysis HR (95% CI) | p |
|---|---|---|---|---|
| TRF (g/L) | | | | |
| ≤2.25 vs >2.25 | 0.611(0.453~0.826) | 0.001 | 0.765(0.551~1.062) | 0.109 |
| TP(g/L) | | | | |
| ≤70.2 vs >70.2 | 1.197(0.898~1.594) | 0.220 | | |
| A/G(g/L) | | | | |
| ≤1.66 vs >1.66 | 1.693(1.210~2.367) | 0.002 | 1.951(1.389~2.740) | **<0.001** |
| PA (mg/L) | | | | |
| ≤216 vs >216 | 0.592(0.445~0.786) | <0.001 | 0.694(0.507~0.949) | **0.022** |
| SF (μg/L) | | | | |
| ≤240 vs >240 | 2.004(1.461~2.748) | <0.001 | 1.254(0.872~1.803) | 0.221 |
| LDH(U/L) | | | | |
| ≤212 vs >212 | 2.620(1.937~3.543) | <0.001 | 1.292(0.907~1.840) | 0.155 |

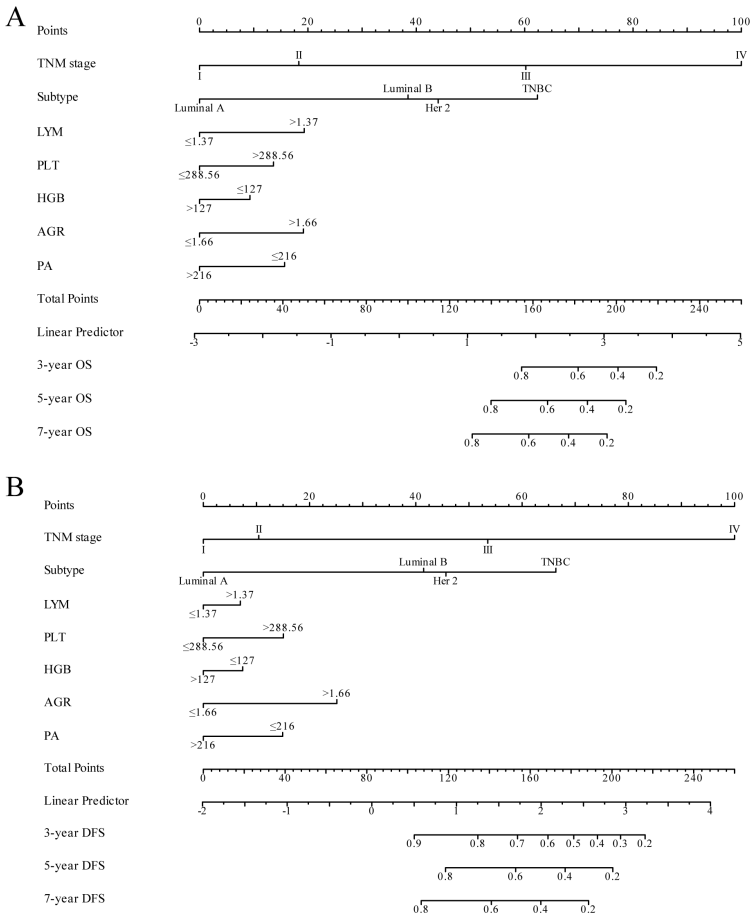



FIGURE 3
Nomogram model for predicting 3-year, 5-year and 7-year OS **(A)** and DFS **(B)** in BC patients within the training cohort. The nomogram was used to sum the points identified on the points scale for each variable. The total points projected on the bottom scales indicate the probability of 3-, 5- and 7-year survival.

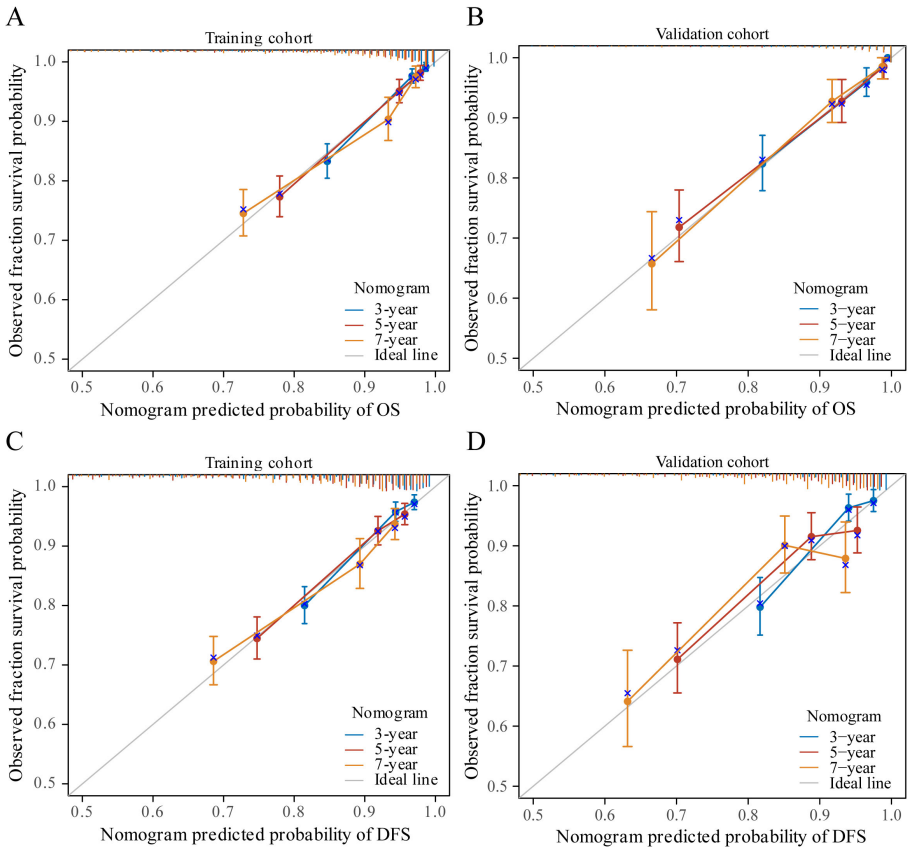



FIGURE 4
The calibration curves for predicting patient OS and DFS at three years, five years and seven years in the training cohort **(A, C)** and at three years, five years and seven years in the validation cohort **(B, D)**.

values across various time points for both OS and DFS. Specifically, in the training cohort, the nomogram achieved AUC values of 0.839, 0.807, and 0.772 for 3-year, 5-year, and 7-year OS, respectively (Figure 6A), compared to AUC values of 0.787, 0.750, and 0.725 for the TNM stage model (Figure 6B). Similarly, for DFS, the nomogram achieved AUC values of 0.780, 0.741, and 0.699 for 3-year, 5-year, and 7-year DFS, respectively (Figure 6E), compared to AUC values of 0.732, 0.700, and 0.683 for the TNM stage model in the training cohort (Figure 6F). These trends were consistent in the validation cohort, where the nomogram demonstrated higher AUC values for both OS and DFS at each time point compared to the TNM stage model (Figures 6C, D, G, H). The ROC curves depicted in Figure 6 further illustrate the enhanced predictive performance of the nomogram over the TNM stage model. In summary, these findings indicate that the nomogram provides superior predictive accuracy and clinical usability in forecasting survival outcomes across different time intervals.

In the training cohort, the C-index of the nomogram was higher than the C-index of the TMN stage, Subtype, and TMN stage + Subtype, respectively. Similarly, consistent results were observed in the validation cohort. The results are shown in Table 3. These findings provide insights into the interrelationships between plasma biomarker levels and various clinical factors in BC patients, highlighting inflammation and nutritional biomarker's potential as prognostic indicators and contributing to predicting the survival of BC patients.

To compare the clinical utility of this approach with traditional TNM staging, a decision curve analysis (DCA) was carried out. The DCA displayed graphically the net benefit of using the nomogram and TNM stage model to predict 5-year OS and DFS in the training and validation cohorts, taking into account a range of various recurrence threshold probabilities on the x-axis. The DCA plots (Figure 7) demonstrated that the nomogram provided a greater net benefit than other prognostic factors including the TNM stage nomogram model across the range of threshold probabilities evaluated. The nomogram showed higher net benefit curves, indicating that using the nomogram for risk stratification resulted in a higher overall net benefit in predicting 5-year OS and DFS. This suggests that the nomogram has a greater clinical utility than the conventional TNM stage model, as it provides superior risk stratification and enhances clinical decision-making. In summary, the DCA results further support the superiority of the nomogram over the TNM stage model in terms of clinical usefulness, as it offers greater net benefit in predicting 5-year OS and DFS across a range of threshold probabilities for recurrence.

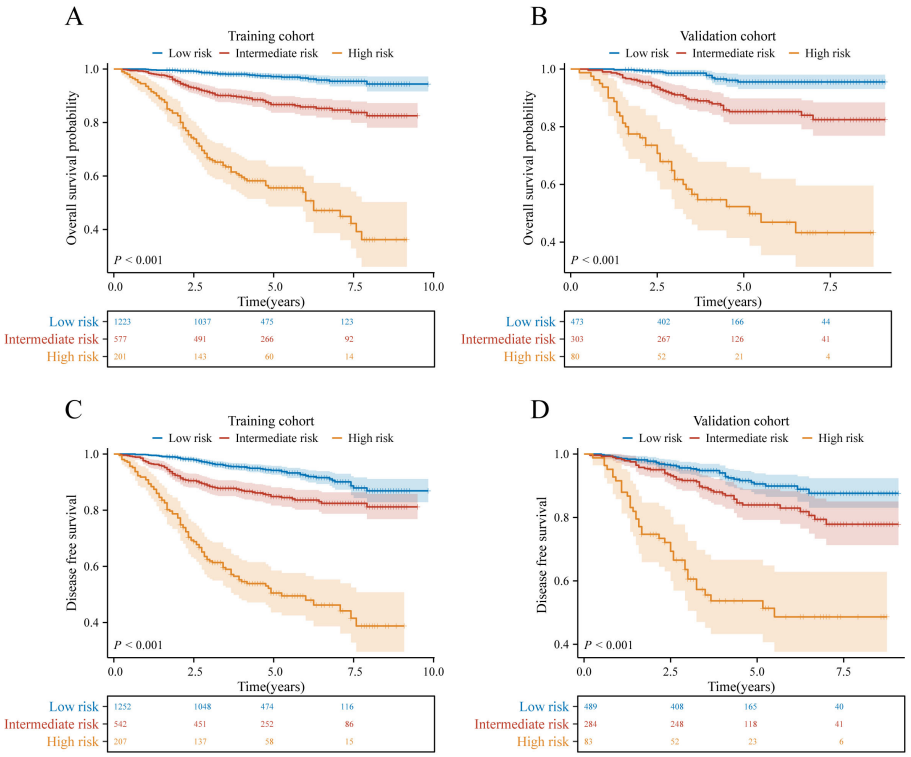



FIGURE 5
Graphs showing the results of Kaplan–Meier curves for all three groups based on the predictor from the nomogram model in the training cohort **(A, C)** and those in the validation cohort **(B, D)**.

## 3.7 External validation of the nomogram

The external validation cohort, consisting of 420 cases, was utilized as an independent validation set to assess the performance of our nomogram model. The C-index of the nomogram in this validation cohort was found to be 0.772 (95% CI: 0.726–0.817) (Figure 8). The validation of a model with a C-index exceeding 0.7 suggests that the model constructed in this study performs well in terms of robustness and reliability, thereby enhancing the credibility and persuasiveness of our research findings.

# 4 Discussion

In the present study, we constructed and confirmed a nomogram model that combines easily accessible inflammation and nutritional factors, and clinicopathological variables to predict OS and DFS in BC patients. The nomogram presented superior predictive accuracy, discriminative ability, and clinical usefulness compared to the traditional TNM stage system. Clinicians can utilize this nomogram to guide treatment decisions, monitor disease progression, and provide personalized patient care.

A growing body of research has demonstrated that inflammation and nutritional status play important roles in both tumor development and patient prognosis (39, 40). Inflammatory responses play a key role in the tumor microenvironment in regulating tumor growth, metastasis and treatment resistance (12). Nutritional status directly affects the immune function, metabolic status and physiological regulation of the patient, resulting in their resistance to tumor and therapeutic response (41). This study found that inflammatory (LYM, PLT) and nutritional (HGB, AGR, PA) indicators were strongly associated with BC prognosis. Watanabe J et al. and Kazuhiro Araki et al. discovered that elevated LYM levels are associated with an improved response to chemotherapies in metastatic BC patients (42, 43). Sung Min Ko et al. reported that LYM was a strong predictor of DFS in BC patients (44), which is consistent with our results. LYM plays a crucial role in anti-tumor immunity by inducing tumor cell apoptosis and is one of the key factors in immune surveillance and immune editing (45, 46).They combat tumors through multiple mechanisms, including the activation of cytotoxic T cells and natural killer (NK) cells, which directly target and eliminate tumor cells. Additionally, lymphocytes secrete cytokines such as interferon-gamma (IFN-γ and tumor necrosis factor-alpha (TNF-α which enhance the anti-tumor activity of other immune cells. These cytokines modulate the tumor microenvironment by promoting an immune-activating milieu and inhibiting tumor growth and metastasis (47, 48). Some studies have also shown that a low LYM may be the cause of inadequate immune response and the result of low survival rates in many types of cancer. This immune deficiency may lead to increased tumor proliferation and metastasis and reduced response to therapeutic interventions (49). Therefore, the immune response to breast tumors could vary depending on the composition

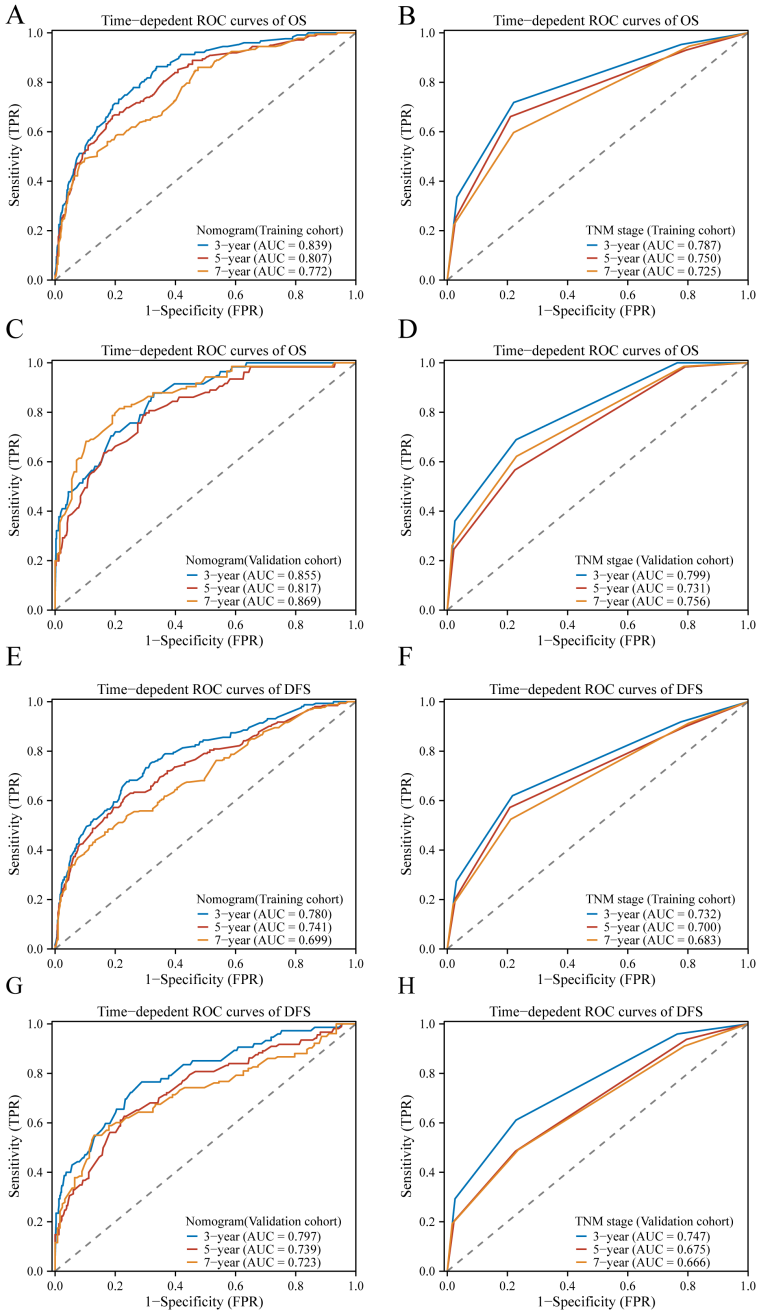



FIGURE 6
The ROC curves of the model to predict BC OS and DFS at 3, 5, and 7 years; In the training cohort, ROC curves comparing the nomogram **(A)** and TNM stage **(B)** for predicting OS, and in the validation cohort **(C, D)**, respectively. Similarly, in the training cohort, ROC curves compare the nomogram **(E)** and TNM stage **(F)** for predicting DFS, and in the validation cohort **(G, H)**, respectively.

of lymphocytes, which ultimately affects prognosis. Further research is needed on the mechanism of peripheral lymphocytes affecting BC. Discoveries have uncovered that PLTs function in inflammatory diseases and malignant tumors (50, 51). PLTs are closely associated with tumor cells and play a crucial role in the key stage of cancer metastasis. PLT regulates immune responses in the tumor microenvironment by secreting a variety of cytokines and growth factors (e.g., transforming growth factor-β, TGF-β). TGF-β not only inhibits the proliferation and function of lymphocytes, weakening the body's immune surveillance and immune editing ability but also promotes the growth and invasion of tumor cells, thus inhibiting the anti-tumor activity of immune cells (52). Through the release of cytokines, tumor cells activate platelets, promoting the extravasation and spread of cancer cells and negatively correlates with survival prognosis (53, 54). Notably, in our study, platelets were an independent prognostic factor of BC, which was also confirmed by the study of Liefaard, M. C. et al. and Graziano, V. et al (55, 56). Changes in LYM and platelets PLT, as indicators of inflammation, not only directly affect the immune response, but also indirectly influence tumor progression by

TABLE 3 The C-indexes of nomograms, TNM stage, Subtype, and TNM stage+ Subtype for prediction of OS and DFS in the training cohort and validation cohort.

| | Training cohort | | Validation cohort | |
|---|---|---|---|---|
| For OS | C-index | HR (95% CI) | C-index | HR (95% CI) |
| **Nomograms** | 0.82 | (0.805–0.350) | 0.838 | (0.818–0.858) |
| **TNM stage** | 0.759 | (0.741–0.778) | 0.772 | (0.749–0.794) |
| **Subtype** | 0.628 | (0.610–0.647) | 0.611 | (0.585–0.637) |
| **TMN stage + Subtype** | 0.801 | (0.785–0.817) | 0.802 | (0.779–0.825) |
| For DFS | | | | |
| **Nomograms** | 0.76 | (0.744–0.776) | 0.755 | (0.730–0.780) |
| **TNM stage** | 0.707 | (0.689–0.725) | 0.708 | (0.684–0.732) |
| **Subtype** | 0.611 | (0.595–0.627) | 0.583 | (0.560–0.607) |
| **TMN stage + Subtype** | 0.744 | (0.728–0.761) | 0.728 | (70.703–0.754) |

affecting the nutritional status. Our research indicates that HGB is a potential prognostic indicator for BC. A study by Michael Henke et al. showed that HGB concentration affects the prognosis of patients with early BC, which corresponds to our results (57). Several investigations indicated anemia and HGB play a pivotal role in malignant progression (58, 59). An important factor contributing to tumor hypoxia is the reduced oxygen transport capacity in the blood resulting from tumor-related and/or treatment-related anemia, which is a frequent complication seen in cancer patients (59, 60). HGB is an important prognostic indicator of nutritional status and hypoxia in cancer patients. Hence, it is essential to elucidate the underlying mechanisms of HGB that affect BC. In cancer patients, changes in the AGR are closely related to prognosis (61). Cancer-related chronic inflammation and malnutrition typically lead to a decrease in AGR, primarily by reducing albumin levels and increasing globulin levels. A low AGR usually indicates poor prognosis, higher recurrence rates, and shorter survival times (62). In the present study, few studies have systematically examined the connection between BC and AGR (63, 64). Basem N. Azab has shown that pretreatment AGR is an independent, significant

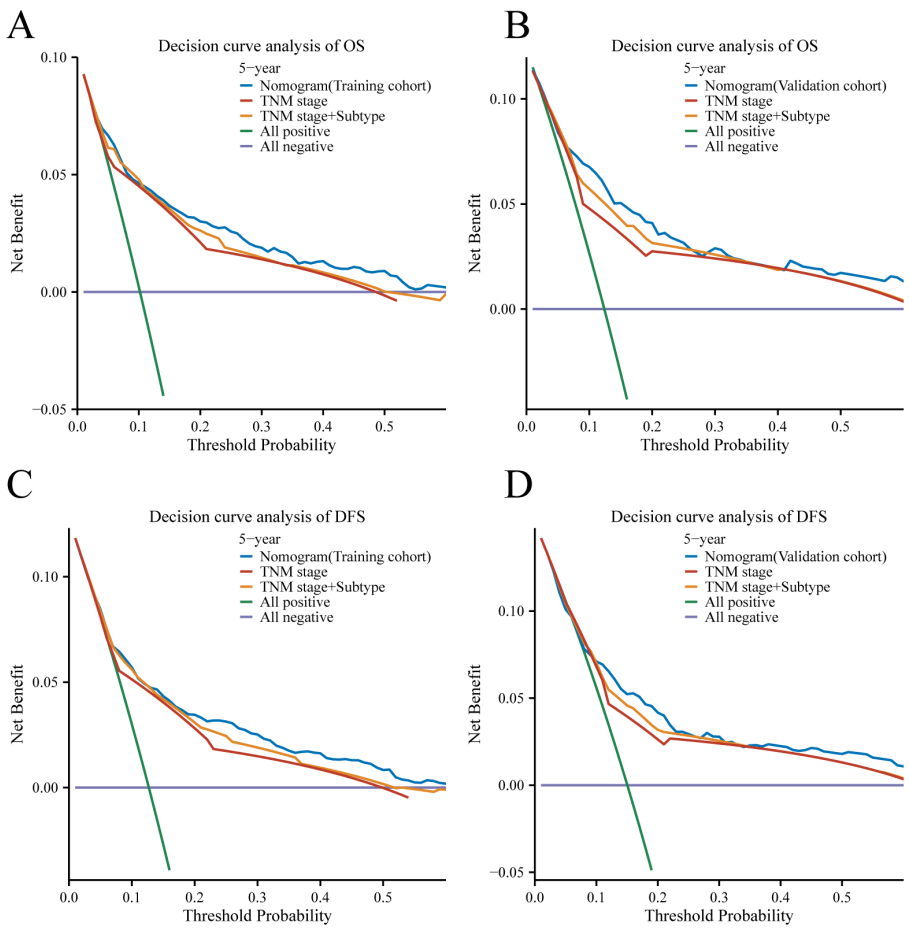



FIGURE 7
Decision curve analysis for 5-year survival predictions. **(A, C)** The decision curve of the training cohort; **(B, D)** The decision curve of the validation cohort.

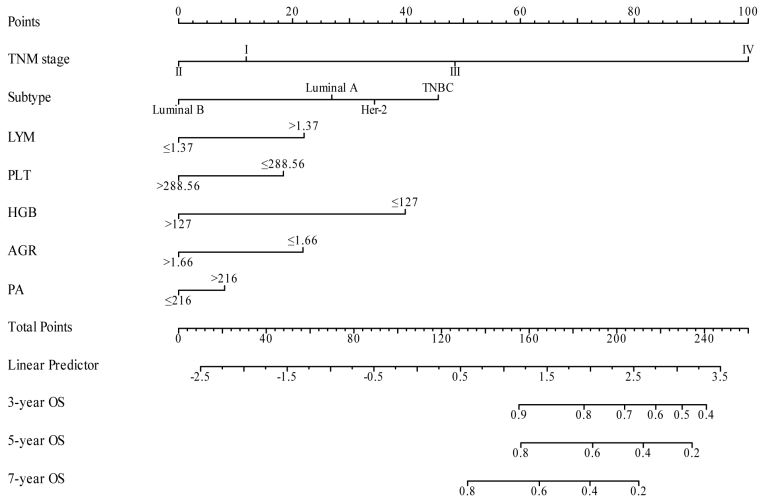



FIGURE 8
Nomogram model for predicting 3-year, 5-year, and 7-year OS in BC patients within the external validation cohort.

predictor of long-term mortality in BC patients (65). Additionally, this research demonstrates that AGR is an independent predictive factor for BC. PA, also known as transthyretin, is a thyroid hormone transport protein synthesized by the liver and partially degraded by the kidneys; its primary function is thyroxine transport. Serum PA concentrations less than 10 mg/dL are associated with malnutrition (66). PA is more sensitive to acute changes in protein balance and reacts to nutrition (67, 68). Numerous studies indicate that PA is a helpful single parameter for assessing protein-energy malnutrition, including postoperative outcomes and recurrence of non-small cell lung cancer (69, 70). Our research also revealed the prognostic significance of PA in BC. We have determined a comprehensive and systematic assessment, combining inflammation and nutritional blood markers, to evaluate their impact on the prognosis of BC.

Our nomogram demonstrated a significant improvement in predicting OS and DFS of BC patients compared to the TNM stage system. The model was further validated in an independent external cohort, confirming its reliability and reproducibility. Currently, several prognostic models are accessible for the clinical assessment of BC patients (71). Jeongmin Lee et al. and Xuanyi Wang et al. both developed a prognostic model based on radiomics to predict the DFS in BC patients, achieving a C-index of 0.63 and 0.82, respectively (72, 73). There are also nomograms based on molecular testing, gene expression profiling, and RNA sequencing data that can provide accurate predictions for BC patients. For instance, Jie Sun et al. created a model to predict BC risk in BRCA gene carriers, but in an empirical investigation, their C index was only 0.711 (74). MammaPrint test on 70 genes proved useful for early-stage BC treatment decisions (75), the C-index of the model for predicting OS was 0.614 (76). Liu Z. et al. developed a nomogram composed of 7-lncRNA signatures associated with immune invasion and tumor mutation burden in BC (77). However, molecular testing, gene expression profiling, and RNA sequencing data were not included in our model due to their requirement for highly specialized testing facilities, which entail high costs and necessitate skilled personnel for operation, thereby limiting their applicability. Our nomogram achieved a C-index of 0.820 for OS and 0.76 for DFS in the training cohort, which is relatively high compared to the analyses described above. Notably, in our study, the acquisition of hematological indicators is generally non-invasive, simpler, and more cost-effective, making them suitable for dynamic monitoring. These indicators can reflect changes in the patient's condition and provide comprehensive information on systemic status, including inflammatory responses, immune function, and nutritional status. In contrast, radiomics may involve the use of radiation or contrast agents, posing certain risks and discomfort. Moreover, radiomics can only provide structural information for specific sites and typically requires longer intervals between repeated assessments. Our prediction models can be cheaper, more accurate, and simpler to use in primary hospitals compared to these models. We developed a nomogram to predict 3-year, 5-year, and 7-year OS and DFS for BC patients in both training and validation cohorts. This tool aids clinicians in estimating individual survival probabilities with greater precision. Our nomogram demonstrates better prognostic accuracy and clinical utility. This prognostic model can be of great clinical value for patient management, risk stratification, therapeutic options, and postoperative monitoring strategies.

Despite the excellent discrimination ability of our nomogram, our research has its limitations. First, as with any retrospective study analysis, there is a potential risk of selection bias. Second, despite the use of an independent external validation cohort in this study, further research involving a multi-center prospective study with a larger dataset is warranted. Third, due to the limitations of our database, we are unable to incorporate genetic variables such as BRCA1/2 and P53 into our current model. We plan to explore and collect additional genetic and molecular marker data in future research to improve predictive accuracy and provide deeper insights into the biological complexity of BC.

## 5 Conclusion

Our study successfully developed a predictive nomogram for OS and DFS in BC patients by incorporating inflammation, nutritional factors, and pathologic, which showed greater precision than the conventional TNM staging system. The nomogram in this study was validated using independent cohorts from different institutions. Independent validation of the model with a C-index greater than 0.7 indicates that our model exhibits good performance in terms of robustness and reliability. The nomogram is a straightforward, low-cost, and useful tool that can assist clinicians with choosing therapies and patient counseling. Further analysis and validation studies are warranted to refine and improve the nomogram, taking into account the limitations mentioned above, and to establish its usefulness in clinical practice.

## 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 authors.

## Ethics statement

The studies involving humans were approved by Guangxi Medical University Cancer Hospital Ethical Review Committee; Guangxi Zhuang Autonomous Region Maternal and Child Health Care Hospital Ethical Review Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

CW: Writing – original draft, Investigation, Methodology, Software. HA: Software, Writing – original draft, Data curation, Formal analysis, Validation. DM: Methodology, Supervision, Visualization, Writing – original draft. PW: Formal analysis, Resources, Writing – original draft, Data curation. LW: Formal analysis, Resources, Writing – original draft. ZL: Writing – original draft, Validation. PL: Validation, Writing – original draft. TH: Writing – original draft, Visualization, Writing – review & editing. ML: Writing – original draft, Writing – review & editing, Conceptualization.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by grants from National Science Foundation of Guangxi (2022GXNSFAA035510), National Science Foundation of China (81760530), and Postdoctoral Science Foundation of China (2021M693803).

## Acknowledgments

The authors gratefully acknowledge each editor and reviewer for their efforts on this study.

## 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. Nolan E, Lindeman GJ, Visvader JE. Deciphering breast cancer: from biology to the clinic. *Cell*. (2023) 186:1708–28. doi: 10.1016/j.cell.2023.01.040

2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. (2023) 73:17–48. doi: 10.3322/caac.21763

3. Mutebi M, Anderson BO, Duggan C, Adebamowo C, Agarwal G, Ali Z, et al. Breast cancer treatment: A phased approach to implementation. *Cancer*. (2020) 126 Suppl 10:2365–78. doi: 10.1002/cncr.32910

4. Blondeaux E, Arecco L, Punie K, Graffeo R, Toss A, De Angelis C, et al. Germline Tp53 pathogenic variants and breast cancer: A narrative review. *Cancer Treat Rev*. (2023) 114:102522. doi: 10.1016/j.ctrv.2023.102522

5. Barzaman K, Karami J, Zarei Z, Hosseinzadeh A, Kazemi MH, Moradi-Kalbolandi S, et al. Breast cancer: biology, biomarkers, and treatments. *Int Immunopharmacol*. (2020) 84:106535. doi: 10.1016/j.intimp.2020.106535

6. Chen MT, Sun HF, Zhao Y, Fu WY, Yang LP, Gao SP, et al. Comparison of patterns and prognosis among distant metastatic breast cancer patients by age groups: A seer population-based analysis. *Sci Rep*. (2017) 7:9254. doi: 10.1038/s41598-017-10166-8

7. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The eighth edition ajcc cancer staging manual: continuing to build a bridge from a population-based to a more "Personalized" Approach to cancer staging. *CA Cancer J Clin*. (2017) 67:93–9. doi: 10.3322/caac.21388

8. Adam MA, Thomas S, Roman SA, Hyslop T, Sosa JA. Rethinking the current american joint committee on cancer Tnm staging system for medullary thyroid cancer. *JAMA Surg*. (2017) 152:869–76. doi: 10.1001/jamasurg.2017.1665

9. Januskeviciene I, Petrikaite V. Heterogeneity of breast cancer: the importance of interaction between different tumor cell populations. *Life Sci*. (2019) 239:117009. doi: 10.1016/j.lfs.2019.117009

10. Turashvili G, Brogi E. Tumor heterogeneity in breast cancer. *Front Med (Lausanne)*. (2017) 4:227. doi: 10.3389/fmed.2017.00227

11. Yeo SK, Guan JL. Breast cancer: multiple subtypes within a tumor? *Trends Cancer*. (2017) 3:753–60. doi: 10.1016/j.trecan.2017.09.001

12. Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y, et al. Inflammation and tumor progression: signaling pathways and targeted intervention. *Signal Transduct Target Ther*. (2021) 6:263. doi: 10.1038/s41392-021-00658-5

13. Xie H, Ruan G, Ge Y, Zhang Q, Zhang H, Lin S, et al. Inflammatory burden as a prognostic biomarker for cancer. *Clin Nutr*. (2022) 41:1236–43. doi: 10.1016/j.clnu.2022.04.019

14. Greten FR, Grivennikov SI. Inflammation and cancer: triggers, mechanisms, and consequences. *Immunity*. (2019) 51:27–41. doi: 10.1016/j.immuni.2019.06.025

15. Manjili SH, Isbell M, Ghochaghi N, Perkinson T, Manjili MH. Multifaceted functions of chronic inflammation in regulating tumor dormancy and relapse. *Semin Cancer Biol*. (2022) 78:17–22. doi: 10.1016/j.semcancer.2021.03.023

16. Takahashi M, Sowa T, Tokumasu H, Gomyoda T, Okada H, Ota S, et al. Comparison of three nutritional scoring systems for outcomes after complete resection of non-small cell lung cancer. *J Thorac Cardiovasc Surg*. (2021) 162:1257–68 e3. doi: 10.1016/j.jtcvs.2020.06.030

17. Lin F, Xia W, Chen M, Jiang T, Guo J, Ouyang Y, et al. A prognostic model based on nutritional risk index in operative breast cancer. *Nutrients*. (2022) 14(18):3783. doi: 10.3390/nu14183783

18. Kubrak C, Martin L, Gramlich L, Scrimger R, Jha N, Debenham B, et al. Prevalence and prognostic significance of malnutrition in patients with cancers of the head and neck. *Clin Nutr*. (2020) 39:901–9. doi: 10.1016/j.clnu.2019.03.030

19. Arends J, Baracos V, Bertz H, Bozzetti F, Calder PC, Deutz NEP, et al. Espen expert group recommendations for action against cancer-related malnutrition. *Clin Nutr*. (2017) 36:1187–96. doi: 10.1016/j.clnu.2017.06.017

20. Dolan RD, McMillan DC. The prevalence of cancer associated systemic inflammation: implications of prognostic studies using the glasgow prognostic score. *Crit Rev Oncol Hematol*. (2020) 150:102962. doi: 10.1016/j.critrevonc.2020.102962

21. Ravasco P. Nutrition in cancer patients. *J Clin Med*. (2019) 8(8):1211. doi: 10.3390/jcm8081211

22. Baracos VE. Cancer-associated malnutrition. *Eur J Clin Nutr*. (2018) 72:1255–9. doi: 10.1038/s41430-018-0245-4

23. Cho U, Park HS, Im SY, Yoo CY, Jung JH, Suh YJ, et al. Prognostic value of systemic inflammatory markers and development of a nomogram in breast cancer. *PloS One*. (2018) 13:e0200936. doi: 10.1371/journal.pone.0200936

24. Deng Q, He B, Liu X, Yue J, Ying H, Pan Y, et al. Prognostic value of pre-operative inflammatory response biomarkers in gastric cancer patients and the construction of a predictive model. *J Transl Med*. (2015) 13:66. doi: 10.1186/s12967-015-0409-0

25. Li B, Deng H, Zhou Z, Tang B. The prognostic value of the fibrinogen to pre-albumin ratio in Malignant tumors of the digestive system: A systematic review and meta-analysis. *Cancer Cell Int*. (2022) 22:22. doi: 10.1186/s12935-022-02445-w

26. Quhal F, Pradere B, Laukhtina E, Sari Motlagh R, Mostafaei H, Mori K, et al. Prognostic value of albumin to globulin ratio in non-muscle-invasive bladder cancer. *World J Urol*. (2021) 39:3345–52. doi: 10.1007/s00345-020-03586-1

27. Tang S, Lin L, Cheng J, Zhao J, Xuan Q, Shao J, et al. The prognostic value of preoperative fibrinogen-to-prealbumin ratio and a novel Ffc score in patients with resectable gastric cancer. *BMC Cancer*. (2020) 20:382. doi: 10.1186/s12885-020-06866-6

28. Xu SS, Li S, Xu HX, Li H, Wu CT, Wang WQ, et al. Haemoglobin, albumin, lymphocyte and platelet predicts postoperative survival in pancreatic cancer. *World J Gastroenterol*. (2020) 26:828–38. doi: 10.3748/wjg.v26.i8.828

29. Chen Y, Chen K, Xiao X, Nie Y, Qu S, Gong C, et al. Pretreatment neutrophil-to-lymphocyte ratio is correlated with response to neoadjuvant chemotherapy as an independent prognostic indicator in breast cancer patients: A retrospective study. *BMC Cancer*. (2016) 16:320. doi: 10.1186/s12885-016-2352-8

30. Lin S, Mo H, Li Y, Guan X, Chen Y, Wang Z, et al. Development and validation of a nomogram for predicting survival of advanced breast cancer patients in China. *Breast*. (2020) 53:172–80. doi: 10.1016/j.breast.2020.08.004

31. Yu Y, Tan Y, Xie C, Hu Q, Ouyang J, Chen Y, et al. Development and validation of a preoperative magnetic resonance imaging radiomics-based signature to predict axillary lymph node metastasis and disease-free survival in patients with early-stage breast cancer. *JAMA Netw Open*. (2020) 3:e2028086. doi: 10.1001/jamanetworkopen.2020.28086

32. Xie X, Tan W, Chen B, Huang X, Peng C, Yan S, et al. Preoperative prediction nomogram based on primary tumor mirnas signature and clinical-related features for axillary lymph node metastasis in early-stage invasive breast cancer. *Int J Cancer*. (2018) 142:1901–10. doi: 10.1002/ijc.31208

33. Hua X, Duan F, Zhai W, Song C, Jiang C, Wang L, et al. A novel inflammatory-nutritional prognostic scoring system for patients with early-stage breast cancer. *J Inflammation Res*. (2022) 15:381–94. doi: 10.2147/JIR.S338421

34. Jiang C, Xiu Y, Yu X, Qiao K, Zhang S, Huang Y. Prognostic value of a modified systemic inflammation score in breast cancer patients who underwent neoadjuvant chemotherapy. *BMC Cancer*. (2022) 22:1249. doi: 10.1186/s12885-022-10291-2

35. Pan X, Yang W, Chen Y, Tong L, Li C, Li H. Nomogram for predicting the overall survival of patients with inflammatory breast cancer: A seer-based study. *Breast*. (2019) 47:56–61. doi: 10.1016/j.breast.2019.05.015

36. Tang Y, Zhang YJ, Zhang N, Shi M, Wen G, Cheng J, et al. Nomogram predicting survival as a selection criterion for postmastectomy radiotherapy in patients with T1 to T2 breast cancer with 1 to 3 positive lymph nodes. *Cancer*. (2020) 126 Suppl 16:3857–66. doi: 10.1002/cncr.32963

37. Huang Z, Shi M, Wang WH, Shen LF, Tang Y, Rong QL, et al. A novel nomogram for predicting locoregional recurrence risk in breast cancer patients treated with neoadjuvant chemotherapy and mastectomy. *Radiother Oncol*. (2021) 161:191–7. doi: 10.1016/j.radonc.2021.06.015

38. Giuliano AE, Edge SB, Hortobagyi GN. Eighth edition of the Ajcc cancer staging manual: breast cancer. *Ann Surg Oncol*. (2018) 25:1783–5. doi: 10.1245/s10434-018-6486-6

39. Borre M, Dam GA, Knudsen AW, Grønbaek H. Nutritional status and nutritional risk in patients with neuroendocrine tumors. *Scand J Gastroenterol*. (2018) 53:284–92. doi: 10.1080/00365521.2018.1430848

40. Fankhauser CD, Sander S, Roth L, Gross O, Eberli D, Sulser T, et al. Systemic inflammatory markers have independent prognostic value in patients with metastatic testicular germ cell tumours undergoing first-line chemotherapy. *Br J Cancer*. (2018) 118:825–30. doi: 10.1038/bjc.2017.467

41. Newsholme P. Cellular and metabolic mechanisms of nutrient actions in immune function. *Nutr Diabetes*. (2021) 11:22. doi: 10.1038/s41387-021-00162-3

42. Araki K, Ito Y, Fukada I, Kobayashi K, Miyagawa Y, Imamura M, et al. Predictive impact of absolute lymphocyte counts for progression-free survival in human epidermal growth factor receptor 2-positive advanced breast cancer treated with pertuzumab and trastuzumab plus eribulin or Nab-paclitaxel. *BMC Cancer*. (2018) 18:982. doi: 10.1186/s12885-018-4888-2

43. Watanabe J, Saito M, Horimoto Y, Nakamoto S. A maintained absolute lymphocyte count predicts the overall survival benefit from eribulin therapy, including eribulin re-administration, in Her2-negative advanced breast cancer patients: A single-institutional experience. *Breast Cancer Res Treat*. (2020) 181:211–20. doi: 10.1007/s10549-020-05626-1

44. Ko SM, Lee J, Bae SJ, Baik SJ, Ji J, Kim D, et al. Body mass index and absolute lymphocyte count predict disease-free survival in Korean breast cancer patients. *Br J Cancer*. (2021) 125:119–25. doi: 10.1038/s41416-021-01391-0

45. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity*. (2004) 21:137–48. doi: 10.1016/j.immuni.2004.07.017

46. Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cell Mol Immunol*. (2020) 17:807–21. doi: 10.1038/s41423-020-0488-6

47. Dupré A, Malik HZ. Inflammation and cancer: what a surgical oncologist should know. *Eur J Surg Oncol*. (2018) 44:566–70. doi: 10.1016/j.ejso.2018.02.209

48. Ménétrier-Caux C, Ray-Coquard I, Blay JY, Caux C. Lymphopenia in cancer patients and its effects on response to immunotherapy: an opportunity for combination with cytokines? *J Immunother Cancer*. (2019) 7:85. doi: 10.1186/s40425-019-0549-5

49. Hoffmann TK, Dworacki G, Tsukihiro T, Meidenbauer N, Gooding W, Johnson JT, et al. Spontaneous apoptosis of circulating T lymphocytes in patients with head and neck cancer and its clinical importance. *Clin Cancer Res*. (2002) 8:2553–62.

50. Stone RL, Nick AM, McNeish IA, Balkwill F, Han HD, Bottsford-Miller J, et al. Paraneoplastic thrombocytosis in ovarian cancer. *N Engl J Med*. (2012) 366:610–8. doi: 10.1056/NEJMoa1110352

51. van der Meijden PEJ, Heemskerk JWM. Platelet biology and functions: new concepts and clinical perspectives. *Nat Rev Cardiol*. (2019) 16:166–79. doi: 10.1038/s41569-018-0110-0

52. Li S, Lu Z, Wu S, Chu T, Li B, Qi F, et al. The dynamic role of platelets in cancer progression and their therapeutic implications. *Nat Rev Cancer*. (2024) 24:72–87. doi: 10.1038/s41568-023-00639-6

53. Marin C, Garcia-Dominguez X, Montoro-Dasi L, Lorenzo-Rebenaque L, Vicente JS, Marco-Jimenez F. Experimental evidence reveals both cross-infection and cross-contamination risk of embryo storage in liquid nitrogen biobanks. *Anim (Basel)*. (2020) 10(4):598. doi: 10.3390/ani10040598

54. Bambace NM, Holmes CE. The platelet contribution to cancer progression. *J Thromb Haemost*. (2011) 9:237–49. doi: 10.1111/j.1538-7836.2010.04131.x

55. Liefaard MC, Moore KS, Mulder L, van den Broek D, Wesseling J, Sonke GS, et al. Tumour-educated platelets for breast cancer detection: biological and technical insights. *Br J Cancer*. (2023) 128:1572–81. doi: 10.1038/s41416-023-02174-5

56. Graziano V, Grassadonia A, Iezzi L, Vici P, Pizzuti L, Barba M, et al. Combination of peripheral neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio is predictive of pathological complete response after neoadjuvant chemotherapy in breast cancer patients. *Breast*. (2019) 44:33–8. doi: 10.1016/j.breast.2018.12.014

57. Henke M, Sindlinger F, Ikenberg H, Gerds T, Schumacher M. Blood hemoglobin level and treatment outcome of early breast cancer. *Strahlenther Onkol*. (2004) 180:45–51. doi: 10.1007/s00066-004-1123-7

58. Vaupel P, Mayer A. Hypoxia and anemia: effects on tumor biology and treatment resistance. *Transfus Clin Biol.* (2005) 12:5–10. doi: 10.1016/j.tracli.2004.11.005

59. Vaupel P, Mayer A, Hockel M. Impact of hemoglobin levels on tumor oxygenation: the higher, the better? *Strahlenther Onkol.* (2006) 182:63–71. doi: 10.1007/s00066-006-1543-7

60. Gilreath JA, Stenehjem DD, Rodgers GM. Diagnosis and treatment of cancer-related anemia. *Am J Hematol.* (2014) 89:203–12. doi: 10.1002/ajh.23628

61. Chen Z, Shao Y, Yao H, Zhuang Q, Wang K, Xing Z, et al. Preoperative albumin to globulin ratio predicts survival in clear cell renal cell carcinoma patients. *Oncotarget.* (2017) 8:48291–302. doi: 10.18632/oncotarget.v8i29

62. Li Q, Meng X, Liang L, Xu Y, Cai G, Cai S. High preoperative serum globulin in rectal cancer treated with neoadjunctive chemoradiation therapy is a risk factor for poor outcome. *Am J Cancer Res.* (2015) 5:2856–64.

63. Liu C, Wang W, Meng X, Sun B, Cong Y, Liu J, et al. Albumin/globulin ratio is negatively correlated with Pd-1 and Cd25 Mrna levels in breast cancer patients. *Onco Targets Ther.* (2018) 11:2131–9. doi: 10.2147/OTT.S159481

64. Xuan Q, Yang Y, Ji H, Tang S, Zhao J, Shao J, et al. Combination of the preoperative albumin to globulin ratio and neutrophil to lymphocyte ratio as a novel prognostic factor in patients with triple negative breast cancer. *Cancer Manag Res.* (2019) 11:5125–31. doi: 10.2147/CMAR.S195324

65. Azab BN, Bhatt VR, Vonfrolio S, Bachir R, Rubinshteyn V, Alkaied H, et al. Value of the pretreatment albumin to globulin ratio in predicting long-term mortality in breast cancer patients. *Am J Surg.* (2013) 206:764–70. doi: 10.1016/j.amjsurg.2013.03.007

66. Keller U. Nutritional laboratory markers in malnutrition. *J Clin Med.* (2019) 8 (6):775. doi: 10.3390/jcm8060775

67. Zhou J, Hiki N, Mine S, Kumagai K, Ida S, Jiang X, et al. Role of prealbumin as a powerful and simple index for predicting postoperative complications after gastric cancer surgery. *Ann Surg Oncol.* (2017) 24:510–7. doi: 10.1245/s10434-016-5548-x

68. Davis CJ, Sowa D, Keim KS, Kinnare K, Peterson S. The use of prealbumin and C-reactive protein for monitoring nutrition support in adult patients receiving enteral nutrition in an urban medical center. *JPEN J Parenter Enteral Nutr.* (2012) 36:197–204. doi: 10.1177/0148607111413896

69. Yu PJ, Cassiere HA, Dellis SL, Manetta F, Kohn N, Hartman AR. Impact of preoperative prealbumin on outcomes after cardiac surgery. *JPEN J Parenter Enteral Nutr.* (2015) 39:870–4. doi: 10.1177/0148607114536735

70. Kawai H, Ota H. Low perioperative serum prealbumin predicts early recurrence after curative pulmonary resection for non-small-cell lung cancer. *World J Surg.* (2012) 36:2853–7. doi: 10.1007/s00268-012-1766-y

71. Ethier JL, Desautels D, Templeton A, Shah PS, Amir E. Prognostic role of neutrophil-to-lymphocyte ratio in breast cancer: A systematic review and meta-analysis. *Breast Cancer Res.* (2017) 19:2. doi: 10.1186/s13058-016-0794-1

72. Lee J, Kim SH, Kim Y, Park J, Park GE, Kang BJ. Radiomics nomogram: prediction of 2-year disease-free survival in young age breast cancer. *Cancers.* (2022) 14 (18):4461. doi: 10.3390/cancers14184461

73. Wang X, Xie T, Luo J, Zhou Z, Yu X, Guo X. Radiomics predicts the prognosis of patients with locally advanced breast cancer by reflecting the heterogeneity of tumor cells and the tumor microenvironment. *Breast Cancer Res: BCR.* (2022) 24:20. doi: 10.1186/s13058-022-01516-0

74. Sun J, Chu F, Pan J, Zhang Y, Yao L, Chen J, et al. Brca-crisk: A contralateral breast cancer risk prediction model for brca carriers. *J Clin Oncol.* (2023) 41:991–9. doi: 10.1200/JCO.22.00833

75. Cardoso F, van't Veer LJ, Bogaerts J, Slaets L, Viale G, Delaloge S, et al. 70-gene signature as an aid to treatment decisions in early-stage breast cancer. *N Engl J Med.* (2016) 375:717–29. doi: 10.1056/NEJMoa1602253

76. Ibraheem A, Olopade OI, Huo D. Propensity score analysis of the prognostic value of genomic assays for breast cancer in diverse populations using the national cancer data base. *Cancer.* (2020) 126:4013–22. doi: 10.1002/cncr.32956

77. Liu Z, Mi M, Li X, Zheng X, Wu G, Zhang L. A Lncrna prognostic signature associated with immune infiltration and tumour mutation burden in breast cancer. *J Cell Mol Med.* (2020) 24:12444–56. doi: 10.1111/jcmm.15762

frontiers | Frontiers in Endocrinology

TYPE Systematic Review
PUBLISHED 29 August 2024
DOI 10.3389/fendo.2024.1360595



OPEN ACCESS

EDITED BY
Zili Zhang,
Nanjing University of Chinese Medicine, China

REVIEWED BY
Fahad Quhal,
King Fahad Specialist Hospital Dammam, Saudi Arabia
Lei Peng,
South China Hospital of Shenzhen University, China

*CORRESPONDENCE
Zhengqing Bao
baozq1101@bjmu.edu.cn

RECEIVED 08 January 2024
ACCEPTED 14 August 2024
PUBLISHED 29 August 2024

CITATION
Bao Z, Li G, He F, Xu X, Liu Z and Wang J (2024) The prognostic value of preoperative plasma fibrinogen in Asian patients with urothelial cancer: a systematic review and meta-analysis.
*Front. Endocrinol.* 15:1360595.
doi: 10.3389/fendo.2024.1360595



# The prognostic value of preoperative plasma fibrinogen in Asian patients with urothelial cancer: a systematic review and meta-analysis

Zhengqing Bao*, Guizhong Li, Feng He, Xiao Xu, Zhenhua Liu and Jianwei Wang

Department of Urology Surgery, Beijing Jishuitan Hospital, Capital Medical University, Beijing, China

**Objective:** We conducted this meta-analysis to comprehensively explore the prognostic value of the preoperative plasma fibrinogen in Asian patients diagnosed with urothelial cancer (UC).

**Methods:** After a systematic search of Web of Science, PubMed, and Embase before May 2024, we included 10 studies in our meta-analysis. The hazard ratios (HRs) with 95% confidence interval (CI) for overall survival (OS), cancer-specific survival (CSS), recurrence-free survival (RFS), and progression free survival (PFS) were estimated using fixed effect model.

**Results:** This meta-analysis included a total of 2875 patients. UC patients with an elevated preoperative plasma fibrinogen had worse OS (pooled HR: 2.13, 95% CI: 1.81-2.51; P<0.001), CSS (pooled HR: 2.22, 95% CI: 1.83-2.70; P<0.001), RFS (pooled HR: 1.90, 95% CI: 1.59-2.27; P<0.001), and PFS (pooled HR: 2.12, 95% CI: 1.36-3.29, P=0.001). No significant heterogeneity or publication bias was found. Additionally, statistically significant pooled HRs were also calculated in subgroup analysis when stratified by cancer type, country, and cut-off value.

**Conclusions:** The presence of elevated preoperative plasma fibrinogen levels is significantly correlated with unfavorable tumor outcomes in UCs.

KEYWORDS

plasma fibrinogen, urothelial cancer, prognosis, meta-analysis, Asian

# 1 Introduction

Urothelial carcinoma (UC) is one of the most common malignancies arising from the entire urinary tract (1), and it mainly includes bladder cancer (BC) and upper tract UC (UTUC). In United States, approximately 168,560 individuals will be diagnosed with UC and 32,590 will die from the disease in 2023 (2). The biological behavior of UC is complicated, making it prone to invasion, recurrence, and metastasis (3). Despite significant improvements in diagnosis and treatment of UC, oncologic outcomes remain poor. The 5-year survival rates for locally advanced UC and metastatic UC were only 34% and 5.4%, respectively (3). Therefore, an effective and applicable biomarker is necessary to accurately predict the prognoses and formulate follow-up strategies based on the stratification of risks for UC patients.

The plasma fibrinogen, serving as a crucial factor in blood coagulation and an indicator of inflammation, plays a pivotal role in maintaining human health (4, 5). Numerous studies have revealed that the coagulation/fibrinolytic system is initiated *in vivo* among cancer patients, and these markers can be employed for predicting tumorigenesis and prognosis (6, 7). Recently, an increasing body of evidence suggests that preoperative plasma fibrinogen can be used as a prognostic predictor in patients with UC, including UTUC (8–13) and BC (14–17). Song et al. (18) conducted a meta-analysis in urological cancers to assess the prognostic value of preoperative plasma fibrinogen. However, their study solely focused on UTUC (no BC, another type of UC) and had limited inclusion of studies. Interestingly, there are well-documented race-based differences in the treatment and outcomes for UC (19). Thus, the present meta-analysis included additional recent studies on UTUC and BC patients to evaluate the prognostic value of preoperative plasma fibrinogen on survival outcomes in UCs among Asian population.

# 2 Materials and methods

## 2.1 Protocol

Before commencing our study, we registered our systematic review project with the International Prospective Register of Systemic Reviews (PROSPERO; http://www.crd.york.ac.uk/PROSPERO/CRD42024496302). This meta-analysis was conducted in accordance with the Meta-analysis of Observational Studies in Epidemiology (MOOSE) criteria (see Supplementary Files).

## 2.2 Literature search

We systematically searched Web of Science, Embase, and PubMed to obtain all available clinical studies published before May 2024 without any language restrictions. We used the terms (fibrinogen, transitional cell carcinoma, upper urinary tract, urothelial carcinoma, ureter cancer, ureteral cancer, ureter carcinoma, ureteral carcinoma, bladder cancer, bladder carcinoma, and bladder tumor) to search for the related articles in the databases (see Supplementary Files). The literature search was independently conducted by two investigators, Zhengqing Bao and Guizhong Li.

## 2.3 Inclusion and exclusion criteria

The literature search, study selection, and validation were independently performed by two authors (Zhengqing Bao and Guizhong Li), and a third author (Jianwei Wang) was consulted to resolve the disagreements.

Studies were considered eligible if they met all of the following criteria: 1) cohort studies on patients with localized UCs reported the association between the preoperative plasma fibrinogen and oncological outcomes, included overall survival (OS), cancer-specific survival (CSS), recurrence-free survival (RFS), or/and progression free survival (PFS), after surgery. The plasma fibrinogen was tested before a definitive operation or diagnostic procedure (a definitive operation was generally performed shortly thereafter); 2) publications provided sufficient information to extract or calculate hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs); 3) all patients classified into low and high plasma fibrinogen groups; and 4) full-text articles. The following studies were excluded based on any of the following criteria: 1) reviews, abstracts, letters, reviews, case reports, editorials, or basic studies; 2) studies with insufficient information for HRs and 95% CIs; 3) sample size<50; 4) non- Asian population; and 5) duplicate or overlapping studies.

## 2.4 Data extraction and quality assessment

The data extraction was independently performed by two investigators, Zhengqing Bao and Guizhong Li. The extracted data included the first author's name, publication year, country, cancer type, sample size, duration time, age, gender, cutoff value, follow-up duration, HRs (95%CI), and analysis method (univariate/multivariate). If both univariate and multivariate analyses were performed, we chose the HRs (95%CI) from multivariate analysis. OS, CSS, RFS and PFS were analyzed. The study quality was systematically evaluated according to the Newcastle-Ottawa Scale (NOS) (see Supplementary Files). A 'star system' has been developed in which a study is judged on three broad perspectives: the selection of the study groups; the comparability of the groups; and the ascertainment of either the exposure or outcome of interest for studies respectively. A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability. Studies with more than 6 stars were considered as high-quality. The quality assessment was independently performed by two investigators (Zhengqing Bao and Guizhong Li), with a third reviewer (Jianwei Wang) consulted to resolve any disagreements.

## 2.5 Statistical analysis

All statistical analyses were conducted using STATA 15.0 (STATA Corporation, College Station, TX, USA). Statistical significance was set as a p-value<0.05. The heterogeneity of included studies was evaluated using Cochran's Q-test and Higgins $I^2$ statistics ($I^2$). The random effects-model was used for the significant heterogeneity, which was indicated by $I^2$>50% or P<0.05. Otherwise, the fixed-effect model was adopted to calculate pooled HRs for no obvious heterogeneity. Subgroup and meta-regression analyses were performed to explore the potential factors for heterogeneity. Sensitivity analysis was conducted using a "one-study removed" model to assess the stability of the overall results. Potential publication bias was assessed by using funnel plots visually, whose results were confirmed by using Begg's and Egger's tests. If significant publication bias was identified, the trim-and-fill method estimated an adjusted effect size.

# 3 Results

## 3.1 Characteristics of the included studies

A total of 656 records were identified through a systematic literature search. After excluding 172 duplicates, the titles and abstracts of the remaining 466 records were screened, resulting in the selection of 18 articles for full-text reading. Out of these 18 records, 8 were excluded: two studies included overlapped data with others; two studies did not have OS, CSS, RFS, and PFS as final outcomes; one study had a sample size <50; one study lacked sufficient information to extract or calculate HRs and their CIs; and two studies were based on non-Asian populations. Finally, 10 studies were included in this meta-analysis (Figure 1; Table 1).

The included studies had 2875 cases. 6 studies examined the prognostic value of plasma fibrinogen in UTUC (including 2017 patients), and 4 in BC (including 858 patients). As for survival outcomes, 8 studies (including 2539 patients) evaluated the prognostic value of plasma fibrinogen in predicting OS, 5 studies (including 1892 patients) evaluated CSS, 6 studies (including 1703 patients) evaluated RFS, and 2 studies (including 336 patients) evaluated PFS. All included studies achieved a minimum score of 7 on the NOS and were deemed to be of high quality.

## 3.2 Overall survival

The presence of elevated preoperative plasma fibrinogen levels was found to be significantly associated with a poorer OS outcome in patients with UCs (fixed effect model, pooled hazard ratio: 2.13, 95% confidence interval: 1.81-2.51; P<0.001) (Figure 2A; Table 2). No heterogeneity across studies was found ($I^2$ = 0.0%, P=0.787). Subgroup analysis based on cancer type revealed that high preoperative plasma fibrinogen was associated with poor OS in both UTUC (fixed effect model, pooled HR: 2.08, 95% CI: 1.74-2.48, P<0.001), and BC (fixed effect model, pooled HR: 2.56, 95% CI: 1.60-4.11, P<0.001) (Figure 2B; Table 2). In subgroup analyses based

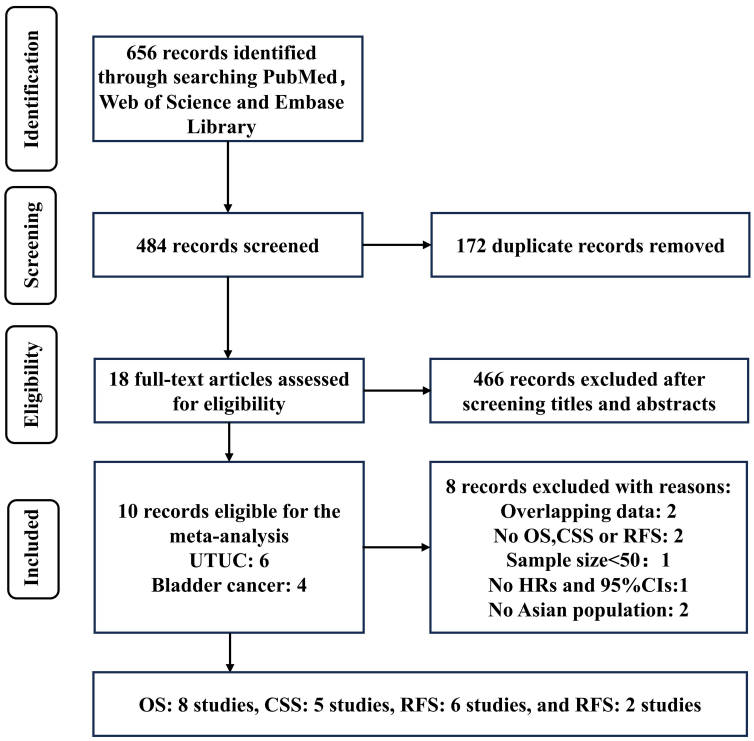



FIGURE 1
Flow diagram of the study selection process.

TABLE 1 Characteristics of all 10 studies included in the meta-analysis.

| Author (year) | Country | Cancer type | N of cases | Duration | Age (years) | Gender (M/F) | Cutoff, mg/dl | Follow-up (months) | HR from | Outcome | NOS score |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tanaka (2015) (11) | Japan | UTUC | 394 | 1995-2011 | 70 (IQR: 63-77) | 289/105 | 390 | 30 (IQR:15-63) | UV/ MV | OS CSS RFS | 8 |
| Huang (2017) (8) | China | UTUC | 481 | 2002-2013 | 65.8 ± 11.1 | 311/170 | 422 | 40 (IQR:24-64) | UV/ MV | OS CSS | 8 |
| Zhang (2016) (13) | China | UTUC | 184 | 2006-2008 | 70 (61-75) | 84/100 | 354 | 78 (34-92) | UV/ MV | OS CSS | 8 |
| Liu (2019) (10) | China | UTUC | 130 | 2009-2017 | 68 (IQR: 59.75-75) | 90/40 | 360.2 | 30 (3-103) | UV/ MV | CSS RFS PFS | 7 |
| Itami (2019) (9) | Japan | UTUC | 125 | 1995-2016 | 72 (38-90) | 96/29 | 340 | 51 (IQR:6-227) | UV/ MV | OS RFS | 7 |
| Xu (2020) (12) | China | UTUC | 703 | 2003-2016 | 67 (IQR: 59-74) | 399/304 | 402.5 | 42 (1-168) | UV/ MV | OS CSS RFS | 8 |
| Li (2019) (15) | China | BC | 206 | 2012-2015 | 62 (19-83) | 165/41 | 356 | 42 (5-72) | UV/ MV | RFS PFS | 7 |
| Yang (2020) (17) | China | BC | 145 | 2014-2019 | 65.92 ± 1016 | 125/20 | 314 | NA | UV/ MV | OS RFS | 7 |
| Gui (2021) (14) | China | BC | 136 | 2005-2016 | 59.5 ± 6.7 | 101/35 | 339 | NA | UV/ MV | OS | 7 |
| Song (2022) (16) | China | BC | 371 | 2013-2019 | 61.30 ± 12.82 | 291/80 | 370 | NA | UV/ MV | OS | 7 |

UTUC, upper tract urothelial carcinoma; BC, Bladder cancer; IQR, interquartile range; MV, multivariate; UV, univariate; OS, overall survival; CSS, cancer-specific survival; RFS, recurrence-free survival; PFS, progression free survival.

on country, high preoperative plasma fibrinogen was associated with poor OS in both Japan (fixed effect model, pooled HR: 1.78, 95% CI: 1.26-2.52, P=0.001), and China (fixed effect model, pooled HR: 2.24, 95% CI: 1.86-2.71, P<0.001) (Table 2). Additionally, statistically significant pooled HRs were also calculated in subgroup analysis when stratified by cutoff value (Table 2).

## 3.3 Recurrence-free survival

The pooled outcome suggested that high preoperative plasma fibrinogen was significantly associated with short RFS in UCs (fixed effect model, pooled HR: 1.90, 95% CI: 1.59-2.27; P<0.001) with no heterogeneity across studies ($I^2$ = 0.0%, P=0.640) (Figure 3A;

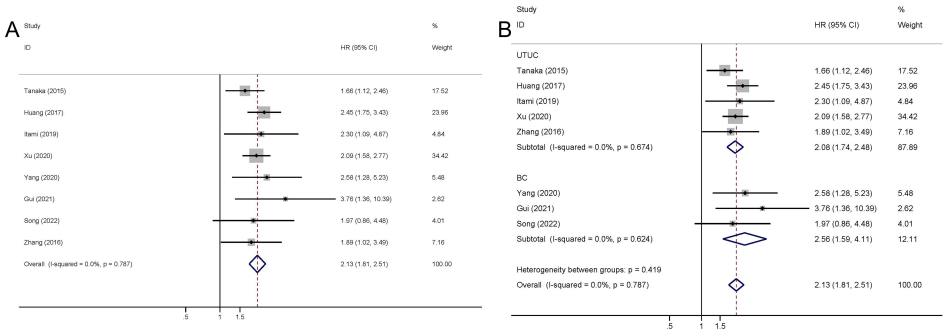



FIGURE 2
Meta-analysis of the association between the preoperative plasma fibrinogen and OS in urothelial cancers. Forest plot of studies evaluating pooled HR for OS in urothelial cancers **(A)** Forest plots of subgroup analyses by cancer type **(B)** for OS.

TABLE 2 HR values for OS according to subgroup analysis.

| Categories | Study (cases) | Model | HR (95% CI) | Z | P_value | Heterogeneity | |
|---|---|---|---|---|---|---|---|
| | | | | | | $I^2$ | $P_H$-value |
| Overall | 8 (2539) | Fixed | 2.13 (1.81-2.51) | 9.01 | <0.001* | 0.0% | 0.787 |
| Cancer type | | | | | | | |
| UTUC | 5 (1887) | Fixed | 2.08 (1.74-2.48) | 8.16 | <0.001* | 0.0% | 0.674 |
| BC | 3 (652) | Fixed | 2.56 (1.60-4.11) | 3.89 | <0.001* | 0.0% | 0.624 |
| Country | | | | | | | |
| Japan | 2 (519) | Fixed | 1.78 (1.26-2.52) | 3.25 | 0.001* | 0.0% | 0.450 |
| China | 6 (2020) | Fixed | 2.24 (1.86-2.71) | 8.48 | <0.001* | 0.0% | 0.842 |
| Cut-off value; mg/dl | | | | | | | |
| <365 | 4 (590) | Fixed | 2.36 (1.63-3.40) | 4.58 | <0.001* | 0.0% | 0.710 |
| ≥365 | 4 (1949) | Fixed | 2.08 (1.73-2.50) | 7.78 | <0.001* | 0.0% | 0.534 |

OS, overall survival; HR, hazard ratio; CI, confidence interval; $P_H$, P for heterogeneity; UTUC, upper tract urothelial carcinoma; BC, bladder cancer.
*P<0.05.

Table 3). Subgroup analysis based on cancer type revealed that high preoperative plasma fibrinogen was associated with poor RFS in both UTUC (fixed effect model, pooled HR: 1.94, 95% CI: 1.58-2.38, P<0.001), and BC (fixed effect model, pooled HR: 1.78, 95% CI: 1.23-2.57, P=0.002) (Figure 3B; Table 3). In subgroup analyses based on country, high preoperative plasma fibrinogen was associated with poor OS in both Japan (fixed effect model, pooled HR: 1.76, 95% CI: 1.28-2.49, P=0.001), and China (fixed effect model, pooled HR: 1.95, 95% CI: 1.59-2.41, P<0.001). Different cut-off value also showed prognostic value of preoperative plasma fibrinogen for RFS (Table 3).

## 3.4 Cancer-specific survival and Progression free survival

The pooled outcome suggested that the high preoperative plasma fibrinogen was significantly associated with short CSS among UC patients (fixed effect model, pooled HR: 2.22, 95% CI: 1.83-2.70; P<0.001) with no heterogeneity across studies ($I^2$ = 0.0%, P=0.814) (Figure 4; Table 4). Additionally, high preoperative plasma fibrinogen was associated with poor PFS in UCs (fixed effect model, pooled HR: 2.12, 95% CI: 1.36-3.29, P=0.001). And no heterogeneity across studies was found ($I^2$ = 0.0%, P=0.900) (Figure 4; Table 4).

## 3.5 Sensitivity analysis

The results of sensitivity analysis for OS, CSS, and RFS outcomes demonstrated that the conclusions for OS, CSS, and RFS remained stable because the pooled HRs were not significantly influenced by excluding any individual study (Supplementary Figure 1).

## 3.6 Publication bias

The presence of publication bias in the included investigations was assessed using Begg's test and Egger's linear regression test. In

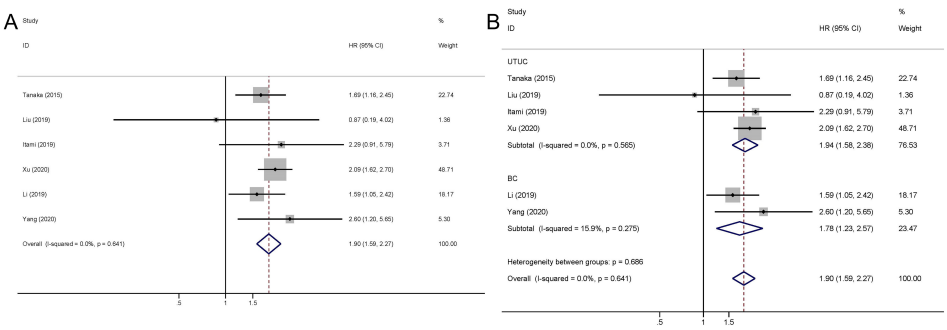



FIGURE 3
Meta-analysis of the association between the preoperative plasma fibrinogen and RFS in urothelial cancers. Forest plot of studies evaluating pooled HR for RFS in urothelial cancers **(A)** Forest plots of subgroup analyses by cancer type **(B)** for RFS.

TABLE 3 HR values for RFS according to subgroup analysis.

| Categories | Study (cases) | Model | HR (95% CI) | Z | P_value | Heterogeneity | |
|---|---|---|---|---|---|---|---|
| | | | | | | $I^2$ | $P_H$-value |
| Overall | 6 (1703) | Fixed | 1.90 (1.59-2.27) | 7.06 | <0.001* | 0.0% | 0.640 |
| Cancer type | | | | | | | |
| UTUC | 4 (1352) | Fixed | 1.94 (1.58-2.38) | 6.38 | <0.001* | 0.0% | 0.565 |
| BC | 2 (351) | Fixed | 1.78 (1.23-2.57) | 3.07 | 0.002* | 15.9% | 0.275 |
| Country | | | | | | | |
| Japan | 2 (519) | Fixed | 1.76 (1.28-2.49) | 3.21 | 0.001* | 0.0% | 0.551 |
| China | 4 (1184) | Fixed | 1.95 (1.59-2.41) | 6.31 | <0.001* | 0.0% | 0.426 |
| Cut-off value, mg/dl | | | | | | | |
| <365 | 4 (606) | Fixed | 1.78 (1.27-2.48) | 6.22 | <0.001* | 0.0% | 0.358 |
| ≥365 | 4 (1097) | Fixed | 1.95 (1.58-2.41) | 3.38 | 0.001* | 0.0% | 0.509 |

RFS, recurrence-free survival; HR, hazard ratio; CI, confidence interval; $P_H$, P for heterogeneity; UTUC, upper tract urothelial carcinoma; BC, bladder cancer.
*P<0.05.

Begg's test, we found that P-value of 0.536 for OS, 0.806 for CSS, and 0.260 for RFS. In Egger's test, the corresponding P-values were found to be 0.480 for OS (Figure 5A), 0.394 for CSS (Figure 5B), and 0.639 for RFS (Figure 5C). Thus, our meta-analysis did not reveal any significant publication bias.

# 4 Discussion

Our meta-analysis incorporated a total of 2875 cases from 10 eligible studies, which were deemed of high quality based on the NOS score system. The results of our study suggest that preoperative plasma fibrinogen levels can serve as a reliable predictor for oncologic outcomes in patients with localized UC. Elevated preoperative plasma fibrinogen levels are associated with unfavorable OS, CSS, RFS, and PFS in patients with UTUC or BC. In subgroup analyses, BC shown a better predictive value for OS, suggesting that preoperative plasma fibrinogen has the best predictive value for OS in BC. UTUC demonstrated a better predictive value for RFS, indicating that preoperative plasma fibrinogen has the best predictive value for RFS in UTUC. Additionally, we found that the preoperative plasma fibrinogen has a better predictive value for OS and RFS among the Chinese population compared to the Japanese population. Therefore, preoperative plasma fibrinogen could serve as a cost-effective and readily accessible prognostic biomarker for urothelial cancers in the Asian population, despite variations in effect sizes.

Although UTUC and BC share some common risk factors, however, they exhibit distinct biological, practical, and clinical characteristics (20), which may account for the difference in prognostic value of preoperative plasma fibrinogen between these two types of UC. The subgroup analysis based on country in our meta-analysis revealed that preoperative plasma fibrinogen exhibited a stronger predictive value within the Chinese population compared to the Japanese population, potentially attributed to limited study availability and inadequate sample sizes. Further studies should be conducted to validate these insignificant results. Sensitivity analyses confirmed the stableness of the pooled outcomes.

Negative associations between preoperative plasma fibrinogen and oncological prognosis have been reported in numerous cancers, not limited to urothelial cancers. These included renal cell carcinoma (21), prostate cancer (22), gastric cancer (23), laryngeal squamous cell carcinoma (24), lung cancer (25),

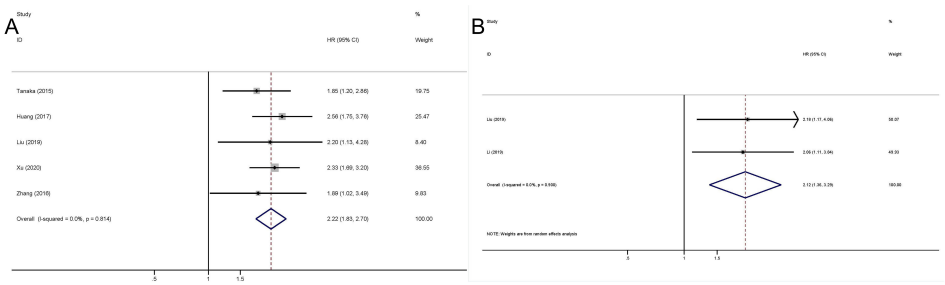



FIGURE 4
Meta-analysis of the association between the preoperative plasma fibrinogen and CSS/RFS in urothelial cancers. Forest plot of studies evaluating pooled HR for CSS **(A)** and for PFS **(B)**.

TABLE 4 HR values for CSS and PFS.

| Categories | Study (cases) | Model | HR (95% CI) | Z | P_value | Heterogeneity | |
|---|---|---|---|---|---|---|---|
| | | | | | | $I^2$ | $P_H$-value |
| CSS | 5 (1892) | Fixed | 2.22 (1.83-2.70) | 8.11 | <0.001* | 0.0% | 0.814 |
| PFS | 2 (336) | Fixed | 2.12 (1.36-3.29) | 3.34 | 0.001* | 0.0% | 0.900 |

CSS, cancer-specific survival; PFS, progression free survival; HR, hazard ratio; CI, confidence interval; $P_H$, P for heterogeneity.
*P<0.05.

hepatocellular carcinoma (26), and pancreatic cancer (27). However, the underlying mechanisms for the associations have not been clearly elucidated. Previous *in vitro* studies have verified that fibrinogen can promote cancer cell proliferation, invasion, epithelial-to-mesenchymal transition (EMT), angiogenesis, and hematogenous dissemination transition (28, 29). Thus, fibrinogen could play an important role in tumor progression. The previous studies have demonstrated the ability of fibrinogen to interact with secreted growth factors, such as transforming growth factor-β (TGF-β), fibroblast growth factor-2 (FGF-2), vascular growth factor (VEGF), and platelet-derived growth factor (PDGF) to stimulate tumor cell proliferation and angiogenesis (30–32). In esophageal squamous cell carcinoma, Zhang et al. (33) have demonstrated that fibrinogen can promote malignant biological tumor behavior involving EMT via the p-AKT/p-mTOR pathway. However, the exact biological mechanism for the relationship between elevated plasma fibrinogen and poor prognosis of UC remains unknow. Further investigations are needed to explore the underlying mechanism.

Despite advancements in the management of cancer, some patients still face a poor prognosis due to local tumor recurrence or distant metastasis. Therefore, novel biomarkers are necessary to predict the prognoses accurately and formulate follow-up strategies based on the stratification of risks for UC patients. Tumor-related immune responses in tumor micro-environment serve as immunological surveillance and contribute to antitumor immune responses, which are closely associated with patients' tumor outcomes (34). Therefore, certain immune-inflammatory indicators, such as C-reactive protein (CRP) (35), platelet-lymphocyte ratio (PLR) (36), neutrophil-lymphocyte ratio (NLR) (37), lymphocyte-monocyte ratio (LMR) (38), albumin (39), and plasma fibrinogen levels (40), have been reported as potential biomarkers for diagnosing and predicting the prognosis of tumor patients. The findings of this meta-analysis indicate that plasma fibrinogen serves as a valuable prognostic biomarker, enabling the identification of high-risk UC patients prior to treatment and subsequently enhancing their tumor outcomes. Esumi et al. (41) also reported that inhibiting coagulation events by using r-hirudin, a highly specific thrombin inhibitor, significantly inhibited lung metastasis in an animal model. Thus, the administration of anticoagulants may potentially mitigate hematogenous metastasis in UC patients exhibiting elevated levels of plasma fibrinogen.

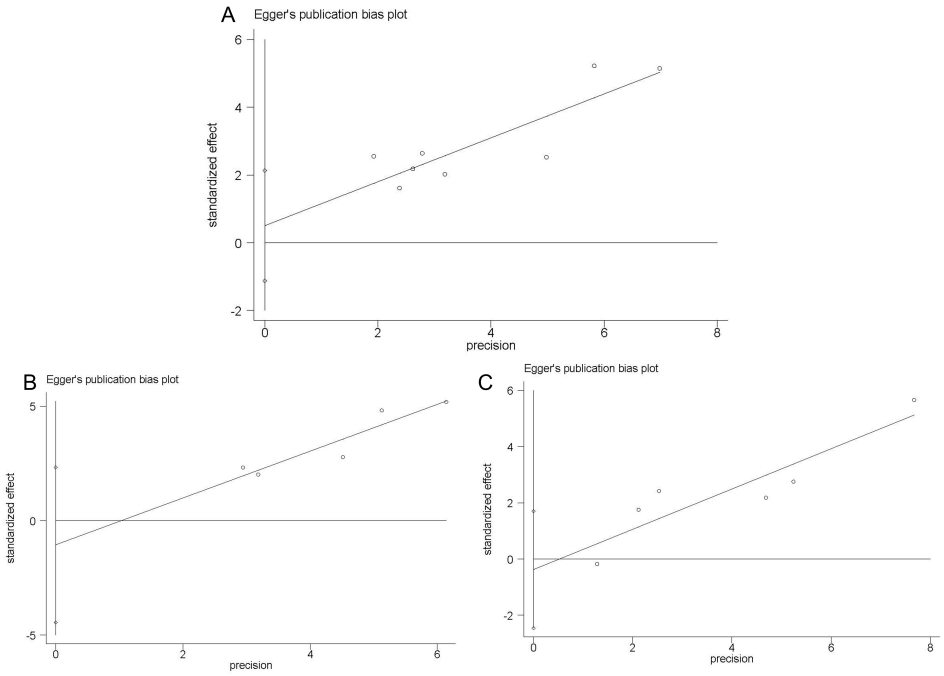



FIGURE 5
Egger's linear regression test. Egger's publication bias plots for OS **(A)** CSS **(B)** and RFS **(C)**.

Although our study comprehensively assessed the prognostic value of the preoperative plasma fibrinogen in UCs with no obvious heterogeneity and publication bias, it had certain limitations. Firstly, some of the included studies only enrolled a small number of patients, which might introduce confounder bias. However, excluding these studies did not significantly affect the overall estimation. Secondly, our focus was primarily on the post-surgical outcomes, thereby excluding consideration of other treatment modalities. Consequently, this led to a paucity of data within the studies included. Additionally, our meta-analysis included a limited number of studies. However, our meta-analysis exhibited no significant heterogeneity and publication bias. Besides, sensitivity analysis confirmed that our findings were stable and reliable. Finally, all the studies included in this meta-analysis were retrospective observational studies with inherent structural defects; therefore, we cannot draw definitive conclusions regarding how preoperative plasma fibrinogen influences oncologic outcomes.

In conclusion, the findings of our meta-analysis indicate a significant association between elevated preoperative plasma fibrinogen levels and unfavorable tumor outcomes in UCs. While further studies are needed, our findings suggested that elevated preoperative plasma fibrinogen could serve as a potential prognostic biomarker for UC patients and may influence clinical decision-making.

## 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/s.

## Author contributions

ZB: Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. GL: Writing – review & editing, Visualization, Validation, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. FH: Writing – review & editing, Visualization, Validation, Methodology, Investigation. XX: Writing – review & editing, Visualization, Validation, Methodology, Investigation. ZL: Writing – review & editing, Visualization, Validation, Methodology, Investigation. JW: Writing – review & editing, Visualization, Validation, Supervision, Project administration, Conceptualization.

## Funding

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

## Conflict of interest

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

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1360595/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Sensitivity analysis for OS **(A)** CSS **(B)** and RFS **(C)**.

## References

1. Rouprêt M, Seisen T, Birtle AJ, Capoun O, Compérat EM, Dominguez-Escrig JL, et al. European association of urology guidelines on upper urinary tract urothelial carcinoma: 2023 update. *Eur Urol.* (2023) 84:49–64. doi: 10.1016/j.eururo.2023.03.013

2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin.* (2023) 73:17–48. doi: 10.3322/caac.21763

3. He W, Chen C, Lin T, Xu Q, Ye C, Du J, et al. Epidemiology, treatments, and related biomarkers of locally advanced or metastatic urothelial carcinoma in Chinese population: A scoping review. *Cancer Med.* (2023) 12:15384–403. doi: 10.1002/cam4.6112

4. Neerman-Arbez M, Casini A. Fifty years of fibrinogen structure and function. *Semin Thromb Hemost.* (2024) 50:148–50. doi: 10.1055/s-0043-1775857

5. Mohammadinejad A, Aleyaghoob G, Nooranian S, Dima L, Moga MA, Badea M. Development of biosensors for detection of fibrinogen: a review. *Anal Bioanal Chem.* (2024) 416:21–36. doi: 10.1007/s00216-023-04976-1

6. Tinholt M, Sandset PM, Iversen N. Polymorphisms of the coagulation system and risk of cancer. *Thromb Res.* (2016) 140 Suppl 1:S49–54. doi: 10.1016/S0049-3848(16)30098-6

7. Sharma BK, Flick MJ, Palumbo JS. Cancer-associated thrombosis: A two-way street. *Semin Thromb Hemost.* (2019) 45:559–68. doi: 10.1055/s-0039-1693472

8. Huang J, Yuan Y, Wang Y, Zhang J, Kong W, Chen H, et al. Prognostic value of preoperative plasma fibrinogen level and platelet-to-lymphocyte ratio (F-PLR) in patients with localized upper tract urothelial carcinoma. *Oncotarget.* (2017) 8:36761–71. doi: 10.18632/oncotarget.13611

9. Itami Y, Miyake M, Tatsumi Y, Gotoh D, Hori S, Morizawa Y, et al. Preoperative predictive factors focused on inflammation-, nutrition-, and muscle-status in patients with upper urinary tract urothelial carcinoma undergoing nephroureterectomy. *Int J Clin Oncol.* (2019) 24:533–45. doi: 10.1007/s10147-018-01381-y

10. Liu R, Zhou X, Zou L, Chen Q, Hu Y, Hu J, et al. Clinicopathological and prognostic significance of preoperative plasma fibrinogen level in patients with upper

urinary tract urothelial carcinoma: A retrospective tumor marker prognostic study. *Int J Surg*. (2019) 65:88–93. doi: 10.1016/j.ijsu.2019.03.022

11. Tanaka N, Kikuchi E, Kanao K, Matsumoto K, Shirotake S, Miyazaki Y, et al. Impact of combined use of blood-based inflammatory markers on patients with upper tract urothelial carcinoma following radical nephroureterectomy: proposal of a cumulative marker score as a novel predictive tool for prognosis. *Eur Urol Focus*. (2015) 1:54–63. doi: 10.1016/j.euf.2015.02.001

12. Xu H, Ai JZ, Tan P, Lin TH, Jin X, Gong LN, et al. Pretreatment elevated fibrinogen level predicts worse oncologic outcomes in upper tract urothelial carcinoma. *Asian J Androl*. (2020) 22:177–83. doi: 10.4103/aja.aja_38_19

13. Zhang B, Song Y, Jin J, Zhou LQ, He ZS, Shen C, et al. Preoperative plasma fibrinogen level represents an independent prognostic factor in a chinese cohort of patients with upper tract urothelial carcinoma. *PloS One*. (2016) 11:e0150193. doi: 10.1371/journal.pone.0150193

14. Gui H, Song Y, Yin Y, Wang H, Rodriguez R, Wang Z. Prognostic value of preoperative inflammation-based predictors in patients with bladder carcinoma after radical cystectomy. *Open Med (Wars)*. (2021) 16:816–25. doi: 10.1515/med-2021-0277

15. Li X, Shu K, Zhou J, Yu Q, Cui S, Liu J, et al. Preoperative plasma fibrinogen and D-dimer as prognostic biomarkers for non-muscle-invasive bladder cancer. *Clin Genitourin Cancer*. (2020) 18:11–19.e11. doi: 10.1016/j.clgc.2019.10.025

16. Song Y, Tian J, Yang L, Zhang Y, Dong Z, Ding H, et al. Prognostic value of preoperative platelet-related parameters and plasma fibrinogen in patients with non-muscle invasive bladder cancer after transurethral resection of bladder tumor. *Future Oncol*. (2022) 18:2933–42. doi: 10.2217/fon-2022-0223

17. Yang S, Guan H, Wang S, Wu H, Sun W, Chen Z, et al. Plasma fibrinogen predicts the prognosis of bladder cancer patients after radical cystectomy. *Cancer manag Res*. (2020) 12:9303–14. doi: 10.2147/CMAR.S269244

18. Song H, Kuang G, Zhang Z, Ma B, Jin J, Zhang Q. The prognostic value of pretreatment plasma fibrinogen in urological cancers: A systematic review and meta-analysis. *J Cancer*. (2019) 10:479–87. doi: 10.7150/jca.26989

19. Jiang C, Perimbeti S, Deng L, Xing J, Chatta GS, Han X, et al. Medicaid expansion and racial disparity in timely multidisciplinary treatment in muscle invasive bladder cancer. *J Natl Cancer Inst*. (2023) 115:1188–93. doi: 10.1093/jnci/djad112

20. Soria F, Shariat SF, Lerner SP, Fritsche HM, Rink M, Kassouf W, et al. Epidemiology, diagnosis, preoperative evaluation and prognostic assessment of upper-tract urothelial carcinoma (UTUC). *World J Urol*. (2017) 35:379–87. doi: 10.1007/s00345-016-1928-x

21. Ni J, Wang Y, Zhang H, Wang K, Song W, Luo M, et al. Combination of preoperative plasma fibrinogen and neutrophil-to-lymphocyte ratio to predict the prognosis for patients undergoing laparoscopic nephrectomy for renal cell carcinoma. *Am J Cancer Res*. (2022) 12:3713–28.

22. Man YN, Chen YF. Systemic immune-inflammation index, serum albumin, and fibrinogen impact prognosis in castration-resistant prostate cancer patients treated with first-line docetaxel. *Int Urol Nephrol*. (2019) 51:2189–99. doi: 10.1007/s11255-019-02265-4

23. Zhang Y, Liu N, Liu C, Cao B, Zhou P, Yang B. High fibrinogen and platelets correlate with poor survival in gastric cancer patients. *Ann Clin Lab Sci*. (2020) 50:457–62.

24. Cai H, Zhang ZH, Zhou YJ, Liu J, Chen HQ, Lin RY. The prognostic value of preoperative plasma fibrinogen and neutrophil-to-lymphocyte ratio in patients with laryngeal squamous cell carcinoma. *Ear Nose Throat J*. (2021) 100:731–6. doi: 10.1177/0145561320920746

25. Zhang K, Xu Y, Tan S, Wang X, Du M, Liu L. The association between plasma fibrinogen levels and lung cancer: a meta-analysis. *J Thorac Dis*. (2019) 11:4492–500. doi: 10.21037/jtd.2019.11.13

26. Dai T, Peng L, Lin G, Li Y, Yao J, Deng Y, et al. Preoperative elevated plasma fibrinogen level predicts tumor recurrence and poor prognosis in patients with hepatocellular carcinoma. *J Gastrointest Oncol*. (2019) 10:1049–63. doi: 10.21037/jgo.2019.09.11

27. Chung KH, Lee JC, Lee J, Cho IK, Kim J, Jang W, et al. Serum fibrinogen as a diagnostic and prognostic biomarker for pancreatic ductal adenocarcinoma. *Pancreatology*. (2020) 20:1465–71. doi: 10.1016/j.pan.2020.06.010

28. Staton CA, Brown N J, Lewis CE. The role of fibrinogen and related fragments in tumour angiogenesis and metastasis. *Expert Opin Biol Ther*. (2003) 3:1105–20. doi: 10.1517/14712598.3.7.1105

29. Shu YJ, Weng H, Bao RF, Wu XS, Ding Q, Cao Y, et al. Clinical and prognostic significance of preoperative plasma hyperfibrinogenemia in gallbladder cancer patients following surgical resection: a retrospective and *in vitro* study. *BMC Cancer*. (2014) 14:566. doi: 10.1186/1471-2407-14-566

30. Sahni A, Simpson-Haidaris PJ, Sahni SK, Vaday GG, Francis CW. Fibrinogen synthesized by cancer cells augments the proliferative effect of fibroblast growth factor-2 (FGF-2). *J Thromb Haemost*. (2008) 6:176–83. doi: 10.1111/j.1538-7836.2007.02808.x

31. Sahni A, Odrljin T, Francis CW. Binding of basic fibroblast growth factor to fibrinogen and fibrin. *J Biol Chem*. (1998) 273:7554–9. doi: 10.1074/jbc.273.13.7554

32. Sahni A, Francis CW. Vascular endothelial growth factor binds to fibrinogen and fibrin and stimulates endothelial cell proliferation. *Blood*. (2000) 96:3772–8. doi: 10.1182/blood.V96.12.3772

33. Zhang F, Wang Y, Sun P, Wang ZQ, Wang DS, Zhang DS, et al. Fibrinogen promotes Malignant biological tumor behavior involving epithelial-mesenchymal transition via the p-AKT/p-mTOR pathway in esophageal squamous cell carcinoma. *J Cancer Res Clin Oncol*. (2017) 143:2413–24. doi: 10.1007/s00432-017-2493-4

34. Sfanos KS, Yegnasubramanian S, Nelson WG, De Marzo AM. The inflammatory microenvironment and microbiome in prostate cancer development. *Nat Rev Urol*. (2018) 15:11–24. doi: 10.1038/nrurol.2017.167

35. Sambataro D, Politi MR, Messina A, Scarpello L, Messina S, Guggino R, et al. Relationship of inflammatory parameters and nutritional status in cancer patients. *Anticancer Res*. (2023) 43:2821–9. doi: 10.21873/anticanres.16451

36. Zhou H, Li J, Zhang Y, Chen Z, Chen Y, Ye S. Platelet-lymphocyte ratio is a prognostic marker in small cell lung cancer-A systemic review and meta-analysis. *Front Oncol*. (2022) 12:1086742. doi: 10.3389/fonc.2022.1086742

37. Wang H, Gong H, Tang A, Cui Y. Neutrophil/lymphocyte ratio predicts lymph node metastasis in patients with gastric cancer. *Am J Transl Res*. (2023) 15:1412–20.

38. Mei P, Feng W, Zhan Y, Guo X. Prognostic value of lymphocyte-to-monocyte ratio in gastric cancer patients treated with immune checkpoint inhibitors: a systematic review and meta-analysis. *Front Immunol*. (2023) 14:1321584. doi: 10.3389/fimmu.2023.1321584

39. Xu H, Zheng X, Ai J, Yang L. Hemoglobin, albumin, lymphocyte, and platelet (HALP) score and cancer prognosis: A systematic review and meta-analysis of 13,110 patients. *Int Immunopharmacol*. (2023) 114:109496. doi: 10.1016/j.intimp.2022.109496

40. Bu F, Cao S, Deng X, Zhang Z, Feng X. Evaluation of C-reactive protein and fibrinogen in comparison to CEA and CA72-4 as diagnostic biomarkers for colorectal cancer. *Heliyon*. (2023) 9:e16092. doi: 10.1016/j.heliyon.2023.e16092

41. Esumi N, Fan D, Fidler IJ. Inhibition of murine melanoma experimental metastasis by recombinant desulfatohirudin, a highly specific thrombin inhibitor. *Cancer Res*. (1991) 51:4549–56.

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 16 September 2024
DOI 10.3389/fendo.2024.1449668



OPEN ACCESS

EDITED BY
Zili Zhang,
Nanjing University of Chinese Medicine, China

REVIEWED BY
Zhuang Yanshuang,
Taizhou Traditional Chinese Medicine Hospital, China
Guochong Jia,
Vanderbilt University Medical Center, United States

*CORRESPONDENCE
Huawei Yang
bcyhw163@163.com

RECEIVED 15 June 2024
ACCEPTED 28 August 2024
PUBLISHED 16 September 2024

CITATION
Song J and Yang H (2024) Identifying new biomarkers and potential therapeutic targets for breast cancer through the integration of human plasma proteomics: a Mendelian randomization study and colocalization analysis.
*Front. Endocrinol.* 15:1449668.
doi: 10.3389/fendo.2024.1449668



# Identifying new biomarkers and potential therapeutic targets for breast cancer through the integration of human plasma proteomics: a Mendelian randomization study and colocalization analysis

Jingshuang Song[1,2] and Huawei Yang[1,3]*

[1]Department of Breast Surgery, Guangxi Medical University Cancer Hospital, Nanning, China, [2]Department of Breast and Thyroid Surgery, Affiliated Hospital of Guilin Medical University, Guilin, China, [3]Laboratory of Breast Cancer Diagnosis and Treatment Research of Guangxi Department of Education, Guangxi Medical University, Nanning, China

**Background:** The proteome is a crucial reservoir of targets for cancer treatment. While some targeted therapies have been developed, there are still significant challenges in early diagnosis and treatment, highlighting the need to identify new biomarkers and therapeutic targets for breast cancer. Therefore, we conducted a comprehensive proteome-wide Mendelian randomization (MR) study to identify novel biomarkers and potential therapeutic targets for breast cancer.

**Methods:** Protein quantitative trait locus (pQTL) data were extracted from two published plasma proteome-wide association studies. Genetic variants associated with breast cancer were obtained from the Breast Cancer Association Consortium, which included 133,384 cases and 113,789 controls, and the Finnish cohort study, comprising 18,786 cases and 182,927 controls. We employed summary-based MR and colocalization methods to identify potential drug targets for breast cancer, which were subsequently validated using a two-sample MR approach. Finally, a protein-protein interaction (PPI) network was constructed to detect interactions between the identified proteins and existing cancer drug targets.

**Results:** Gene-predicted levels of ten proteins were associated with breast cancer risk. Decreased levels of CASP8, DDX58, CPNE1, ULK3, PARK7, and BTN2A1, as well as increased levels of TNFRSF9, TNXB, DNPH1, and TLR1, were linked to an elevated risk of breast cancer. Among these, CASP8 and DDX58 were supported by tier-one evidence, while CPNE1, ULK3, PARK7, and TNFRSF9 received tier-two evidence support. The remaining proteins, TNXB, BTN2A1, DNPH1, and TLR1, were supported by tier-three evidence. CASP8, DDX58, CPNE1, ULK3, PARK7, and TNFRSF9 have already been identified as targets in drug development and potential therapeutic targets for breast cancer treatment. Additionally, ULK3 showed promise as a prognostic biomarker for breast cancer.

**Conclusions:** The present study identified several novel potential drug targets and biomarkers for breast cancer, providing new insights into its diagnosis and treatment. The integration of PPI and druggability evaluations enhances the prioritization of these therapeutic targets, paving the way for future drug development efforts.

KEYWORDS

breast cancer, proteomics, biomarkers, drug targets, Mendelian randomization, colocalization analysis

# 1 Introduction

Breast cancer is one of the most prevalent malignancies among women worldwide, with an incidence that continues to rise. In 2023, the United States alone had some 300,000 new cases, accounting for approximately 15.32% of all newly diagnosed cancers. Simultaneously, around 43,000 deaths due to breast cancer were recorded, constituting 7.2% of all cancer-related mortalities. Breast cancer profoundly affects patients' quality of life and overall health (1, 2), and despite the advancements in treatment modalities, significant challenges, including the inadequacy of early diagnosis, the unpredictability of treatment outcomes, and the development of drug resistance, remain (3). As a result, identifying novel biomarkers and therapeutic targets has become a critical focus of contemporary breast cancer research.

Proteomics is a high-throughput technology that can reflect normal physiological processes and cancer pathobiology. Researchers can discover novel cancer-associated biomarkers by analyzing protein expression profiles in tumor tissues or body fluids, offering theoretical support for personalized patient treatment (4). Previous observational studies have identified specific circulating proteins associated with breast cancer risk (5–8); however, reverse causality or confounding factors may obscure the conclusions drawn from traditional observational research.

Mendelian randomization (MR) is a method employed to estimate causal effects within specific hypothetical contexts based on the principle that genes are randomly assigned from parents to offspring during gametogenesis and conception. Unlike traditional observational studies, MR is not susceptible to the biases of reverse causality or confounding (9). Consequently, several studies using the MR approach have uncovered various circulating proteins linked to breast cancer risk. For example, Jia and colleagues employed a two-sample MR approach to assess the association between 1,142 proteins and breast cancer risk, identifying 22 proteins linked to this risk (10). Similarly, Shu et al. utilized the same method to examine 2,994 proteins, uncovering 56 associated with breast cancer risk (11). They further explored the relationship between 1,890 circulating proteins and various breast cancer subtypes, identifying 98 proteins significantly associated with one or more subtypes (12). Additionally, Mälarstig and colleagues used a two-sample MR approach to identify five proteins potentially causally linked to breast cancer (13). However, these studies often relied on a single analytical method or faced limitations in protein coverage and sample size, generating inconsistent findings that have hindered a comprehensive understanding of the relationship between protein expression and breast cancer risk. Two recent studies have further advanced this field by employing bidirectional MR and colocalization analyses to systematically explore potential drug targets between plasma proteins and breast cancer. Colocalization analysis effectively distinguishes causal relationships from linkage disequilibrium (LD) within the genome, thereby enhancing the reliability of the results. This multifaceted approach offers crucial insights into identifying potential drug targets for breast cancer and lays a solid foundation for future drug development (14, 15).

The present study utilized Summary-based Mendelian Randomization (SMR), an advanced extension of traditional MR methods. The SMR approach integrates independent genome-wide association study (GWAS) summary data with quantitative trait locus (QTL) data, thereby prioritizing potential causal genes identified in GWAS. Unlike conventional MR methods, SMR can more accurately distinguish potential causal associations from LD within the genome, yielding more reliable causal inferences (16). By combining SMR with colocalization analysis, we systematically investigated the relationship between the human plasma proteome and breast cancer risk. This innovative approach enabled us to address some of the limitations of previous studies, providing more robust supporting evidence (17). Given the limitations of evidence from a single methodological approach, we further employed a two-sample MR approach for validation,

**Abbreviations:** MR, Mendelian randomization; SMR, Summarized data-based Mendelian Randomization; GWAS, genome-wide association studies; QTL, Quantitative Trait Locus; pQTL, protein quantitative trait locus; LD, linkage disequilibrium; IVW, inverse variance weighting; BCAC, Breast Cancer Association Consortium; SNPs, single nucleotide polymorphisms; FDR, false discovery rate; OR, odds ratio; CI, confidence interval; PPI, protein-protein interaction.

systematically assessing the potential of proteins as novel biomarkers and therapeutic targets for breast cancer. Future research should integrate multi-omics data, including expression quantitative trait locus (eQTL) and methylation quantitative trait locus (mQTL), by combining SMR and two-sample MR methods, as such an approach could offer new perspectives on the molecular mechanisms of breast cancer and provide critical insights for identifying targets in personalized therapy.

# 2 Materials and methods

The present research adhered to the guidelines outlined in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) (Supplementary STROBE-MR checklist table) (18).

## 2.1 Study design

Both preliminary and validation analyses were conducted in the present study. Protein quantitative trait locus (pQTL) data from two large-scale proteomic studies were used, and the SMR method was employed to evaluate the association between proteome and breast cancer. Positive findings from this initial assessment were subjected to Bayesian colocalization analysis. For proteins that met the criteria after colocalization analysis, the causal relationship with breast cancer was further validated using a two-stage MR framework, which included discovery and replication phases and was supplemented by sensitivity analyses. The MR analysis adhered rigorously to the following fundamental assumptions: (i) relevance assumption: single nucleotide polymorphisms (SNPs) are significantly associated with the exposure (protein expression levels); (ii) independence assumption: SNPs are independent of confounding factors, meaning they are not associated with variables that influence both the exposure and the disease outcome; (iii) exclusion restriction assumption: SNPs affect breast cancer risk exclusively through protein expression levels and not through other pathways (9) (Figure 1).

## 2.2 Proteomic data source

The pQTL data for the proteomic studies were obtained from research conducted by Ferkingstad et al. and Sun et al. The former study assessed the plasma protein levels in 35,559 Icelandic individuals using the SomaScan multiplex aptamer assay. They profiled 4,719 proteins and identified pQTL data for 18,084 protein quantitative trait loci (19). Sun et al. performed proteomic profiling of plasma samples from 54,219 participants in the UK Biobank using the antibody-based Olink Explore 3072 PEA technology. They profiled 2,923 distinct proteins and yielded pQTL data for 14,287 protein quantitative trait loci (20) (Supplementary Table S1).

## 2.3 Study population

The genetic data relevant to breast cancer were obtained from the Breast Cancer Association Consortium (BCAC) and the FinnGen Biobank. The BCAC consortium combined three datasets: iCOGS (38,349 cases and 37,818 controls), OncoArray (80,125 cases and 58,383 controls), and additional GWAS studies (14,910 cases and 17,588 controls), resulting in a total of 133,384 breast cancer cases and 113,789 controls (21). The FinnGen Biobank dataset includes 18,786 cases and 182,927 controls (22). In order to ensure the robustness of the study and minimize bias, the datasets used were exclusively derived from populations of European ancestry.

This study utilized publicly available databases, with all participant involvement ethically approved by their respective review boards and informed consent obtained from all subjects in the original studies (Supplementary Table S2).

## 2.4 Instrumental variable selection

The selection of genetic instruments for pQTL analysis adhered to the following criteria: first, genome-wide significant associations with a p-value of $<5\times10^{-8}$ were established, and SNPs significantly associated with any protein were extracted; an $r^2$ value of 0.001 and a distance of 10,000 kb were used to exclude SNPs in linkage disequilibrium (LD) (23, 24). Second, the F statistic ( $F = (\frac{R^2}{1-R^2})(\frac{n-k-1}{k}), R^2 = 2 \times (1 - MAF) \times MAF \times (\frac{\beta}{sd})^2$) was calculated to evaluate the strength of the association between SNPs and IVs, with an $F > 10$ indicating a sufficiently robust association to effectively mitigate bias from weak IVs (25). Third, SNPs within 1 Mb of the transcription start site of genes encoding proteins were classified as cis pQTL, whereas those outside this region were categorized as trans pQTL. Due to the considerable pleiotropy associated with trans pQTL, only cis pQTL were selected as IVs for this study (26).

## 2.5 Statistical analysis

The preliminary analysis utilized SMR, an extension of the MR concept, to investigate whether the effect sizes of SNPs on phenotypes are mediated by gene expression. This approach prioritizes GWAS hits for genes, facilitating subsequent functional investigations. These methodologies are applicable to various molecular quantitative trait loci data, including DNA methylation quantitative trait loci and pQTL. The present study employed SMR software with default settings via the command line for the analysis. The effect size (β) of variants indicated the direction of protein expression changes (16). The p-values from the results were adjusted using false discovery rate (FDR) correction, with associations possessing a PFDR < 0.05 considered statistically significant (27).

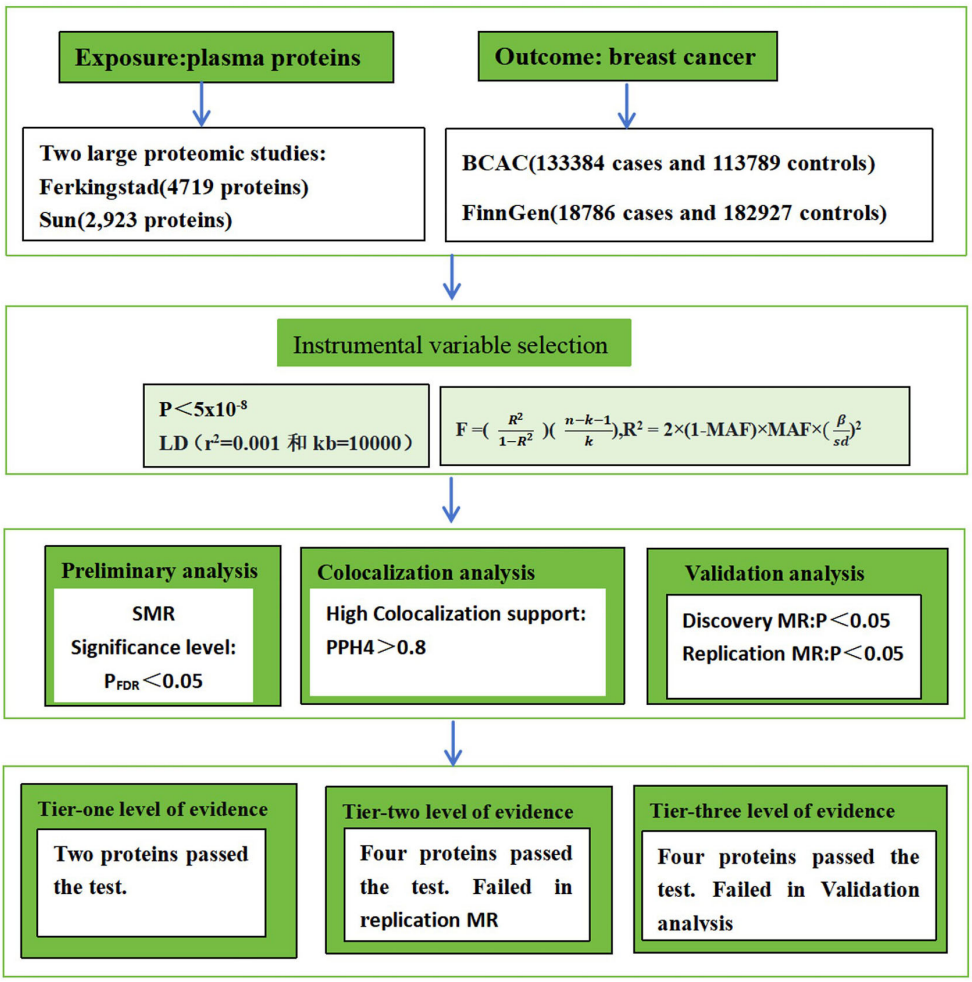



FIGURE 1
The workflow of study design. BCAC stands for Breast Cancer Association Consortium. LD stands for Linkage Disequilibrium. MR stands for Mendelian randomization. SMR stands for Summary-data-based MR.

## 2.6 Colocalization analysis

In order to optimize outcomes, Bayesian colocalization analysis was used to evaluate whether protein expression and breast cancer are influenced by the same causal variant, thereby discerning confounding effects due to linkage LD. Bayesian colocalization analysis involved five hypotheses: H0, no significant association exists between protein expression and breast cancer with any SNP locus within a genomic region; H1, protein expression is significantly associated with SNP loci within a genomic region; H2, breast cancer is significantly associated with SNP loci within a genomic region; H3, protein expression, and breast cancer are significantly associated with SNP loci within a genomic region, but driven by different causal variants; and H4, protein expression and breast cancer are significantly associated with SNP loci within a genomic region, driven by the same causal variant (28). Colocalization analysis was conducted on all SNPs within ±500 kb of gene start sites, utilizing default parameters: P1 = $1\times10^{-4}$ (prior probability of SNP association with protein), P2 = $1\times10^{-4}$ (prior probability of SNP association with breast cancer), and P12 = $1\times10^{-5}$ (prior probability of SNP association with both protein expression and breast cancer). Posterior probabilities were used to assess the support for each hypothesis, with a posterior probability of PP.H4 > 80% considered compelling evidence of colocalization (17).

## 2.7 Validation analysis

Validation analysis was conducted using a two-stage (discovery and replication) MR approach. In the Two-Sample MR analysis, the inverse variance weighted (IVW) method (29), the weighted median method (30), and the MR-Egger method (31) were employed as the primary analytical techniques. The IVW method, which has the highest statistical power, assumes the absence of an intercept term and that all genetic variants are valid IVs. On the other hand, the MR-Egger method accounts for the presence of an intercept term, though its testing efficiency may be less precise compared to the IVW method. As a complement to MR-Egger, the weighted median method allows for including some invalid

variants, provided that at least half of them are valid IVs. In both the discovery and replication stages of MR, individual protein-level data (https://www.decode.com) were acquired, and separate two-sample MR analyses for breast cancer were conducted. A P value < 0.05 indicated a statistically significant association.

## 2.8 Sensitivity analysis

Cochran's Q test was employed to evaluate heterogeneity among genetic variants. If the P-value of Cochran's Q test was < 0.05, a random effects model was used for MR analysis; otherwise, a fixed effects model was applied (32). Additionally, the MR-Egger and MR-PRESSO methods were used to detect the presence of horizontal pleiotropy. By identifying and correcting for pleiotropy, MR-PRESSO can reduce bias caused by pleiotropy and provide more reliable causal effect estimates. Moreover, MR-PRESSO offers correction methods and evaluates the robustness of causal estimates through sensitivity analysis (33). We also used forest plots to assess the causal effect of each SNP and compared these with the causal estimates from the IVW and MR-Egger methods. A leave-one-out analysis was conducted by removing each SNP individually to evaluate whether a single variant drives the association between the exposure and outcome variables (34).

Ultimately, the selected proteins that met the criteria were categorized into three tiers based on the strength of evidence. Tier one included proteins meeting all standards in SMR, discovery MR, replication MR, and colocalization. Tier two included proteins meeting standards in SMR, discovery MR, and colocalization. Tier three included proteins meeting standards in SMR and colocalization.

The SMR tool version 0.1.3 (https://yanglab.westlake.edu.cn/software/SMR/#Overview) was utilized. All MR analyses were performed in R software version 4.3.1, using the Two Sample MR (version 0.5.9), MR-PRESSO (version 1.0), dplyr (version 1.1.3), circlize (version 0.4.15), stringr (version 1.5.0), ComplexHeatmap (version 2.15.4), and coloc (version 5.2.3) packages.

# 3 Results

## 3.1 Preliminary SMR and colocalization results

Following thorough IV processing, 7,981 cis pQTLs were utilized for SMR analysis, detecting significant associations with breast cancer susceptibility across 64 proteins (PFDR < 0.05) (Supplementary Table S3).

Next, colocalization analysis was conducted separately for these proteins in relation to breast cancer. The results indicated that 10 proteins, CPNE1, TNXB, ULK3, CASP8, BTN2A1, PARK7, DNPH1, DDX58, TNFRSF9, and TLR1, had PP.H4 results of > 80% (Table 1; Figure 2).

## 3.2 Validation analysis

In the discovery phase of MR, CPNE1 exhibited an inverse association with breast cancer risk (IVW: odds ratio (OR) = 0.94, 95% confidence interval (CI): 0.90-0.98, P = 0.005); ULK3 demonstrated a negative correlation with breast cancer risk (IVW: OR = 0.78, 95% CI: 0.65-0.93, P = 0.005); CASP8 revealed a negative correlation with breast cancer risk (IVW: OR = 0.83, 95% CI: 0.77-0.91, P = 2.74E-05); PARK7 manifested a negative correlation with breast cancer risk (IVW: OR = 0.84, 95% CI: 0.74-0.95, P = 0.005); DDX58 indicated an inverse association with breast cancer risk (IVW: OR = 0.84, 95% CI: 0.72-0.98, P = 0.023); and TNFRSF9 exhibited a positive association with breast cancer risk (IVW: OR = 1.18, 95% CI: 1.07-1.30, P =7.10E-04). However, TNXB, BTN2A1,

TABLE 1 The summary of SMR results for the ten proteins that meet colocalization criteria with breast cancer.

| Protein | Protein full name | SMR | | | Colocalization |
|---|---|---|---|---|---|
| | | Beta | $P_{FDR}$ | OR(95%CI) | PP.H4 |
| CASP8 | caspase 8 | -0.15 | 8.34E-05 | 0.86(0.81-0.91) | 0.97 |
| DDX58 | DExD/H-box helicase 58 (also known as RIG-I, Retinoic acid-Inducible Gene I) | -0.12 | 5.06E-04 | 0.89(0.85-0.93) | 0.99 |
| CPNE1 | Copine 1 | -0.04 | 5.34E-03 | 0.96(0.94-0.98) | 0.90 |
| ULK3 | unc-51 like kinase 3 | -0.37 | 0.01 | 0.69(0.59-0.81) | 0.97 |
| PARK7 | Parkinsonism associated deglycase | -0.19 | 5.06E-04 | 0.83(0.77-0.90) | 0.93 |
| TNFRSF9 | TNF receptor superfamily member 9 | 0.19 | 0.00 | 1.21(1.11-1.32) | 0.82 |
| TNXB | tenascin XB | 0.04 | 0.04 | 1.04(1.02-1.07) | 0.81 |
| BTN2A1 | butyrophilin subfamily 2 member A1 | -0.14 | 3.06E-05 | 0.87(0.83-0.92) | 0.97 |
| DNPH1 | 2'-deoxynucleoside 5'-phosphate N-hydrolase 1 | 0.09 | 0.00 | 1.10(1.05-1.15) | 0.94 |
| TLR1 | toll like receptor 1 | 0.10 | 2.56E-09 | 1.10(1.07-1.13) | 0.85 |

SMR, Summary-data-based Mendelian randomization, PP.H4 values were all higher than 0.80 under priors (p12 = 1e−5) and windows (± 500kb).

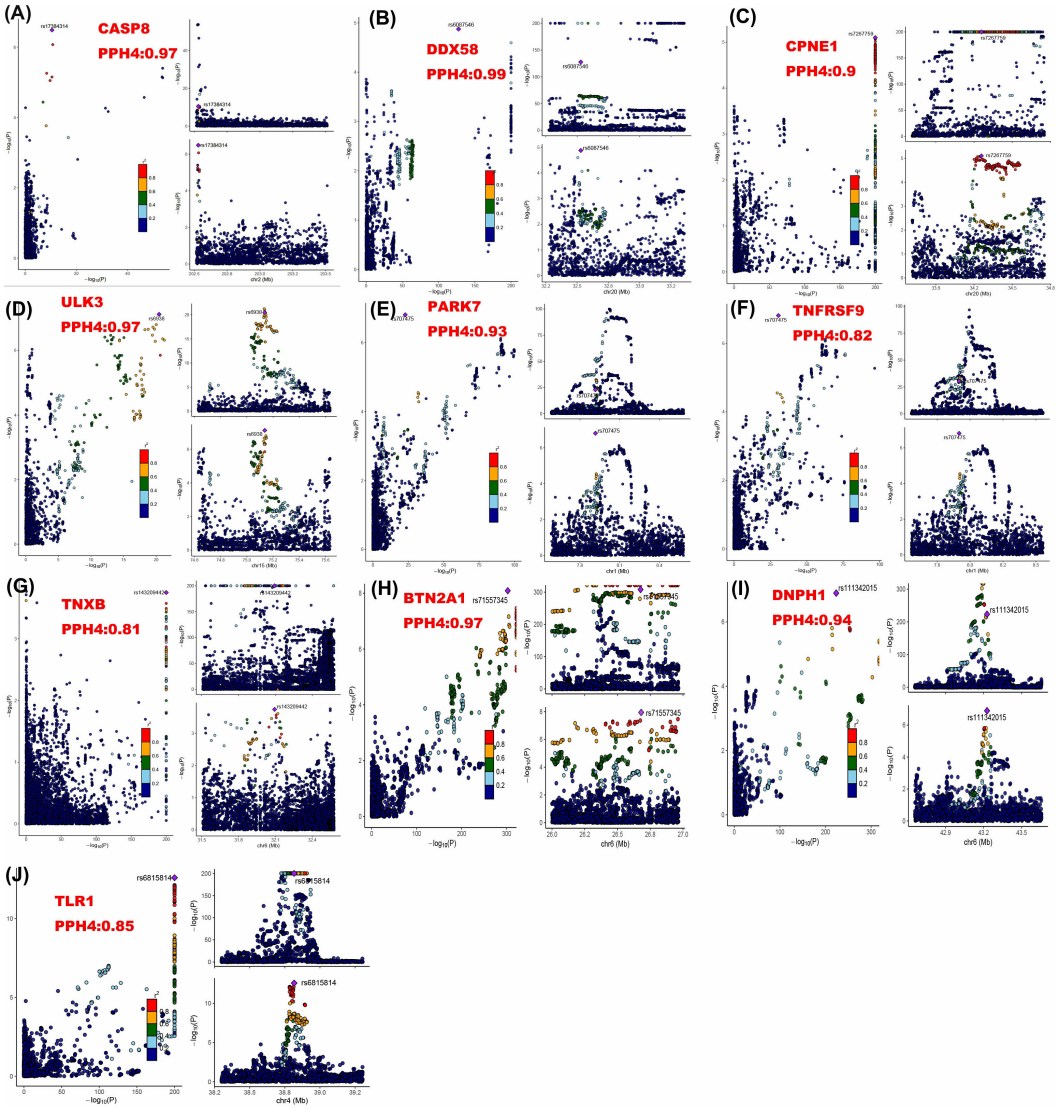



FIGURE 2
Regional association plots for colocalization analysis of ten proteins with breast cancer risk. The lead SNP is shown as a purple diamond. SNPs within ±500 kb of the protein quantitative trait locus were included; p12 = 1e-5, prior probability a SNP is associated with both protein and breast cancer.

DNPH1, and TLR1 had no significant correlation with breast cancer ($P > 0.05$ for all) (Figure 3; Supplementary Table S4).

In the replication stage of MR, CASP8 continued to show a negative correlation with breast cancer risk (IVW: OR = 0.75, 95% CI: 0.62-0.89, P = 0.001), and DDX58 maintained an inverse association with breast cancer risk (IVW: OR = 0.80, 95% CI: 0.67-0.94, P = 0.007). However, CPNE1, ULK3, PARK7, and TNFRSF9 did not replicate ($P > 0.05$ for all) (Figure 3; Supplementary Table S4).

## 3.3 Sensitivity analysis

Both MR-Egger regression and IVW methods detected heterogeneity in CASP8, DNPH1, and ULK3, prompting the use of a random effects model for MR analysis. Meanwhile, MR-Egger and MR-PRESSO methods detected no horizontal pleiotropy (Figure 3). To ensure the stability of the study results, the symmetrical distribution of SNPs in the funnel plot was confirmed. A leave-one-out analysis was performed to assess the influence of individual SNPs on the results, revealing no significant impact from any single SNP. Additionally, to gain a more comprehensive understanding of the data, scatter plots were generated to illustrate the causal relationships between proteins and breast cancer (Supplementary Figure S1-S6).

## 3.4 PPI and drug evaluation

The STRING database was used to construct a protein-protein interaction (PPI) network to elucidate the connections among TLR1, CASP8, and DDX58 proteins, as well as the interactions

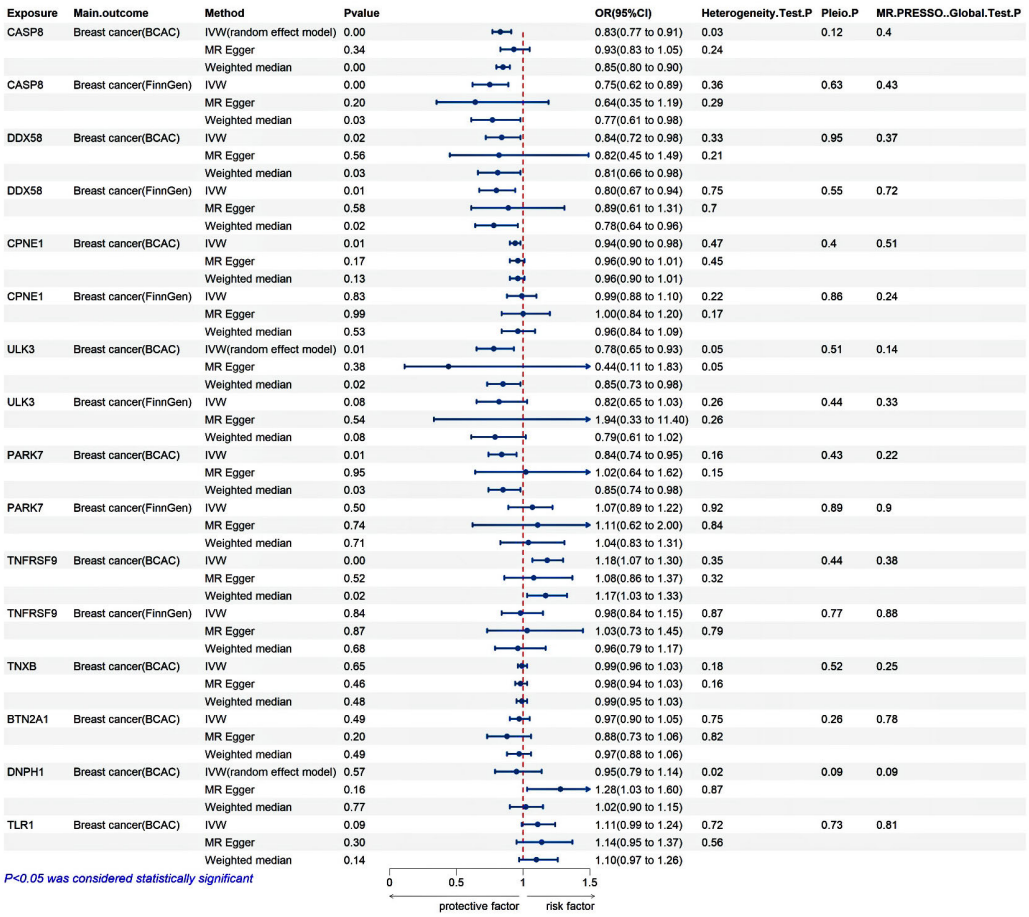



FIGURE 3
Validation analysis. The discovery MR is derived from the BCAC database, while the replication MR is derived from the FinnGen database.

involving CASP8 and PARK7 (Figure 4). Drug evaluation, conducted through platforms such as DGIdb4.0 (35) and DrugBank5.0 (36), identified CASP8, DDX58, CPNE1, ULK3, PARK7, and TNFRSF9 as notable targets for pharmacological exploration. Currently, therapeutic agents targeting CASP8 include bardoxolone, under investigation for lymphoma and solid tumor management; bryostatin 1, explored for HIV infection and Alzheimer's disease intervention; AN-9, scrutinized for liver cancer, lung cancer, melanoma, and leukemia; trichostatin A; oleandrin, assessed for lung cancer therapy and chemotherapy-induced adverse effects. Pharmaceutical candidates targeting DDX58 include INARIGIVIR SOPROXIL, which is used as an immunomodulator and antiviral agent. Theophylline is a medication tailored for CPNE1 and used to mitigate symptoms associated with reversible airflow obstruction in conditions such as asthma, chronic obstructive pulmonary disease, and other pulmonary ailments. Fostamatinib, a spleen tyrosine kinase inhibitor, targets ULK3, providing therapeutic relief for chronic immune thrombocytopenia following alternative interventions. Therapeutic strategies aimed at PARK7 include copper, a transition metal present in various supplements and vitamins, and intravenous infusion solutions used in total parenteral nutrition. TNFRSF9 is targeted by Urelumab, currently under investigation for its efficacy against leukemia, multiple myeloma, malignant tumors, solid tumors, and B-cell non-Hodgkin's lymphoma (Supplementary Table S5).

## 4 Discussion

The present study used publicly available large-sample pQTL and GWAS databases to analyze the causal relationship between 2,385 proteins and breast cancer, identifying 10 proteins associated with breast cancer risk. Among these, decreased levels of CASP8, DDX58, CPNE1, ULK3, PARK7, and BTN2A1 were found, alongside increased levels of TNFRSF9, TNXB, DNPH1, and TLR1. CASP8 and DDX58 exhibit the highest evidence strength, while CPNE1, ULK3, PARK7, and TNFRSF9 show secondary evidence strength. TNXB, BTN2A1, DNPH1, and TLR1 demonstrated the strength of the tertiary evidence. Druggability assessments prioritized six protein biomarkers already developed as drug targets for various chronic diseases or cancers, suggesting their potential repurposing as therapeutic targets for breast cancer.

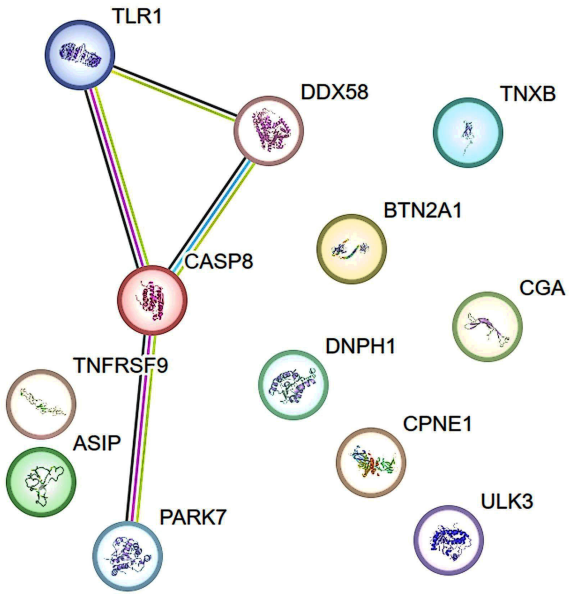



FIGURE 4
The Protein-protein interaction (PPI) network of proteins identified by proteome-wide Mendelian randomization. Lines represent interactions between proteins. Green line indicates gene neighborhood and predicted interaction; Blue line indicates known interaction from curated databases; Fuchsia line indicates known interaction that is experimentally determined. Black line indicates co-expression. Data information was from STRING database.

Our study identified several candidate proteins previously linked to breast cancer, including CASP8, DDX58, CPNE1, PARK7, BTN2A1, TNFRSF9, TNXB, DNPH1, and TLR1, with CASP8 and DDX58 supported by the most robust evidence. CASP8, also known as caspase 8, is a critical initiator enzyme in the apoptosis pathway, which is vital in regulating programmed cell death (37, 38). Beyond its apoptotic function, CASP8 influences various cellular signaling pathways involved in inflammatory and immune responses (39). In oncology, CASP8 is recognized as a significant tumor suppressor gene, with aberrant expression or dysfunction linked to the onset, invasion, and metastasis. Experimental evidence indicates that CASP8 induces PD-L1 degradation by upregulating TNFAIP3 (A20) expression, and reduced CASP8 expression may predict sensitivity to anti-PD-L1/PD-1 immunotherapy (40). Previous studies have also suggested that the CASP8 D302H polymorphism decreases breast cancer risk associated with *BRCA1* and *BRCA2* mutations, delaying cancer onset (41). A preliminary study on Iranian breast cancer patients further reported significantly decreased CASP8 expression (42), which is consistent with our findings. Our preliminary SMR analysis and validation MR analysis both supported the protective role of elevated CASP8 levels against breast cancer risk (Table 1; Figure 3). Notably, a meta-analysis further substantiated the role of CASP8 in cancer susceptibility. This study assessed the association between CASP8 rs3834129 and rs1045485 polymorphisms with the risk of breast cancer and other malignancies, revealing that these polymorphisms significantly reduced the risk of breast cancer and several other cancers, particularly in Asian and Caucasian populations (43). These findings provide compelling evidence supporting the protective role of elevated CASP8 protein levels against breast cancer risk, underscoring the importance of CASP8 as a tumor suppressor gene in breast cancer. Additionally, the potential of CASP8 as a therapeutic target in other cancers, such as liver cancer, lung cancer, melanoma, and leukemia, has been explored, highlighting its broader applicability in cancer treatment (Supplementary Table S5). These studies emphasize the relevance of CASP8 in drug development, bolstering the case for considering CASP8 as a potential target in breast cancer therapy. While our results strongly support the protective effect of elevated CASP8 protein levels against breast cancer risk, the literature presents contrasting findings. For instance, a prospective observational study indicated that increased CASP8 levels might be associated with poorer prognosis in patients with metastatic breast cancer (44). This discrepancy could be due to differences in study populations, such as the distinction between metastatic patients and those with early-stage breast cancer, which may involve significant variations in disease progression and immune response. Alternatively, it may stem from differing research methodologies or analytical strategies. These divergences further underscore the importance of exploring the role of CASP8 across different cancer stages and subtypes.

DDX58, also known as RIG-I, is a critical intracellular pattern recognition receptor pivotal in immune responses. Cao et al. have shown that deficiencies in RIG-I contribute to chemotherapy resistance in triple-negative breast cancer by impeding apoptosis mediated through type I IFN signaling. They also found that patients with diminished DDX58 expression have lower rates of achieving pathological complete response and exhibit poorer prognosis (45). Additionally, studies focusing on innate immune strategies for activating breast cancer cells and the tumor microenvironment have shown that RIG-I activation within breast tumors enhances tumor-infiltrating lymphocytes while diminishing tumor growth and metastasis (46). These findings underscore the robust immunogenicity and therapeutic potential of RIG-I agonists when delivered to tumors, particularly in the context of less immunogenic breast cancers (46). Consistent with these observations, a previous study demonstrated that the active metabolite of tamoxifen (TAM), 4-hydroxytamoxifen (4-OH-TAM), regulates the expression of multiple genes, including the upregulation of DDX58 in estrogen receptor-positive (ER+) breast cancer MCF-7 cells. This research revealed that DDX58 and other genes were upregulated following 4-OH-TAM treatment, underscoring its role in both estrogen receptor-dependent and independent pathways (47). Our study indicates that lower levels of DDX58 protein were associated with an increased risk of breast cancer, which is consistent with previous foundational research (Table 1; Figure 3). Herein, we provided robust genetic evidence supporting the protective role of DDX58 against breast cancer risk. Currently, DDX58 is under investigation for its potential use as an immune modulator and antiviral agent, indicating its promise as a novel therapeutic target for breast cancer (Supplementary Table S5).

In the present study, CPNE1, PARK7, and TNFRSF9 were supported by secondary evidence strength. CPNE1 (Copine-1) is a calcium-binding protein with crucial roles in cellular signal transduction, adhesion, and apoptosis (48). Our research indicated

that decreased circulating levels of CPNE1 are associated with an increased risk of breast cancer, which is consistent with findings by Ren et al. (14). However, multiple studies have also shown that CPNE1 promotes aerobic glycolysis and metastasis in triple-negative breast cancer (TNBC) through the PI3K/AKT/HIF-1α signaling pathway, thereby accelerating tumor progression (49). Additionally, other research has demonstrated that CPNE1 is overexpressed in TNBC tissues and cell lines, closely associated with tumor size, distant metastasis, and the survival rates of TNBC patients. CPNE1 also promotes tumorigenesis and radioresistance in TNBC cells by activating the AKT signaling pathway (50). Although most foundational studies suggest that CPNE1 has a pro-tumor role in cancer progression, our study and Ren's research, employing MR, provide robust evidence from a causal perspective that CPNE1 may have a protective role in breast cancer. Our findings indicated a negative association between CPNE1 and breast cancer risk (OR: 0.94, 95% CI: 0.90-0.98), and Ren's study yielded similar results (OR: 0.96, 95% CI: 0.94-0.98). This discovery suggests that a reduction in CPNE1 levels may increase the risk of breast cancer, which contradicts the tumor-promoting role of CPNE1 supported by conventional basic research. Therefore, while existing research predominantly focuses on the oncogenic role of CPNE1 in cancer, our and Ren's study provide causal evidence through MR analysis, revealing the potential protective function of CPNE1. This causal insight offers a new perspective on CPNE1 as a potential therapeutic target in breast cancer and suggests that future research should further explore the dual mechanisms of CPNE1 to gain a more comprehensive understanding of its role in breast cancer progression.

PARK7, also known as DJ-1 protein, exhibits findings similar to those of CPNE1. PARK7 is widely expressed intracellularly and is involved in regulating cellular responses to oxidative stress, protecting mitochondrial function, maintaining cellular redox balance, and inhibiting apoptosis (51). The present study suggests that decreased circulating levels of PARK7 are associated with an increased risk of breast cancer (Figure 3), which is in line with findings by Wang et al. (52). In their retrospective study, Tsuchiya and colleagues demonstrated that DJ-1 protein expression in invasive ductal carcinoma (IDC) tissues was lower than in adjacent non-cancerous epithelial tissues despite higher mRNA levels. Among IDC patients, lower DJ-1 protein expression was significantly associated with shorter disease-free survival ($P = 0.015$) and overall survival ($P = 0.020$) (53). However, an observational study indicated that DJ-1 is upregulated in HR+ breast cancer and significantly correlates with poor prognosis (54). These discrepancies may stem from differences in the breast cancer molecular subtypes used in our analysis compared to traditional epidemiological studies, or they may underscore limitations in adjusting for confounding factors and reverse causation in traditional epidemiological research. In summary, the relationship between PARK7 and breast cancer risk remains inconclusive. PARK7 is supported by secondary evidence strength in our study, suggesting its potential as a therapeutic target for breast cancer. However, further experimental studies are needed to clarify the directionality of the associations between PARK7 and breast cancer.

TNFRSF9, also known as 4-1BB, is a protein that belongs to the tumor necrosis factor receptor superfamily and has a critical role in immune regulation, particularly in the activation and proliferation of T cells. When the 4-1BB receptor binds with its ligand, 4-1BBL, it triggers various signaling pathways, including AKT, NF-kB, and MAPK, promoting T cell proliferation, survival, and function (55). Currently, monoclonal antibodies targeting 4-1BB, such as urelumab and utomilumab, have been used in the treatment of B-cell non-Hodgkin lymphoma, lung cancer, breast cancer, soft tissue sarcoma, and other solid tumors (56). Our findings suggest that elevated levels of TNFRSF9 protein increase the risk of breast cancer (Figure 3), supported by secondary evidence strength. Our research enhances the genetic evidence linking TNFRSF9 elevation to an increased risk of breast cancer. Moreover, in their study, Harao et al. demonstrated that 4-1BB-enhanced expansion of CD8+ TILs can significantly promote the growth of these T cells within TNBC tumors. This approach can be used to identify immunogenic mutations within autologous TNBC tumor tissues. These findings underscore the potential application of 4-1BB in immunotherapy and offer a novel perspective on adoptive immunotherapy for TNBC (57). Our research further corroborates the role of TNFRSF9 in breast cancer and provides new genetic evidence supporting its potential as a therapeutic target.

ULK3 has emerged from our study as a novel prognostic biomarker for breast cancer. ULK3, or Unc-51 like autophagy activating kinase 3, primarily regulates the autophagy pathway within cells (58). Previous studies have identified the upregulation of ULK3 in squamous cell carcinoma of the skin and head and neck (59), and its potential as a prognostic biomarker in colon cancer (60, 61). However, there are currently no basic experimental research reports on the association between ULK3 and breast cancer. A preliminary SMR analysis conducted in the present study indicated a negative correlation between ULK3 and the risk of breast cancer (Table 1). This finding was confirmed by validation MR analyses (Figure 3). Notably, fostamatinib, a spleen tyrosine kinase inhibitor used for chronic immune thrombocytopenia after other treatments, targets ULK3 (Supplementary Table S5), which shows promise as a new therapeutic target for breast cancer treatment and as a prognostic biomarker.

In the present study, preliminary SMR analysis suggested potential causal relationships between TNXB, BTN2A1, DNPH1, CGA, TLR1, and breast cancer; however, these associations were not validated in the MR analysis, resulting in only tertiary evidence support, which indicates insufficient strength of evidence. Further research is necessary to confirm their associations with breast cancer.

This study has several strengths. Firstly, we systematically explored the relationship between plasma protein levels and breast cancer risk using a two-stage proteome-wide MR design. This approach benefits from a large sample size and comprehensive coverage. It also mitigates the risks of reverse causation and confounding biases. Secondly, our study included preliminary and validation studies, encompassing discovery and replication MR analyses, thereby providing robust evidence for our findings. Thirdly, we employed colocalization methods to minimize false positives arising from LD and horizontal pleiotropy. Additionally, our study samples were drawn from European populations, reducing potential biases related to racial differences in research outcomes. Fourthly, PPI and druggability assessments offer insights into the potential pathogenic roles of candidate proteins in breast cancer, aiding in the prioritization of druggable targets. Notably,

proteins such as CASP8 and DDX58, which are already targeted for other diseases, exhibit promising potential as novel therapeutic targets for breast cancer.

However, the present study also has several limitations. Firstly, we did not investigate the relationship between circulating proteins and specific breast cancer subtypes due to the absence of cross-validated databases. This limitation underscores the necessity for future research to thoroughly explore the roles of these proteins across different breast cancer subtypes. Secondly, our study samples were exclusively from European populations, potentially limiting the generalizability of our findings to other ethnic groups. Further studies are needed to determine the applicability of these results across diverse racial populations. Thirdly, while we identified causal associations between relevant proteins and breast cancer, we could not conduct comprehensive biological experiments due to financial constraints. Future research could incorporate animal models and cell line experiments by addressing this gap to provide more robust evidence supporting our findings.

## 5 Conclusion

Using the MR combined colocalization method, we identified several plasma proteins associated with breast cancer risk, notably CASP8 and DDX58, which are promising targets for developing screening biomarkers and therapeutic drugs for breast cancer. Based on our findings, future experimental and clinical studies are essential to assess the efficacy and validate the potential of these candidate drugs.

## 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.

## Author contributions

JS: Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. HY: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The work was supported by the Program of National Natural Science Foundation of China(No.81860464).

## Acknowledgments

The study's feasibility owes gratitude to the creators of the IEU GWAS and FinnGen database and the authors who diligently uploaded the data, as their valuable contributions have been instrumental.

## Conflict of interest

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

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1449668/full#supplementary-material

## References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. (2023) 73:17–48. doi: 10.3322/caac.21763

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

3. McDonald ES, Clark AS, Tchou J, Zhang P, Freedman GM. Clinical diagnosis and management of breast cancer. *J Nucl Med*. (2016) 57 Suppl 1:9S–16S. doi: 10.2967/jnumed.115.157834

4. Hanash SM, Pitteri SJ, Faca VM. Mining the plasma proteome for cancer biomarkers. *Nature*. (2008) 452:571–9. doi: 10.1038/nature06916

5. Peila R, Rohan TE. Circulating levels of biomarkers and risk of ductal carcinoma in *situ* of the breast in the UK Biobank study. *Int J Cancer*. (2024) 154:1191–203. doi: 10.1002/ijc.34795

6. Thomas CE, Dahl L, Byström S, Chen Y, Uhlén M, Mälarstig A, et al. Circulating proteins reveal prior use of menopausal hormonal therapy and increased risk of breast cancer. *Transl Oncol*. (2022) 17:101339. doi: 10.1016/j.tranon.2022.101339

7. Byström S, Eklund M, Hong MG, Fredolini C, Eriksson M, Czene K, et al. Affinity proteomic profiling of plasma for proteins associated to area-based mammographic breast density. *Breast Cancer Res*. (2018) 20:14. doi: 10.1186/s13058-018-0940-z

8. Monson KR, Goldberg M, Wu HC, Santella RM, Chung WK, Terry MB. Circulating growth factor concentrations and breast cancer risk: a nested case-control study of IGF-1, IGFBP-3, and breast cancer in a family-based cohort. *Breast Cancer Res*. (2020) 22:109. doi: 10.1186/s13058-020-01352-0

9. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet*. (2014) 23:R89–98. doi: 10.1093/hmg/ddu328

10. Jia G, Yang Y, Ping J, Xu S, Liu L, Guo X, et al. Identification of target proteins for breast cancer genetic risk loci and blood risk biomarkers in a large study by integrating genomic and proteomic data. *Int J Cancer*. (2023) 152:2314–20. doi: 10.1002/ijc.34472

11. Shu X, Bao J, Wu L, Long J, Shu XO, Guo X, et al. Evaluation of associations between genetically predicted circulating protein biomarkers and breast cancer risk. *Int J Cancer*. (2020) 146:2130–8. doi: 10.1002/ijc.32542

12. Shu X, Zhou Q, Sun X, Flesaker M, Guo X, Long J, et al. Associations between circulating proteins and risk of breast cancer by intrinsic subtypes: a Mendelian randomisation analysis. *Br J Cancer*. (2022) 127:1507–14. doi: 10.1038/s41416-022-01923-2

13. Mälarstig A, Grassmann F, Dahl L, Dimitriou M, McLeod D, Gabrielson M, et al. Evaluation of circulating plasma proteins in breast cancer using Mendelian randomisation. *Nat Commun*. (2023) 14:7680. doi: 10.1038/s41467-023-43485-8

14. Ren F, Jin Q, Liu T, Ren X, Zhan Y. Proteome-wide mendelian randomization study implicates therapeutic targets in common cancers. *J Trans Med*. (2023) 21:646. doi: 10.1186/s12967-023-04525-5

15. Sun J, Luo J, Jiang F, Zhao J, Zhou S, Wang L, et al. Exploring the cross-cancer effect of circulating proteins and discovering potential intervention targets for 13 site-specific cancers. *J Natl Cancer Inst*. (2024) 116:565–73. doi: 10.1093/jnci/djad247

16. Zhu Z, Zhang F, Hu H, Bakshi A, Robinson MR, Powell JE, et al. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. *Nat Genet*. (2016) 48:481–7. doi: 10.1038/ng.3538

17. Giambartolomei C, Vukcevic D, SChadt EE, Franke L, Hingorani AD, Wallace C, et al. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genet*. (2014) 10:e1004383. doi: 10.1371/journal.pgen.1004383

18. Cuschieri S. The STROBE guidelines. *Saudi J Anaesth*. (2019) 13:S31–4. doi: 10.4103/sja.SJA_543_18

19. Ferkingstad E, Sulem P, Atlason BA, Sveinbjornsson G, Magnusson MI, Styrmisdottir EL, et al. Large-scale integration of the plasma proteome with genetics and disease. *Nat Genet*. (2021) 53:1712–21. doi: 10.1038/s41588-021-00978-w

20. Sun BB, Chiou J, Traylor M, Benner C, Hsu YH, Richardson TG, et al. Plasma proteomic associations with genetics and health in the UK Biobank. *Nature*. (2023) 622:329–38. doi: 10.1038/s41586-023-06592-6

21. Zhang H, Ahearn TU, Lecarpentier J, Barnes D, Beesley J, Qi G, et al. Genome-wide association study identifies 32 novel breast cancer susceptibility loci from overall and subtype-specific analyses. *Nat Genet*. (2020) 52:572–81. doi: 10.1038/s41588-020-0609-2

22. Kurki MI, Karjalainen J, Palta P, Sipilä TP, Kristiansson K, Donner K, et al. FinnGen: Unique genetic insights from combining isolated population and national health register data. *medRxiv*. (2022) 03:1–56. doi: 10.1101/2022.03.03.22271360

23. Luo J, Le Cessie S, Blauw GJ, Franceschi C, Noordam R, van Heemst D. Systemic inflammatory markers in relation to cognitive function and measures of brain atrophy: a Mendelian randomization study. *GeroScience*. (2022) 44:2259–70. doi: 10.1007/s11357-022-00602-7

24. Burgess S, Davey Smith G, Davies NM, Dudbridge F, Gill D, Glymour MM, et al. Guidelines for performing Mendelian randomization investigations: update for summer 2023. *Wellcome Open Res*. (2023) 4:186. doi: 10.12688/wellcomeopenres.15555.3

25. Burgess S, Thompson SG; CRP CHD Genetics Collaboration. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol*. (2011) 40:755–64. doi: 10.1093/ije/dyr036

26. Sun BB, Maranville JC, Peters JE, Stacey D, Staley JR, Blackshaw J, et al. Genomic atlas of the human plasma proteome. *Nature*. (2018) 558:73–9. doi: 10.1038/s41586-018-0175-2

27. Shi X, Wei T, Hu Y, Wang M, Tang Y. The associations between plasma soluble Trem1 and neurological diseases: a Mendelian randomization study. *J Neuroinflamm*. (2022) 19:218. doi: 10.1186/s12974-022-02582-z

28. Burgess S, Bowden J, Fall T, Ingelsson E, Thompson SG. Sensitivity analyses for robust causal inference from Mendelian randomization analyses with multiple genetic variants. *Epidemiology*. (2017) 28:30–42. doi: 10.1097/EDE.0000000000000559

29. Sun J, Zhao J, Jiang F, Wang L, Xiao Q, Han F, et al. Identification of novel protein biomarkers and drug targets for colorectal cancer by integrating human plasma proteome with genome. *Genome Med*. (2023) 15:75. doi: 10.1186/s13073-023-01229-9

30. Slob EAW, Burgess S. A comparison of robust Mendelian randomization methods using summary data. *Genet Epidemiol*. (2020) 44:313–29. doi: 10.1002/gepi.22295

31. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. (2016) 40:304–14. doi: 10.1002/gepi.21965

32. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur J Epidemiol*. (2017) 32:377–89. doi: 10.1007/s10654-017-0255-x

33. Greco M FD, Minelli C, Sheehan NA, Thompson JR. Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome. *Stat Med*. (2015) 34:2926–40. doi: 10.1002/sim.6522

34. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. (2018) 50:693–8. doi: 10.1038/s41588-018-0099-7

35. Freshour SL, Kiwala S, Cotto KC, Coffman AC, McMichael JF, Song JJ, et al. Integration of the Drug-Gene Interaction Database (DGIdb 4.0) with open crowdsource efforts. *Nucleic Acids Res*. (2021) 49:D1144–51. doi: 10.1093/nar/gkaa1084

36. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res*. (2018) 46:D1074–82. doi: 10.1093/nar/gkx1037

37. Fritsch M, Günther SD, Schwarzer R, Albert MC, Schorn F, Werthenbach JP, et al. Caspase-8 is the molecular switch for apoptosis, necroptosis and pyroptosis. *Nature*. (2019) 575:683–7. doi: 10.1038/s41586-019-1770-6

38. Newton K, Wickliffe KE, Maltzman A, Dugger DL, Reja R, Zhang Y, et al. Activity of caspase-8 determines plasticity between cell death pathways. *Nature*. (2019) 575:679–82. doi: 10.1038/s41586-019-1752-8

39. Lehle AS, Farin HF, Marquardt B, Michels BE, Magg T, Li Y, et al. Intestinal inflammation and dysregulated immunity in patients with inherited caspase-8 deficiency. *Gastroenterology*. (2019) 156:275–8. doi: 10.1053/j.gastro.2018.09.041

40. Zou J, Xia H, Zhang C, Xu H, Tang Q, Zhu G, et al. Casp8 acts through A20 to inhibit PD-L1 expression: The mechanism and its implication in immunotherapy. *Cancer Sci*. (2021) 112:2664–78. doi: 10.1111/cas.14932

41. Palanca Suela S, Esteban Cardeñosa E, Barragán González E, de Juan Jiménez I, Chirivella González I, Segura Huerta A, et al. CASP8 D302H polymorphism delays the age of onset of breast cancer in BRCA1 and BRCA2 carriers. *Breast Cancer Res Treat*. (2010) 119:87–93. doi: 10.1007/s10549-009-0316-2

42. Aghababazadeh M, Dorraki N, Javan FA, Fattahi AS, Gharib M, Pasdar A. Downregulation of Caspase 8 in a group of Iranian breast cancer patients - A pilot study. *J Egypt Natl Canc Inst*. (2017) 29:191–5. doi: 10.1016/j.jnci.2017.10.001

43. Hashemi M, Aftabi S, Moazeni-Roodi A, Sarani H, Wiechec E, Ghavami S. Association of CASP8 polymorphisms and cancer susceptibility: A meta-analysis. *Eur J Pharmacol*. (2020) 881:173201. doi: 10.1016/j.ejphar.2020.173201

44. Gunnarsdottir FB, Bendahl PO, Johansson A, Benfeitas R, Rydén L, Bergenfelz C, et al. Serum immuno-oncology markers carry independent prognostic information in patients with newly diagnosed metastatic breast cancer, from a prospective observational study. *Breast Cancer Res*. (2023) 25:29. doi: 10.1186/s13058-023-01631-6

45. Cao S, Long X, Xiao L, Zhang P, Shen M, Chen F, et al. DDX58 deficiency leads to triple negative breast cancer chemotherapy resistance by inhibiting Type I IFN-mediated signalling apoptosis. *Front Oncol*. (2024) 14:1356778. doi: 10.3389/fonc.2024.1356778

46. Elion DL, Jacobson ME, Hicks DJ, Rahman B, Sanchez V, Gonzales-Ericsson PI, et al. Therapeutically active RIG-I agonist induces immunogenic tumor cell killing in breast cancers. *Cancer Res*. (2018) 78:6183–95. doi: 10.1158/0008-5472.CAN-18-0730

47. Fang Q, Yao S, Luo G, Zhang X. Identification of differentially expressed genes in human breast cancer cells induced by 4-hydroxyltamoxifen and elucidation of their pathophysiological relevance and mechanisms. *Oncotarget*. (2017) 9:2475–501. doi: 10.18632/oncotarget.23504

48. Tang H, Zhu J, Du W, Liu S, Zeng Y, Ding Z, et al. CPNE1 is a target of miR-335-5p and plays an important role in the pathogenesis of non-small cell lung cancer. *J Exp Clin Cancer Res*. (2018) 37:131. doi: 10.1186/s13046-018-0811-6

49. Cao J, Cao R, Liu Y, Dai T. CPNE1 mediates glycolysis and metastasis of breast cancer through activation of PI3K/AKT/HIF-1α signaling. *Pathology Res practice*. (2023) 248:154634–4. doi: 10.1016/j.prp.2023.154634

50. Shao Z, Ma X, Zhang Y, Sun Y, Lv W, He K, et al. CPNE1 predicts poor prognosis and promotes tumorigenesis and radioresistance via the AKT singling pathway in triple-negative breast cancer. *Mol Carcinog*. (2020) 59:533–44. doi: 10.1002/mc.23177

51. Kim RH, Peters M, Jang Y, Shi W, Pintilie M, Fletcher GC, et al. DJ-1, a novel regulator of the tumor suppressor PTEN. *Cancer Cell*. (2005) 7:263–73. doi: 10.1016/j.ccr.2005.02.010

52. Wang Y, Yi K, Chen B, Zhang B, Gao J. Elucidating the susceptibility to breast cancer: an in-depth proteomic and transcriptomic investigation into novel potential plasma protein biomarkers. *Front Mol Biosci*. (2024) 10. doi: 10.3389/fmolb.2023.1340917

53. Tsuchiya B, Iwaya K, Kohno N, Kawate T, Akahoshi T, Matsubara O, et al. Clinical significance of DJ-1 as a secretory molecule: retrospective study of DJ-1 expression at mRNA and protein levels in ductal carcinoma of the breast. *Histopathology*. (2012) 61:69–77. doi: 10.1111/j.1365-2559.2012.04202.x

54. Xie Y, Li Y, Yang M. DJ-1: A potential biomarker related to prognosis, chemoresistance, and expression of microenvironmental chemokine in HR-positive breast cancer. *J Immunol Res*. (2023) 2023:1–15. doi: 10.1155/2023/5041223

55. Sanmamed MF, Etxeberría I, Otano I, Melero I. Twists and turns to translating 4-1BB cancer immunotherapy. *Sci Trans Med*. (2019) 11:eaax4738. doi: 10.1126/scitranslmed.aax4738

56. Shen X, Zhang R, Nie X, Yang Y, Hua Y, Peng Lü. 4-1BB targeting immunotherapy: mechanism, antibodies, and chimeric antigen receptor T. *Cancer biotherapy radiopharmaceuticals*. (2023) 38:431–44. doi: 10.1089/cbr.2023.0022

57. Harao M, Forget MA, Roszik J, Gao H, Babiera GV, Krishnamurthy S, et al. 4-1BB-enhanced expansion of CD8+ TIL from triple-negative breast cancer unveils mutation-specific CD8+ T cells. *Cancer Immunol Res*. (2017) 5:439–45. doi: 10.1158/2326-6066.CIR-16-0364

58. Young ARJ, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JF, et al. Autophagy mediates the mitotic senescence transition. *Genes Dev*. (2009) 23:798–803. doi: 10.1101/gad.519709

59. Goruppi S, Clocchiatti A, Bottoni G, Di Cicco E, Ma M, Tassone B, et al. he ULK3 kinase is a determinant of keratinocyte self-renewal and tumorigenesis targeting the arginine methylome. *Nat Commun*. (2023) 14:887. doi: 10.1038/s41467-023-36410-6

60. Xu J, Dai S, Yuan Y, Xiao Q, Ding K. A prognostic model for colon cancer patients based on eight signature autophagy genes. *Front Cell Dev Biol*. (2020) 8. doi: 10.3389/fcell.2020.602174

61. Wang Y, Lin K, Xu T, Wang L, Fu L, Zhang G, et al. Development and validation of prognostic model based on the analysis of autophagy-related genes in colon cancer. *Aging*. (2021) 13:19028–47. doi: 10.18632/aging.v13i14

frontiers | Frontiers in Endocrinology

TYPE Mini Review
PUBLISHED 08 October 2024
DOI 10.3389/fendo.2024.1484815



OPEN ACCESS

EDITED BY
Min Tu,
Nanjing Medical University, China

REVIEWED BY
Qiong Lu,
Central South University, China
Zhengrui Li,
Shanghai Jiao Tong University, China

*CORRESPONDENCE
Xi-Qiao Zhou
zhouxiqiao@njucm.edu.cn
Jing Sun
sunjing@njucm.edu.cn

†These authors have contributed equally to this work

RECEIVED 22 August 2024
ACCEPTED 19 September 2024
PUBLISHED 08 October 2024

CITATION
Huai J-X, Wang F, Zhang W-H, Lou Y, Wang G-X, Huang L-J, Sun J and Zhou X-Q (2024) Unveiling new chapters in medullary thyroid carcinoma therapy: advances in molecular genetics and targeted treatment strategies.
*Front. Endocrinol.* 15:1484815.
doi: 10.3389/fendo.2024.1484815



# Unveiling new chapters in medullary thyroid carcinoma therapy: advances in molecular genetics and targeted treatment strategies

Jia-Xuan Huai[1,2†], Fang Wang[3†], Wen-Hui Zhang[1,2], Yan Lou[1], Gao-Xiang Wang[1], Li-Ji Huang[1], Jing Sun[1,2*] and Xi-Qiao Zhou[1,2*]

[1]Department of Endocrinology, Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, China, [2]The First School of Clinical Medicine, Nanjing University of Chinese Medicine, Nanjing, China, [3]Department of Otolaryngology, Xinyang Central Hospital, Xinyang, China

Medullary Thyroid Carcinoma (MTC), a neuroendocrine malignancy that arises from the calcitonin-secreting parafollicular C-cells of the thyroid, constitutes a minor yet impactful fraction of thyroid malignancies. Distinguished by its propensity for aggressive growth and a pronounced tendency for metastasis, MTC poses formidable obstacles to the early diagnosis and therapeutic intervention. The molecular genetics of MTC, particularly the role of the RET gene and the RAS gene family, have been extensively studied, offering insights into the pathogenesis of the disease and revealing potential therapeutic targets. This comprehensive review synthesizes the latest advancements in the molecular genetics of MTC, the evolution of precision therapies, and the identification of novel biomarkers. We also discuss the implications of these findings for clinical practice and the future direction of MTC research.

KEYWORDS

**medullary thyroid carcinoma, molecular genetics, RET gene, RAS genes, targeted therapy, biomarkers, immunotherapy, multi-kinase inhibitors**

## 1 Introduction

Medullary thyroid carcinoma (MTC), a highly invasive neuroendocrine neoplasm, originates from the thyroid's parafollicular C-cells, which originate from the neural crest and are characterized by their secretion of calcitonin (CTN)-a pivotal biomarker for the identification and surveillance of MTC (1). The disease occurs in both hereditary and sporadic forms. The inherited form, which constitutes 25% of cases, typically exhibits autosomal dominant inheritance and is associated with multiple endocrine neoplasia type 2 (MEN2) syndromes, and the sporadic form makes up the remaining 75% of cases (2).

Three distinct variants of the MEN2 spectrum, categorized as MEN2A, MEN2B (alternatively designated as MEN3), and Familial Medullary Thyroid Carcinoma (FMTC), are recognized based on the presence or absence of associated conditions like hyperparathyroidism and pheochromocytoma, along with distinctive clinical manifestations (3). Although MTC represents only 1-2% of all thyroid malignancies, it is linked to considerable morbidity and mortality, characterized by a high incidence of metastasis at the time of diagnosis and a lack of recent advancements in early detection or patient survival rates (4). Extensive research into the molecular underpinnings of MTC has enhanced our comprehension of its etiology and uncovered possible targets for therapeutic intervention.

# 2 Molecular genetics research

## 2.1 RET gene mutations

The RET proto-oncogene, localized on the chromosomal region 10q11.2, translates into a receptor tyrosine kinase crucial for a multitude of intracellular signaling cascades. This kinase plays a pivotal role in the morphogenesis of diverse organs, encompassing the parathyroid glands, the urogenital system, and tissues that originate from the neural crest, such as the cerebral structures, neural ganglia, intestinal ganglia, adrenal medulla, and the calcitonin-producing C cells of the thyroid (5). Given its widespread influence, it is not unexpected that dysregulation of RET is implicated in the tumorigenesis observed in multiple endocrine neoplasia (MEN) syndromes, where these organs are commonly affected (6). Within the RET proto-oncogene, a preponderance of identified mutations are missense in nature, predominantly occurring within exons 10 and 11, which form the extracellular portion of the RET protein, or exons 13 to 16, encompassing the tyrosine kinase domain. Furthermore, mutations are not restricted to these regions; exons 3, 5, and 8 are also implicated in the mutational spectrum (1).

In the realm of MTC, the spectrum of over a hundred gain-of-function mutations within the RET gene has been characterized, with germline mutations present in hereditary cases and somatic mutations in sporadic cases (7).

In the context of sporadic medullary thyroid carcinoma (MTC), somatic mutations in the RET gene have been detected in a substantial proportion, ranging from 43 to 71%. Intriguingly, this prevalence escalates dramatically to 85% in cases characterized by advanced metastasis, with the M918T mutation being particularly prevalent (8, 9), followed by C634 within exon 11 (10). RET-mutant MTC cases harboring RET mutations are observed to display a more virulent phenotype, which encompasses an elevated likelihood of lymph node invasion, propensity for distant metastasis, and a generally unfavorable prognosis when juxtaposed with MTC cases featuring alternative mutations (11, 12). This aberrant RET signaling enhances cell proliferation, a critical mechanism for tumor growth (13–15). For a detailed breakdown of the risk levels, mutations, associated diagnoses, and management strategies, please refer to Table 1.

In hereditary medullary thyroid carcinoma (HMTC), the most common mutations in MEN2A are found in exons 10 and 11, particularly the codon 634 in exon 11, accounting for 85% of mutations in this exon. In MEN2B, 95% of cases exhibit the M918T mutation located in exon 16. This specific mutation is not only linked to the most severe prognosis but also exhibits an exceptionally high rate of heritability, approaching 95% (16).

## 2.2 RAS gene family

Beyond the already recognized RET proto-oncogene, the frontiers of genomic investigation have disclosed a spectrum of supplementary genetic alterations that are instrumental in the etiological framework of MTC.

The trio of N-RAS, H-RAS, and K-RAS genes, collectively known as the RAS gene family, is recognized as a critical oncogenic driver in sporadic MTC, second only to the RET oncogene. Approximately 70% of tumors with a wild-type RET status exhibit RAS gene mutations, predominantly involving HRAS, followed by less frequent KRAS mutations, and with NRAS mutations being quite uncommon (12).These genes encode for 21 kDa GTPase proteins that are instrumental in regulating signal transduction pathways essential for cell proliferation, metabolism, migration, and other cellular activities (17). Significantly, the RAS proteins fulfill their regulatory roles via pivotal signaling cascades, including the Raf/MEK/ERK and PI3K/Akt pathways, which play essential roles in both normal cellular functions and disease states (18, 19).

RAS gene mutations are prevalent in various types of cancer, making up approximately 30% of all somatic mutations found in comprehensive studies of MTC (20). These mutations are significant because they can lead to the dysregulation of the normal functioning of RAS proteins, potentially resulting in uncontrolled cell growth and tumor development (12).

## 2.3 Other genetic mutations

Advanced sequencing techniques, including whole-exome sequencing and next-generation sequencing (NGS), have revealed that in tumors without mutations in the RET and RAS genes, the prevalence of additional recurrent genetic mutations is significantly scarce (20, 21). Nonetheless, some research has pointed to the involvement of alterations in alternative pathways, along with evidence of epigenetic changes in sporadic MTC.

In a subset of sporadic MTC tumors, Furthermore, activation of the mTOR pathway has been identified in sporadic MTC, with a particular increase noted in cases with lymph node metastases. This activation is also observed in RAS-mutant MTC, suggesting a potential role in the disease's progression (22, 23). Loss of somatic copy number of the CDKN2C gene has been correlated with detrimental clinical consequences, such as an increased propensity for distant metastasis and a markedly diminished overall survival rate. In the absence of this genetic aberration, the median overall survival extends to 18.3 years, which stands in stark contrast to the abbreviated survival of 4.1 years noted among patients with this mutation (24). Furthermore, heightened expression of hepatocyte growth factor (HGF) and its receptor

10.3389/fendo.2024.1484815

TABLE 1 Summary of RET mutations and associated clinical features in Multiple Endocrine Neoplasia Type 2 (MEN2).

| Risk level | RET mutation | RET exon | Possible Diagnoses | incidence of PEHO | incidence of HPTH | CLA | HD | Follow-up | Prophylactic Thyroidectomy Recommendations |
|---|---|---|---|---|---|---|---|---|---|
| Highest Risk (HST) | M918T | 16 | MEN2B | +++ | - | N | Y | Physical exam, neck US, serum Ctn, and serum CEA every 6 mos first year, then annually; begin screening for pheochromocytoma at age 11 yr | Within the first year of life or the first months of life based upon specialist and parental discussions. The ability to identify and preserve or transplant parathyroid glands determines level VI dissection. |
| High Risk (H) | A883F | 15 | MEN2A | +++ | ++ | N | N | Physical exam, neck US, serum Ctn, and serum CEA every 6 mos first year, then annually. Begin screening for pheochromocytoma at age 11. | At or before age 5 yr, to be determined on the basis of serum Ctn |
| | C634W/Y | 11 | | +++ | - | Y | N | | |
| Moderate Risk (MOD) | G533C | 8 | MEN2A | + | - | N | N | Evaluate every 6 months for 1 year. Annual follow-ups thereafter if serum Ctn is normal or undetectable. Begin screening for pheochromocytoma at age 16 yr | When serum Ctn becomes elevated or in childhood to avoid lengthy evaluation period. |
| | C609F/G/R/S/Y | 10 | | +/++ | + | N | Y | | |
| | C611F/G/S/Y/W | | | +/++ | + | N | Y | | |
| | C618F/R/S | | | +/++ | + | N | Y | | |
| | C620F/R/S | | | +/++ | + | N | Y | | |
| | C630R/Y | 11 | | +/++ | + | N | N | | |
| | D631Y | | | +++ | - | N | N | | |
| | K666E | | | + | - | N | N | | |
| | E768D | 13 | | - | - | N | N | | |
| | L790F | | | + | - | N | N | | |
| | V804L/M | 14 | | + | + | N/Y | N | | |
| | S891A | 15 | | + | + | N | N | | |
| | R912P | 16 | | - | - | N | N | | |

PHEO, Pheochromocytoma - a tumor of the adrenal gland that overproduces catecholamines.
HPTH, Hyperparathyroidism - a condition where there is excessive production of parathyroid hormone.
CLA, Cutaneous Lichen Amyloidosis - a skin condition characterized by amyloid deposits in the skin.
HD, Hirschsprung's Disease - a congenital condition affecting the large intestine due to the absence of nerve cells.
N, No
Y, Yes
+++, High incidence
++, Moderate incidence
+, Low incidence
-, No incidence
Varies: Incidence varies based on specific mutation.
Various: Applies to multiple mutations and exons.

MET has been identified in MTC, potentially indicating a correlation with the occurrence of multifocal tumorigenesis (25).

## 2.4 Epigenetic and post-transcriptional modifications

Epigenetic and post-transcriptional modifications are increasingly recognized as pivotal forces in the pathophysiology of MTC. In a study encompassing 41 individuals with sporadic MTC, a pronounced elevation in the levels of histone methyltransferases EZH2 and SMYD3 was noted in tumors with a more aggressive phenotype. This upregulation was observed irrespective of the mutational landscape of the RET and RAS genes, indicating that epigenetic mechanisms may significantly contribute to the advancement of MTC through pathways distinct from those involving RET and RAS (26).

An assessment of global DNA methylation among peripheral blood leukocytes in individuals with either sporadic or hereditary MTC disclosed that the sporadic variant is marked by an increased

level of methylation. This elevated methylation in sporadic MTC might be attributed to environmental factors rather than germline mutations, which are characteristic of hereditary MTC (27).

While mutations in the TERT promoter region are infrequently identified in MTC, an upsurge in TERT gene copy number and the methylation of its promoter region have been documented. Such promoter methylation correlates with heightened TERT expression and telomerase activation, which in turn are associated with diminished disease-free intervals and overall patient survival rates in MTC (28).

MicroRNAs (miRNAs), a subset of non-coding RNA molecules ranging from 18 to 25 nucleotides in length, are recognized as pivotal regulators of gene expression at the post-transcriptional level (29). These miRNAs have been associated with the intricate control of a diverse array of cellular mechanisms and display unique expression signatures that vary between physiological and pathological conditions, as well as in response to a spectrum of therapeutic interventions (30, 31).

Within the realm of MTC, miRNAs have risen to prominence as influential modulators, with particular miRNAs, including miR-21, miR-375, and miR-183, being correlated with adverse clinical prognoses and the perpetuation of metastatic malignancy. In contrast, miR-224 has demonstrated a positive association with the presence of non-invasive disease states and the achievement of biochemical remission (32, 33).

Divergent miRNA expression patterns have been documented between sporadic and hereditary forms of MTC, with a significant reduction in miR-127 levels observed specifically in sporadic cases harboring somatic RET mutations, in contrast to those with the wild-type RET genotype (34). Furthermore, the overexpression of genes involved in microRNA biogenesis, such as DICER, Additionally, an upregulation of genes pivotal to miRNA biogenesis, including DICER, DGCR8, and XPO5, has been identified in tumors with RET mutations; however, this overexpression pattern does not appear to be linked to the mutational status of RAS genes (35).

Emerging research underscores the profound influence of miRNAs on the cellular phenotypes within Medullary Thyroid Carcinoma (MTC). Notably, the suppression of miR-200b and miR-200c in MTC cell cultures has been correlated with the induction of mesenchymal transition, conferring heightened invasive properties. In parallel, the activation of TGFb-1 and the repression of miR-183 have been associated with diminished cellular viability, coupled with an upregulation of the light chain 3B of microtubule-associated protein 1, a key player in autophagy (36). Collectively, these observations illuminate the intricate dynamics of miRNAs in the etiology and advancement of MTC, indicating their potential to act as either promoters or inhibitors of tumorigenesis.

Moreover, the discovery of circulating miRNAs as potential biomarkers in bodily fluids has introduced the concept of "liquid biopsy" for non-invasive disease monitoring (37, 38). These extracellular miRNAs, released from cells into the bloodstream, offer a promising avenue for early detection, treatment response assessment, and monitoring of disease progression in MTC and potentially other cancers (39).

# 3 Molecular genetics therapies

## 3.1 Immunotherapies

Despite initial studies indicating promising outcomes, the efficacy of immunotherapy in patients with MTC has been observed to be relatively subdued compared to its impact on other types of cancer. Recent findings indicate that the immunological characteristics of the MTC tumor microenvironment could be more subdued, or less reactive, than what was initially thought.

In an expanded patient sample of 200 individuals, a thorough examination of co-inhibitory receptors, including PD-1, TIM-3, CTLA-4, LAG-3, and TIGIT, demonstrated a correlation between elevated structural recurrence rates and the presence of TIM-3 and CTLA-4, in conjunction with the concurrent expression of PD-1 and its ligand, PD-L1 (40). Moreover, research by Pozdeyev and colleagues has illuminated the intricate immunological landscape of Medullary Thyroid Carcinoma (MTC), revealing that organized immune cell infiltration is a common feature, identified in nearly half of the primary MTC tumor cases and, more markedly, in an overwhelming majority—90%—of metastatic sites (41). The presence of CD8+ T lymphocytes and B cells was commonly noted, whereas regulatory T cells (Tregs) were identified in less than 5% of the surrounding non-cancerous cells. Recent studies have uncovered CD276 as a prospective immunotherapeutic target in MTC through immune profiling of these tumors (42). CD276 may enhance the efficacy of other immune checkpoint inhibitors. Consequently, the deployment of combination immunotherapy, involving both anti-CD276 antibodies and anti-PD1/PD-L1 antibodies, holds potential as a novel therapeutic strategy in cancer treatment (43).

The immune microenvironment in MTC significantly influences the efficacy of immunotherapies due to its distinctive characteristics. The tumor's immune cell infiltration patterns, which vary among individuals, are critical for immunotherapy responses, emphasizing the need for personalized treatment approaches tailored to specific immune contexts. Future research should delve deeper into the immunological features of MTC, particularly the distribution and functionality of tumor-infiltrating immune cells, to enhance the potential of immunotherapeutic interventions and optimize treatment outcomes.

## 3.2 Targeted therapies

Multi-kinase inhibitors (MKIs) are therapeutic agents that possess the capability to inhibit multiple tyrosine kinase receptors (TKRs), which are crucial in cellular mechanisms encompassing growth, differentiation, and angiogenesis. These TKRs are integral components of signaling pathways that regulate cell proliferation, including the PI3K/AKT/mTOR and the MAP kinase/ERK pathways. In the context of oncology, TKR mutations or overexpression are commonly observed in cancer cells, contributing to malignant transformation and tumor progression (44, 45). The process of angiogenesis, characterized by the formation of new blood vessels, is essential for tumorigenesis and

metastasis, as it supplies vital nutrients and aids in the dissemination of malignant cells to extranodal regions (46). Central to this process are the Vascular Endothelial Growth Factor (VEGF) and its cognate receptor, VEGF-R, which are frequently observed in elevated levels within neoplastic tissues. This overexpression is particularly pertinent to MTC, a malignancy distinguished by an abundant vascular architecture (47, 48). The heightened levels of VEGF and its receptor, VEGFR, observed in MTC, especially in tumors with RET mutations, reinforce the therapeutic rationale for the use of MKIs, alternatively termed anti-angiogenic medications, in the clinical management of advanced-stage MTC.

Selective RET kinase inhibitors are a novel class of highly selective targeted drugs, which are more efficient and safer in clinical application. Notable among the drugs approved in 2020 are selpercatinib and pralsetinib, which have shown remarkable effectiveness in treating patients with prior exposure to tyrosine kinase inhibitors (TKIs) (49). Due to their high selectivity and lower incidence and severity of adverse reactions, selective RET kinase inhibitors are currently a hot topic in the research of MTC treatment.

The pivotal phase 3 LIBRETTO-531 clinical trial has affirmed the superiority of selpercatinib as a premier therapeutic option for individuals afflicted with RET-mutant MTC, demonstrating superior efficacy and safety over cabozantinib or vandetanib. At a median follow-up duration of 12 months, selpercatinib has been shown to notably prolong both progression-free survival and treatment failure-free survival, achieving an 86.8% rate of progression-free survival and an 86.2% rate of treatment failure-free survival at the one-year mark. Moreover, the overall response rate within the selpercatinib cohort peaked at 69.4%, significantly eclipsing the 38.8% response rate documented in the comparator group. Additionally, selpercatinib was associated with fewer dose reductions and treatment discontinuations due to adverse events, highlighting its favorable safety profile in this patient population (50).

## 4 Conclusion

In conclusion, the molecular basis of MTC has been substantially clarified, with key genetic drivers such as RET and RAS gene mutations being identified. The advent of selective RET kinase inhibitors, particularly selpercatinib, has significantly improved treatment outcomes in terms of progression-free survival and safety. However, challenges including therapeutic resistance necessitate ongoing research into resistance mechanisms and the development of innovative combination therapies to enhance MTC treatment efficacy.

While the complexity of MTC's molecular landscape suggests the presence of additional targets for therapy, the immune microenvironment's role in MTC also warrants further investigation to refine immunotherapeutic strategies. Overcoming therapeutic resistance is pivotal for the enduring success of targeted therapies, and innovative clinical trials along with combination therapy approaches will be key in this endeavor.

The translation of these molecular insights into clinical practice is crucial for optimizing patient care. As our comprehension of MTC's molecular genetics deepens, our therapeutic strategies must evolve in tandem. It is expected that ongoing research will yield increasingly personalized and potent treatments, leading to improved patient outcomes and survival rates.

## Author contributions

J-XH: Writing – review & editing, Writing – original draft, Visualization. FW: Writing – review & editing. W-HZ: Writing – review & editing, Data curation. YL: Writing – review & editing, Investigation, Conceptualization. G-XW: Writing – review & editing, Data curation, Conceptualization. L-JH: Writing – review & editing, Investigation, Conceptualization. JS: Writing – review & editing, Project administration, Conceptualization. X-QZ: Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The work was supported by the National Natural Science Foundation of China (No. 82203850).

## 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. Wells SA Jr, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid*. (2015) 25:567–610. doi: 10.1089/thy.2014.0335

2. Fagin JA, Wells SA Jr. Biologic and clinical perspectives on thyroid cancer. *N Engl J Med*. (2016) 375:2307. doi: 10.1056/NEJMc1613118

3. Pelizzo MR, Boschin IM, Bernante P, Toniato A, Piotto A, Pagetta C, et al. Natural history, diagnosis, treatment and outcome of medullary thyroid cancer: 37 years experience on 157 patients. *Eur J Surg Oncol*. (2007) 33:493–7. doi: 10.1016/j.ejso.2006.10.021

4. Barletta JA, Nosé V, Sadow PM. Genomics and epigenomics of medullary thyroid carcinoma: from sporadic disease to familial manifestations. *Endocr Pathol*. (2021) 32:35–43. doi: 10.1007/s12022-021-09664-3

5. Falvo L, Catania A, Sorrenti S, D'Andrea V, Berni A, De Stefano M, et al. Prognostic significance of the age factor in the thyroid cancer: statistical analysis. *J Surg Oncol*. (2004) 88:217–22. doi: 10.1002/jso.20140

6. Mulligan LM. RET revisited: expanding the oncogenic portfolio. *Nat Rev Cancer*. (2014) 14:173–86. doi: 10.1038/nrc3680

7. Conzo G, Calò PG, Gambardella C, Tartaglia E, Mauriello C, Della Pietra C, et al. Controversies in the surgical management of thyroid follicular neoplasms. Retrospective analysis of 721 patients. *Int J Surg*. (2014) 12 Suppl 1:S29–34. doi: 10.1016/j.ijsu.2014.05.013

8. Moura MM, Cavaco BM, Pinto AE, Domingues R, Santos JR, Cid MO, et al. Correlation of RET somatic mutations with clinicopathological features in sporadic medullary thyroid carcinomas. *Br J Cancer*. (2009) 100:1777–83. doi: 10.1038/sj.bjc.6605056

9. Dvorakova S, Vaclavikova E, Sykorova V, Domingues R, Santos JR, Cid MO, et al. Somatic mutations in the RET proto-oncogene in sporadic medullary thyroid carcinomas. *Mol Cell Endocrinol*. (2008) 284:21–7. doi: 10.1016/j.mce.2007.12.016

10. Romei C, Ciampi R, Elisei R. A comprehensive overview of the role of the RET proto-oncogene in thyroid carcinoma. *Nat Rev Endocrinol*. (2016) 12:192–202. doi: 10.1038/nrendo.2016.11

11. Romei C, Ciampi R, Casella F, Tacito A, Torregrossa L, Ugolini C, et al. RET mutation heterogeneity in primary advanced medullary thyroid cancers and their metastases. *Oncotarget*. (2018) 9:9875–84. doi: 10.18632/oncotarget.23986

12. Ciampi R, Romei C, Ramone T, Prete A, Tacito A, Cappagli V, et al. Genetic landscape of somatic mutations in a large cohort of sporadic medullary thyroid carcinomas studied by next-generation targeted sequencing. *iScience*. (2019) 20:324–36. doi: 10.1016/j.isci.2019.09.030

13. Illini O, Hochmair MJ, Fabikan H, Weinlinger C, Tufman A, Swalduz A, et al. Selpercatinib in RET fusion-positive non-small-cell lung cancer (SIREN): a retrospective analysis of patients treated through an access program. *Ther Adv Med Oncol*. (2021) 13:17588359211019675. doi: 10.1177/17588359211019675

14. Mulligan LM. RET revisited: expanding the oncogenic portfolio. *Nat Rev Cancer*. (2014) 14:173–86. doi: 10.1038/nrc3680

15. Ban K, Feng S, Shao L, Ittmann M. RET signaling in prostate cancer. *Clin Cancer Res*. (2017) 23:4885–96. doi: 10.1158/1078-0432.CCR-17-0528

16. De Falco V, Carlomagno F, Li HY, Santoro M. The molecular basis for RET tyrosine-kinase inhibitors in thyroid cancer. *Best Pract Res Clin Endocrinol Metab*. (2017) 31:307–18. doi: 10.1016/j.beem.2017.04.013

17. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. *Nat Rev Cancer*. (2011) 11:761–74. doi: 10.1038/nrc3106

18. Plowman SJ, Hancock JF. Ras signaling from plasma membrane and endomembrane microdomains. *Biochim Biophys Acta*. (2005) 1746:274–83. doi: 10.1016/j.bbamcr.2005.06.004

19. Lau KS, Haigis KM. Non-redundancy within the RAS oncogene family: insights into mutational disparities in cancer. *Mol Cells*. (2009) 28:315–20. doi: 10.1007/s10059-009-0143-7

20. Chang YS, Chang CC, Huang HY, Lin CY, Yeh KT, Chang JG. Detection of molecular alterations in Taiwanese patients with medullary thyroid cancer using whole-exome sequencing. *Endocr Pathol*. (2018) 29:324–31. doi: 10.1007/s12022-018-9543-6

21. Heilmann AM, Subbiah V, Wang K, Sun JX, Elvin JA, Chmielecki J, et al. Comprehensive genomic profiling of clinically advanced medullary thyroid carcinoma. *Oncology*. (2016) 90:339–46. doi: 10.1159/000445978

22. Lyra J, Vinagre J, Batista R, Pinto V, Prazeres H, Rodrigues F, et al. mTOR activation in medullary thyroid carcinoma with RAS mutation. *Eur J Endocrinol*. (2014) 171:633–40. doi: 10.1530/EJE-14-0389

23. Tamburrino A, Molinolo AA, Salerno P, Chernock RD, Raffeld M, Xi L, et al. Activation of the mTOR pathway in primary medullary thyroid carcinoma and lymph node metastases. *Clin Cancer Res*. (2012) 18:3532–40. doi: 10.1158/1078-0432.CCR-11-2700

24. Grubbs EG, Williams MD, Scheet P, Vattathil S, Perrier ND, Lee JE, et al. Role of CDKN2C copy number in sporadic medullary thyroid carcinoma. *Thyroid*. (2016) 26:1553–62. doi: 10.1089/thy.2016.0224

25. Papotti M, Olivero M, Volante M, Negro F, Prat M, Comoglio PM, et al. Expression of hepatocyte growth factor (HGF) and its receptor (MET) in medullary carcinoma of the thyroid. *Endocr Pathol*. (2000) 11:19–30. doi: 10.1385/ep:11:1:19

26. Sponziello M, Durante C, Boichard A, Dima M, Puppin C, Verrienti A, et al. Epigenetic-related gene expression profile in medullary thyroid cancer revealed the overexpression of the histone methyltransferases EZH2 and SMYD3 in aggressive tumours. *Mol Cell Endocrinol*. (2014) 392:8–13. doi: 10.1016/j.mce.2014.04.016

27. Ceolin L, Goularte APP, Ferreira CV, Romitti M, Maia AL. Global DNA methylation profile in medullary thyroid cancer patients. *Exp Mol Pathol*. (2018) 105:110–4. doi: 10.1016/j.yexmp.2018.06.003

28. Wang N, Kjellin H, Sofiadis A, Fotouhi O, Juhlin CC, Bäckdahl M, et al. Genetic and epigenetic background and protein expression profiles in relation to telomerase activation in medullary thyroid carcinoma. *Oncotarget*. (2016) 7:21332–46. doi: 10.18632/oncotarget.7237

29. Chu YH, Lloyd RV. Medullary thyroid carcinoma: recent advances including microRNA expression. *Endocr Pathol*. (2016) 27:312–24. doi: 10.1007/s12022-016-9449-0

30. Besharat ZM, Abballe L, Cicconardi F, Bhutkar A, Grassi L, Le Pera L, et al. Foxm1 controls a pro-stemness microRNA network in neural stem cells. *Sci Rep*. (2018) 8:3523. doi: 10.1038/s41598-018-21876-y

31. Catanzaro G, Sabato C, Russo M, Rosa A, Abballe L, Besharat ZM, et al. Loss of miR-107, miR-181c and miR-29a-3p Promote Activation of Notch2 Signaling in Pediatric High-Grade Gliomas (pHGGs). *Int J Mol Sci*. (2017) 18:2742. doi: 10.3390/ijms18122742

32. Calò PG, Erdas E, Medas F, Pisano G, Barbarossa M, Pomata M, et al. Late Bleeding after Total Thyroidectomy: Report of Two Cases occurring 13 Days after Operation. *Clin Med Insights Case Rep*. (2013) 6:165–70. doi: 10.4137/CCRep.S13024

33. Calò PG, Tuveri M, Pisano G, Tatti A, Medas F, Donati M, et al. Il gozzo recidivo. Nostra esperienza [Recurrent goitre: our experience. *Chir Ital*. (2009) 61:545–9.

34. Wei S, LiVolsi VA, Montone KT, Morrissette JJ, Baloch ZW. Detection of molecular alterations in medullary thyroid carcinoma using next-generation sequencing: an institutional experience. *Endocr Pathol*. (2016) 27:359–62. doi: 10.1007/s12022-016-9446-3

35. Mian C, Pennelli G, Fassan M, Balistreri M, Barollo S, Cavedon E, et al. MicroRNA profiles in familial and sporadic medullary thyroid carcinoma: preliminary relationships with RET status and outcome. *Thyroid*. (2012) 22:890–6. doi: 10.1089/thy.2012.0045

36. Puppin C, Durante C, Sponziello M, Verrienti A, Pecce V, Lavarone E, et al. Overexpression of genes involved in miRNA biogenesis in medullary thyroid carcinomas with RET mutation. *Endocrine*. (2014) 47:528–36. doi: 10.1007/s12020-014-0204-3

37. De Smaele E, Ferretti E, Gulino A. MicroRNAs as biomarkers for CNS cancer and other disorders. *Brain Res*. (2010) 1338:100–11. doi: 10.1016/j.brainres.2010.03.103

38. Macías M, Alegre E, Díaz-Lagares A, Patiño A, Pérez-Gracia JL, Sanmamed M, et al. Liquid biopsy: from basic research to clinical practice. *Adv Clin Chem*. (2018) 83:73–119. doi: 10.1016/bs.acc.2017.10.003

39. Sozzi G, Boeri M, Rossi M, Verri C, Suatoni P, Bravi F, et al. Clinical utility of a plasma-based miRNA signature classifier within computed tomography lung cancer screening: a correlative MILD trial study [published correction appears in J Clin Oncol. *J Clin Oncol*. (2014) 32:768–73. doi: 10.1200/JCO.2013.50.4357

40. Shi X, Li CW, Tan LC, Wen SS, Liao T, Zhang Y, et al. Immune co-inhibitory receptors PD-1, CTLA-4, TIM-3, LAG-3, and TIGIT in medullary thyroid cancers: A large cohort study. *J Clin Endocrinol Metab*. (2021) 106:120–32. doi: 10.1210/clinem/dgaa701

41. Pozdeyev N, Erickson TA, Zhang L, Ellison K, Rivard CJ, Sams S, et al. Comprehensive immune profiling of medullary thyroid cancer. *Thyroid*. (2020) 30:1263–79. doi: 10.1089/thy.2019.0604

42. Hińcza-Nowak K, Kowalik A, Walczyk A, Pałyga I, Gąsior-Perczak D, Płusa AV, et al. Immune profiling of medullary thyroid cancer-an opportunity for immunotherapy. *Genes (Basel)*. (2021) 12:1534. doi: 10.3390/genes12101534

43. Liu S, Liang J, Liu Z, Zhang C, Wang Y, Watson AH, et al. The role of CD276 in cancers. *Front Oncol*. (2021) 11:654684. doi: 10.3389/fonc.2021.654684

44. Crossman SH, Janovjak H. Light-activated receptor tyrosine kinases: Designs and applications. *Curr Opin Pharmacol*. (2022) 63:102197. doi: 10.1016/j.coph.2022.102197

45. Salokas K, Liu X, Öhman T, Chowdhury I, Gawriyski L, Keskitalo S, et al. Physical and functional interactome atlas of human receptor tyrosine kinases. *EMBO Rep*. (2022) 23:e54041. doi: 10.15252/embr.202154041

46. Tan A, Xia N, Gao F, Mo Z, Cao Y. Angiogenesis-inhibitors for metastatic thyroid cancer. *Cochrane Database Syst Rev*. (2010) 2010:CD007958. doi: 10.1002/14651858.CD007958.pub2

47. Rosen LS. Clinical experience with angiogenesis signaling inhibitors: focus on vascular endothelial growth factor (VEGF) blockers. *Cancer Control*. (2002) 9:36–44. doi: 10.1177/107327480200902S05

48. Capp C, Wajner SM, Siqueira DR, Brasil BA, Meurer L, Maia AL. Increased expression of vascular endothelial growth factor and its receptors, VEGFR-1 and VEGFR-2, in medullary thyroid carcinoma. *Thyroid*. (2010) 20:863–71. doi: 10.1089/thy.2009.0417

49. Zheng X, Ji Q, Sun Y, Ge M, Zhang B, Cheng Y, et al. Efficacy and safety of selpercatinib in Chinese patients with advanced RET-altered thyroid cancers: results from the phase II LIBRETTO-321 study. *Ther Adv Med Oncol*. (2022) 14:17588359221119318. doi: 10.1177/17588359221119318

50. Hadoux J, Elisei R, Brose MS, Hoff AO, Robinson BG, Gao M, et al. Phase 3 trial of selpercatinib in advanced RET-mutant medullary thyroid cancer. *N Engl J Med*. (2023) 389:1851–61. doi: 10.1056/NEJMoa2309719

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 31 October 2024
DOI 10.3389/fonc.2024.1448687



OPEN ACCESS

EDITED BY
Min Tu,
Nanjing Medical University, China

REVIEWED BY
Loredana Mauro,
University of Calabria, Italy
Sreeram Vallabhaneni,
Harvard Medical School, United States

*CORRESPONDENCE
Lihua Kang
Kanglh@jlu.edu.cn

RECEIVED 14 June 2024
ACCEPTED 09 October 2024
PUBLISHED 31 October 2024

CITATION
Gao Y, Yu Y, Zhang M, Yu W and Kang L (2024) Mechanisms of endocrine resistance in hormone receptor-positive breast cancer. *Front. Oncol.* 14:1448687. doi: 10.3389/fonc.2024.1448687



# Mechanisms of endocrine resistance in hormone receptor-positive breast cancer

Yuan Gao, Yang Yu, Mingqing Zhang, Wenjun Yu and Lihua Kang*

Cancer Center, The First Hospital of Jilin University, Changchun, Jilin, China

Hormone receptor-positive breast cancer may recur or metastasize years or decades after its diagnosis. Furthermore, hormone receptor expression may persist in relapsed or metastatic cancer cells. Endocrine therapy is one of the most efficacious treatments for hormone receptor-positive breast cancers. Nevertheless, a considerable proportion of patients develop resistance to endocrine therapy. Previous studies have identified numerous mechanisms underlying drug resistance, such as epigenetic abnormalities in the estrogen receptor (ER) genome, activation of ER-independent ligands, and alterations in signaling pathways including PI3K/AKT/mTOR, Notch, NF-κB, FGFR, and IRE1-XBP1. This article reviews the mechanisms of endocrine resistance in hormone receptor-positive advanced breast cancer, drawing from previous studies, and discusses the latest research advancements and prospects.

KEYWORDS

**breast cancer, hormone receptor-positive, endocrine treatment, resistance, mechanisms**

## 1 Introduction

Statistics from the Global Cancer Research Institute indicate that in 2020, female breast cancer surpassed lung cancer as the most common malignant tumor, with approximately 2.3 million new cases diagnosed annually, and approximately 700,000 women dying from the disease each year (1). In 2013, the St. Gallen International Breast Cancer Conference released its pathological molecular classification, which can be divided into Luminal A, Luminal B, HER-2 overexpression, and basal-like subtypes. Luminal A is hormone-sensitive, effective for endocrine therapy, and has a better prognosis than the other types (2). Approximately 70% of breast cancers express estrogen receptors (3). Estrogen is a steroid hormone that binds to receptors besides its impact on the reproductive system. It also exerts effects on other aspects of its physiological role, including cardiovascular, water, and salt metabolism, as well as the central and motor systems (4). Traditional endocrine therapies include selective estrogen receptor (ER) modulators (SERMs), selective ER degraders (SERDs), and aromatase inhibitors (AIs). The treatment strategy involves a blockade of the biological functions of estrogen and estrogen receptors.

This blockade provides a significant survival benefit for such patients. Endocrine therapy has demonstrated an effective rate of 40–80% in patients with hormone receptor-positive disease (5). Although approximately 30% of patients with early breast cancer respond to endocrine therapy, subsequent drug resistance is inevitable, and approximately 50% of patients with advanced breast cancer and metastasis do not respond to endocrine therapy (6). Endocrine therapy remains a significant challenge in patients who can overcome resistance. Primary endocrine resistance refers to recurrence and metastasis within 24 months of adjuvant endocrine therapy, or disease progression within 6 months of first-line endocrine therapy for metastatic breast cancer (7). Secondary endocrine resistance refers to other endocrine resistance conditions that do not conform to primary endocrine resistance (7). Endocrine drug resistance results from the interplay between multiple mechanisms. This review primarily focuses on the current nature or possible mechanisms and recent progress in drug resistance.

## 2 Estrogen receptor and ESR1

ERα and ERβ are expressed in numerous tissues, including the uterus, ovary, breast, prostate, lung, and brain (8). The DNA-binding domains (DBD) of ERα and ERβ exhibit 96% homology, whereas the ligand-binding domain (LBD) displays 53% sequence similarity (8). The primary distinction lies in their respective N-terminal hormone-independent transcriptional activation (AF-1) domains (8). In the context of breast cancer, ERα represents the primary manifestation and can be activated by 17-β-E2, which plays a pivotal role in regulating cell growth, proliferation, and migration, as well as other biological functions. ERβ is likely to be a protective factor that inhibits cell proliferation and plays an antitumor role (9). ERα is a 66 kDa ligand-dependent transcription factor composed of 595 amino acids, including one central DNA-binding region, one ligand-binding region, and two trans-active domains (10) (Figure 1). The A/B domain, at the amino terminus, encompasses the ligand-independent activation region AF-1, which is regulated by phosphorylation (11). The C domain is responsible for binding to estrogen response elements (ERE) and the DNA sequence of the target gene (11). The D-domain is a hinge region that contributes to the specificity and nuclear localization of DNA-binding (12). The E domain represents the ligand-dependent activation region of the LBD and the AF-2 region, and is regulated by estrogen or SERMs (11). The C-terminal helix H12 in the LBD is a pivotal component of AF-2 cleavage and determines the agonist or antagonist status of the receptor (13). The F domain was identified at the carboxyl-terminal end. The ER is activated to regulate the expression of numerous genes by directly binding to EREs within the nuclear genome or interacting with other transcription factors (11).

ERα and ERβ are located on different chromosomes and are encoded by ESR1 and ESR2, respectively. Mutations in these genes appear to be one of the main mechanisms underlying secondary endocrine resistance (14). In approximately 30% of metastatic hormone receptor-positive breast cancers, ESR1 mutations enhance the active conformational stability of ERα, particularly in patients who have been treated with AIs (15). An ESR1 mutation is an acquired mutation that results in ligand-independent ER activation (16). Studies have reported that mutations in the LBD region of ESR1 can be detected by next-generation sequencing in histological specimens of recurrent and metastatic hormone receptor-positive breast cancer (17, 18). The most common types of point mutations include the D538G (15%-20%), Y537S (5%-10%), and E380Q (5%-10%) mutations, which are in the LBD region of ERα (19, 20). Both the D538G and Y537S point mutations alter the H11-12 ring in the LBD region of ERα. The altered spatial conformation of H11-12 results in a structure that is more similar to that of the wild-type ERα-E2 complex, which maintains the receptor in an excited state. This pattern simulates the activated ligand-binding receptor pattern and blocks the binding of SERMs or SERDs to the receptor (21). These mutations result in structural changes at the protein level, which leads to a reduction in ligand affinity for the receptor-binding domain (21, 22). Keren Merenbakh-Lamin et al. conducted genetic analysis on tumor samples from 13 patients with metastatic breast cancer and examined the capacity of 538G-ERα to stimulate MCF-7 cell proliferation (23). The results demonstrated that compared to WT-ERα, 538G-ERα exhibited a 33% increase in cell proliferation in the untreated group and a 28% increase in the E2-treated group. Furthermore, 538G-ERα is more prone to distant metastasis (23). Previous studies have demonstrated that D538G and Y537S mutation models induced by doxycycline promote tumor metastasis. However, tumor cells in metastatic foci retreat after the inducer is withdrawn, which indirectly indicates that tumor metastasis is caused by mutations (24). Other studies have demonstrated elevated TCA activity in

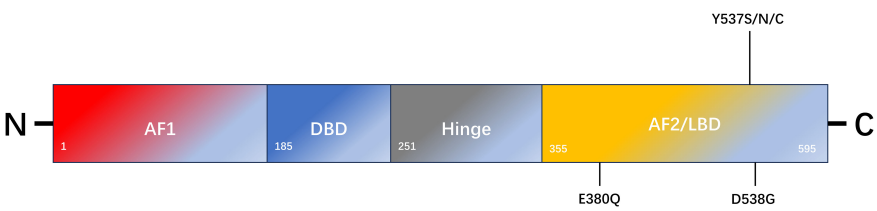



FIGURE 1
Schematic representation of ESR1 encoding ERα and the most common mutation sites of endocrine resistance. ERα comprises 595 amino acids. The structural domains of ERα include the transcription activation function 1 domain (AF1), DNA-binding domain (DBD), ligand-binding domain (LBD), AF2 domain, and flexible-hinge domain.

537S-ERα mutants, which are not only glucose dependent but also use glutamine as an alternative carbon source compared to WT-ERα cells, which are primarily glucose dependent (25). Consequently, the mutants exhibited a heightened biological capacity to invade and metastasize, which may account for the prevalence of ESR1 mutations in metastatic breast cancer but not in early breast cancer. The Paloma-2 trial demonstrated that patients continued to accumulate the Y537S mutation throughout treatment with fulvestrant alone or combined with Palbociclib (26). In the Paloma-3 trial, patients with the Y537S mutation were treated with fulvestrant. The results demonstrated that the Y537S mutation had a worse clinical outcome than the D538G mutation (26). The combination of AI and mTOR inhibitors administered to patients with the D538G mutation has been shown to result in more favorable therapeutic outcomes than the Y537S mutation (20). In contrast, Jeselsohn et al. demonstrated that SERM or SERD treatment of the Y537S mutant exhibited a more pronounced anti-growth inhibition effect than that of the D538G mutant and wild-type ERα (24). This study revealed that cell lines treated with SERM or SERD for an extended period did not develop ESR1 mutations, whereas most mutations occurred during the withdrawal of AI drugs (24, 27). Spoerke et al. examined ESR1 mutants in 37% (57/153) of ctDNAs from patients before and after the progression of AI drug use using liquid biopsy (28). The researchers compared ctDNA with matched tumor tissue data and found that ESR1 mutations (0/81), (3/31), and (12/19) were present in tumor tissues collected at the initial diagnosis, before AI treatment, and after AI treatment progression, respectively. Furthermore, the content of ESR1 mutations in ctDNA is often higher than that in matched tumor tissues (28). Survival analysis revealed that the overall survival rate of individuals harboring the Y537S or D538G mutant was lower than individuals harboring the wild-type form of ERα (20.7 months vs. 32.1 months) (29). Nevertheless, preclinical studies indicate these mutations elicit disparate responses to SERMs and SERDs. For instance, they exhibit reduced sensitivity to fulvestrant, although this depends on dosage (30).

Transcriptional regulatory nucleoprotein 1 (NUPR1, P8, and COM-1) is a transcription co-regulatory factor induced by tamoxifen (TAM) in a time- and dose-dependent manner. Wang et al. demonstrated that NUPR1 can bind to ESR1 and regulate the transcription of BECN1, GREB1, RAB31, PGR, CYP1B1, and other genes involved in autophagy and drug resistance. Furthermore, they observed that the level of NUPR1 was significantly elevated in TAMR cells, and that its expression level was significantly correlated with postoperative survival time (31).

Gene rearrangement is a pivotal driver of a multitude of solid tumors (32). Similarly, in advanced hormone receptor-positive breast cancer, the fusion of non-coding promoter genes is a factor in relapse resistance (33). In 2018, Hartmaier et al. applied a novel algorithm to target sequencing genome structure rearrangement (RES) in three breast cancer cell lines and identified gene fusion transcripts using DNA pairing and RNA sequencing. The intra-frame fusion transcripts ESR1-DAB2 and ESR1-GYG1 were found in patients with supraclavicular lymph node metastasis and bone metastasis, respectively (34). Subsequently, the ctDNA of 9,542 breast cancer patients was subjected to further analysis, which revealed other concentrated gene fusion transcripts and transcripts with higher abundance in ER-positive metastatic breast cancer. Researchers postulated that at least 1% of MBC cases were associated with ESR1 gene fusion, with a 10-fold increase in ctDNA (34). To ascertain its function, further analysis of ESR1-DAB2, ESR1-GYG1, and ESR1-SOX9 fusion genes revealed that they all exhibited ligand-independent characteristics. Compared to the wild-type, ER with ESR1-DAB2 and ESR1-SOX9 fusion genes exhibited tenfold greater activity, whereas the ER activity of the mutant with LBD deletion was lower than the wild-type. However, these activities are independent of ligands (34). Elimination of the LBD region by the ESR1 fusion protein, which carries multiple 3' chaperone genes, may be the mechanism underlying endocrine relapse drug resistance. Furthermore, drug therapies for ER are insensitive.

## 3 Cell cycle pathway: CDK4/6

Cyclin-dependent kinase 4/6 (CDK4/6) has been identified as a key driver of ER-positive breast cancer growth and proliferation (35). Amplification and overexpression of cyclin D1 (encoded by CCND) are common in breast malignancies, with a particularly high prevalence in the Luminal A (29%) and Luminal B (58%) subtypes. In contrast, CDK4 is amplified in Luminal A (14%) and Luminal B (58%) subtypes (36). CyclinD1 binds to CDK4/6 to form a holoenzyme complex. In this process, the KIP/CIP protein (encoded by CDKN1) is required to assist in the assembly of the complex, whereas KIP/CIP inhibits the CDK1/2 complex (37). The holoenzyme complex phosphorylates a subset of the retinoblastoma protein (Rb) family, including P107 (encoded by RBL1), P110 (encoded by RB1), and P130 (encoded by RB2) during the G1 phase. The CDK2-cyclin complex then phosphorylates Rb (38), releasing the E2F transcription factor and inducing cyclin E (encoded by CCNE) to form a complex with other CDK1-3, which induces a series of biological reactions. Concurrently, the cyclin-CDK4/6 complex can directly phosphorylate the transcription factor FOXM1 (39), facilitating the transition of cells from G1 to S phase (40, 41) (Figure 2).

The activity of CDK is regulated by endogenous inhibitors; however, it requires the involvement of fully functional Rb proteins rather than incomplete or disabled Rb proteins (42). CDK inhibitors belong to the CDK-interacting protein/kinase inhibitor (CIP/KIP) family, which exerts both activating and inhibitory effects and influences the activity of cyclin-CDK complexes (43). This family of proteins includes p21CIP1, p27KIP1, and p57KIP2 (43). They are inhibitors of CDK2, both *in vitro* and under conditions of cell growth arrest (44). They are essentially disordered proteins that fold sequentially into cyclin and CDK to form a complex (45). Mice lacking p21 or p27 are susceptible to tumor formation (46, 47). Some studies have demonstrated that low expression of P21 can facilitate the formation of the CDK4 complex, whereas high expression of P21 exerts an inhibitory effect (48). Guiley et al. demonstrated that p27 allosterically activates the CDK4-Cyclin complex through a remodeling kinase. The recombinant CDK4-cyclinD complex containing p27 activity is insensitive to inhibitors such as

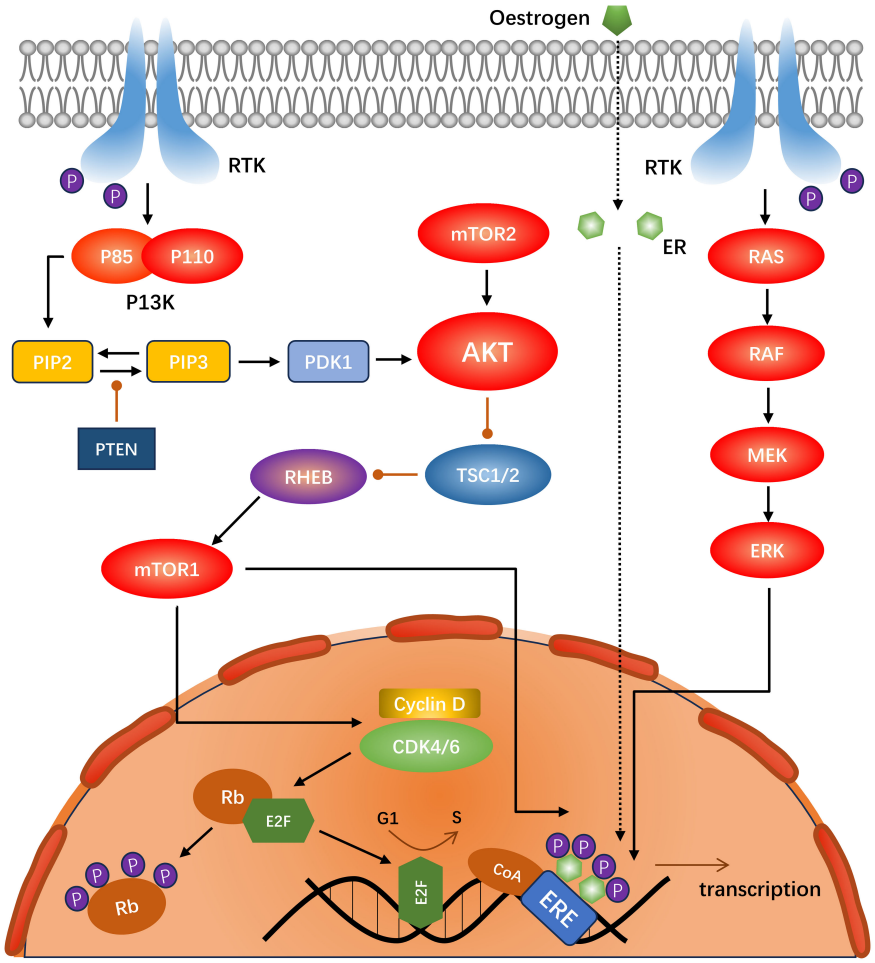



FIGURE 2
Schematic of the interactions between the PI3K-Akt-mTOR, RAS-RAF-MEK-ERK, and CDK4/6 pathways in ER-positive, HER2-negative breast cancer.

Palbociclib, whereas p21 exhibits relatively low activity (49). The INK4 family of proteins, including p16INK4A, p14ARF, p15INK4B, p18INK4C, and p19INK4D, specifically interacts with the catalytic domain of CDK4/6. This process is initiated by inhibition of the binding of the aforementioned proteins to cyclin D, which inhibits the kinase activity of CDK4/6 (43). This results in the release of E2F and subsequent cell cycle arrest in the G1 phase (50). In HR+ breast cancer, there is often concomitant inactivation of RB1, amplification of cyclinD1 gene, and inactivation of CDKN2. However, RB1 inactivation is rare (51). Loss of RB1 may be a mechanism of resistance to CDK4/6 inhibitors in tumors that have lost RB1 function. Furthermore, alterations in the overexpression of cyclin D or cyclin E are expected to reduce therapeutic responsiveness in tumors that retain functional Rb (52). The loss of RB1 function and high levels of CCNE1 expression resulted in a decrease in ESR1 and PRG expression levels and hormone-dependent reactivity, which demonstrates that RB1 status is related to the growth and proliferation of hormone-dependent tumor cells (53, 54). Cyclin D levels are regulated by multiple cellular signaling pathways and can also be involved in cell cycle regulation as independent kinases, such as interactions with hormone receptors and transcription factors (55). Studies have demonstrated that the expression level of cyclin D1 is elevated in breast cancer stromal cells, prompting fibroblasts to secrete pro-inflammatory factors and osteopontin, facilitating tumorigenesis (56). The inhibitory protein encoded by CDKN2 competitively binds to CDK4/6 and induces conformational changes. Inactivation of the inhibitory gene results in the indirect enhancement of CDK4/6 activity (41), increasing the sensitivity threshold of cancer cells to CDK4/6 inhibitors, which induces drug resistance (57). Several resistances mechanisms have been implicated, with hormone-dependent cell signaling being the most susceptible to CDK4/6 inhibitors (51). CDKN1 has both inhibitory and activating effects on CDK4/6 cells. Previous studies have indicated that loss of ERα expression and ESR1 gene mutations are frequently found in drug-resistant tissues treated with CDK4/6 inhibitors combined with anti-estrogen drugs (58). However, these studies also suggested that the sensitivity of tumors with endocrine resistance to CDK4/6 is not related to ESR1 status (59).

CDK4/6 inhibitors play a positive role in tumor immunity. Goel et al. identified a mechanism related to the involvement of CDK4/6 inhibitors in immune evasion, which enhances antitumor immunity (60). The expression of HLA in CCND1-amplified tumor cells was upregulated by CDK4/6 inhibitors, which also activated the expression of retroviral elements in tumor cells treated with

Abemaciclib. This results in increased dsRNA levels, driving interferon-stimulating gene (ISG) production, increasing the production of type III IFN, upregulating IL-2 to inhibit Tregs, and decreasing the number of peripheral Tregs and Treg/CD8+ ratio (60, 61). These regulatory methods are independent of tumorigenesis and enhance the ability of antigen-presenting and tumor-killing CD8+ T cells. The combination of Abemaciclib with anti-PD-L1 demonstrated enhanced immune effects, including dendritic cell maturation, cytokine activation, Th1/2 pathway activation, antigen presentation, and cell enhancement (60, 62).

Some alterations in the targets of the P13K-AKT/RAS-ERK signaling pathway are related to the resistance to CDK4/6 inhibitors. Amplification and mutation of AKT1 and AKT3 in the AKT pathway represent one such pathway, whereas PIK3CA mutation does not constitute a mechanism of drug resistance. The combination of CDK4/6 and PI3K inhibitors resulted in the regression of breast cancer grafts with PIK3CA mutations, and the combination of PI3K, AKT, and mTORC1/2 inhibitors demonstrated increased efficacy in multiple preclinical models of breast cancer. Wander et al. performed whole-exome sequencing of 59 samples and identified genomic alterations associated with hormone receptor-positive breast cancer, including 27 (65.9%) of 41 CDK4/6 inhibitor-resistant biopsies. In 9% of 41 CDK4/6 inhibitor-resistant biopsies, at least one of the following eight changes was observed: activating mutations and/or amplification of AKT1, KRAS/HRAS/NRAS, FGFR2, and ERBB2; amplification of CCNE2 or AURKA; biallelic disruption of RB1; and loss of ER (63). These changes were still present in small amounts in the susceptible cohort but were less abundant than in the resistant cohort (63). These novel findings provide insights into the potential mechanisms underlying CDK4/6 resistance.

CDK4/6 inhibitors (including Palbociclib, Ribociclib, and Abemaciclib) can block the cell cycle by inhibiting downstream signaling. Both domestic and foreign guidelines to treat hormone receptor-positive breast cancer recommend a CDK4/6 inhibitor combined with endocrine therapy as the preferred initial treatment for patients with luminal-type breast cancer following the development of endocrine therapy. A summary of clinical studies evaluating CDK4/6 inhibitors in HR-positive/HER2-negative breast cancers is shown in Table 1.

According to the findings of the PALOMA-1 clinical study, the Food and Drug Administration (FDA) approved Palbociclib in February 2015 (57). Subsequently, in a Phase III clinical trial of a CDK4/6 inhibitor combined with an aromatase inhibitor, Palbociclib, Ribociclib, and Abemaciclib demonstrated superior clinical efficacy in progression-free survival (PFS), establishing the first-line treatment

TABLE 1 Summary of randomized phase II/III clinical trials evaluating CDK4/6 inhibitors in HR-positive/HER2-negative Advanced or Metastatic BC (36).

| Trial Name | Phase | Population | Treatment Arms | Sample Size | Primary Outcome (Exp vs. Ctrl Arm) HR (95% CI) |
|---|---|---|---|---|---|
| MONALEESA-2 | III | AI-sensitive postmenopausal women with HR-positive/HER2-negative advanced or metastatic BC; no previous systemic therapy for ABC | Ribociclib + Letrozole | | PFS 25.3 vs. 16 months |
| | | | vs. | 668 | (HR 0.568; 95% CI |
| | | | Letrozole + Placebo | | 0.457–0.704) |
| MONALEESA-3 | III | AI-sensitive/resistant postmenopausal women with HR-positive/HER2-negative advanced or metastatic BC; 0-1 line of ET for ABC | Ribociclib + Fulvestrant | | PFS 20.5 vs. 12.8 months |
| | | | vs. | 726 | (HR 0.593; 95% CI |
| | | | Fulvestrant + Placebo | | 0.480–0.732) |
| MONALEESA-7 | III | AI-sensitive peri/premenopausal women with HR-positive/HER2-negative advanced or metastatic BC; no previous ET and up to 1 line of CT for ABC | Ribociclib + TAM/NSAI | | PFS 23.8 vs. 13.3 months |
| | | | vs. | 672 | (HR 0.553: 95% CI |
| | | | TAM or NSAI + Placebo | | 0.441–0.694) |
| MONARCH-2 | III | AI-resistant pre/postmenopausal women with HR-positive/HER2-negative advanced BC that progressed after ET; no previous CT for ABC | Abemaciclib + Fulvestrant | | PFS 16.4 vs. 9.3 months |
| | | | vs. | 669 | (HR 0.553; 95% CI |
| | | | Placebo + Fulvestrant | | 0.449–0.681) |
| MONARCH-3 | III | AI-sensitive postmenopausal women with HR-positive/HER2-negative advanced or metastatic BC; no previous systemic therapy for ABC | Abemaciclib + NSAI | | PFS 28.1 vs. 14.7 months |
| | | | vs. | 493 | (HR 0.540; CI |
| | | | Placebo + NSAI | | 0.418–0.698) |

*(Continued)*

TABLE 1 Continued

| Trial Name | Phase | Population | Treatment Arms | Sample Size | Primary Outcome (Exp vs. Ctrl Arm) HR (95% CI) |
|---|---|---|---|---|---|
| PALOMA-1/TRIO-18 | II | AI-sensitive postmenopausal women with HR-positive/HER2-negative advanced or metastatic BC; no previous systemic therapy for ABC | Palbociclib + Letrozole | | PFS 20.2 vs. 10.2 months |
| | | | vs. | 165 | (HR 0.488; 95% CI |
| | | | Letrozole | | 0.319–0.748) |
| PALOMA-2 | III | AI-sensitive postmenopausal women with HR-positive/HER2-negative advanced or metastatic BC; no previous systemic therapy for ABC | Palbociclib + Letrozole | | PFS 27.6 vs. 14.5 months |
| | | | vs. | 666 | (HR 0.563; 95% CI |
| | | | Letrozole | | 0.461–0.687) |
| PALOMA-3 | III | AI-resistant pre/postmenopausal women with HR-positive/HER2-negative advanced or metastatic breast cancer that progressed after ET | Palbociclib + Fulvestrant | | PFS 9.5 vs. 4.6 months (HR 0.46; 95% CI 0.36–0.59) |
| | | | vs. | 521 | |
| | | | Fulvestrant + Placebo | | |
| PEARL | III | AI-resistant postmenopausal women with HR-positive, HER2-negative metastatic BC | Palbociclib + ET | | PFS 7.5 vs. 10 months (HR 1.09; 95% CI 0.83-1.44) |
| | | | vs. | 601 | |
| | | | Capecitabine | | |
| MAINTAIN | II | Pre/postmenopausal women or men with HR-positive/ HER2-negative advanced or metastatic BC who have progressed on an AI plus a CDK4/6 inhibitor (either Palbociclib or Ribociclib) | Palbociclib or Ribociclib) vs. Fulvestrant + Placebo | 119 | PFS 5.29 vs. 2.76 months (HR 0.57; 95% CI 0.39–0.85) |
| PACE | II | Pre/postmenopausal women or men with HR-positive/ HER2-negative advanced or metastatic BC who have progressed on an ET plus a CDK4/6 inhibitor and up to 1 line of CT for ABC | Fulvestrant vs. | 220 | PFS 4.8 vs. 4.6 (HR 1.11; 95% CI 0.79-1.55) vs. 8.1 months (HR 0.75; 95% CI 0.50-1.12) |
| | | | Palbociclib + Fulvestrant | | |
| | | | vs. | | |
| | | | Palbociclib + Fulvestrant + | | |
| | | | Avelumab | | |

Exp, experimental; Ctrl, control; HR, hazard ratio; CI, confidence interval; AI, aromatase inhibitor; HR, hormone receptor; HER2, human epidermal growth factor 2; BC, breast cancer; ABC, advanced breast cancer; PFS, progression-free survival; ET, endocrine therapy; CT, chemotherapy; TAM, tamoxifen; NSAI, nonsteroidal aromatase inhibitors.

status of AI combined with CDK4/6. The Phase III clinical PALOMA-2 study demonstrated that the PFS in patients treated with Palbociclib plus letrozole was significantly longer than in those treated with letrozole alone (24.8 months vs. 14.5 months; hazard ratio (HR) = 0.56; $P < 0.001$). Furthermore, the objective response rate (ORR) was higher in the combination therapy group (55.3% vs. 44.4%) (64, 65). The Phase III MONALEESA-2 evaluation of first-line Ribociclib + letrozole demonstrated that the median PFS in the Ribociclib + letrozole group was significantly longer than that in the placebo group (25.3 months vs. 16.0 months; HR = 0.57; $P < 0.001$), indicating an improvement (66). The MONARCH 3 clinical study demonstrated that the combination of Abemaciclib and AI significantly prolonged PFS in patients with advanced breast cancer who had not previously received systemic therapy (28.2 months vs. 14.8 months; HR = 0.54; $P < 0.001$) and ORR (61% vs. 46%) (67).

# 4 Cell signaling pathways

## 4.1 PI3K-AKT-mTOR

The PI3K-AKT-mTOR pathway plays a pivotal role in the regulation of numerous physiological processes. It is also one of the most prevalent signal transduction pathways in malignant tumors, as evidenced by numerous studies (68). PI3K (encoded by PIK3CA), a dimer composed of the regulatory subunit P85 and catalytic subunit P110, is activated by the receptor tyrosine kinases (RTKs) and GPCRs (69, 70), which phosphorylate PIP2 to PIP3. This process is inhibited by the negative regulator PTEN, resulting in an increased intracellular PIP3 concentration (71). This prompts PDK1 to phosphorylate threonine on AKT (depending on mTORC2) (71). Activation of AKT inhibits dimerization of Tuberous Sclerosis Complex 1/2

(TSC1/2), a negative regulator of mTORC1. Consequently, AKT activates mTORC1 indirectly (70). The principal nodes in the P13K-AKT-mTOR signaling pathway are shown in Figure 2.

PIK3CA mutations are present in 20% to 50% of patients with breast cancer, including 35% of hormone receptor-positive patients. In contrast, AKT and PTEN mutations are more common in hormone-receptor-positive patients (72). PIK3CA mutations are primarily observed in specific regions within exons 9 and 20, which encode PI3K helix and kinase domains, respectively (73). The mutation of exon 9 enables P110 α to circumvent the inhibitory function of P85 through the SH2 domain; however, the mechanism of exon 20 remains unclear (74). Loss of PTEN protein is more prevalent than loss of PTEN mutations in patients with breast cancer (75, 76). Stemke-Hale et al. demonstrated that the AKT1-E17K mutation is restricted to breast cancers expressing both ER and PR, and confirmed that AKT1-E17K, PIK3CA, and PTEN mutations are mutually exclusive in breast cancer cell lines, similarly to other tumor types (72). However, PTEN loss and PIK3CA mutations were not mutually exclusive, which is consistent with previous results (77). PIK3CA mutation is associated with high expression of AKT1 and cyclinD1, whereas PIK3CA, AKT mutation, and PTEN loss seem associated with a favorable clinical prognosis (72, 77). In wild-type PIK3CA, the loss of PTEN protein was associated with increased AKT activation, whereas PIK3CA mutation was not significantly associated with the phosphorylation of PTEN protein or its downstream substrate (72). However, PIK3CA mutations were not significantly associated with prognosis in patients with ERα-positive breast cancer treated with tamoxifen (72, 77). Furthermore, Wander et al. observed alterations in PIK3CA in both sensitive and drug-resistant hormone receptor-positive breast cancer biopsies, suggesting that PIK3CA is unlikely to cause drug resistance (63). Despite the absence of evidence that PTEN loss causes PI3K activation, Stommel et al. found that RTK inhibitors can downregulate AKT, suggesting that AKT activation may be associated with the absence of PTEN (78). In a previous retrospective study conducted by Tokunaga et al., endocrine therapy was significantly less effective in AKT-negative patients ($P < 0.01$) than in 12 AKT-positive patients (33.4%) (79), suggesting that AKT activation is associated with poor clinical outcomes. Both PI3K and mTOR belong to the PI3K-related kinase (PIKK) superfamily and share similar domains, which allows for the simultaneous targeting of these two kinases by some inhibitory drugs (70). Previous studies have also observed that the P13K-AKT-mTOR pathway is activated in MCF-7 cells with stable 537S-ER expression or transient 538G-ER expression and that phosphorylation of AKT, mTOR, and downstream S6K is enhanced (25). This is a downstream marker of mTOR activation and predicts lower survival in breast cancer patients with high expression of hormone receptor-positive breast cancer undergoing endocrine therapy (80). The combination of mTOR and AIs has been the standard of care for ER+ advanced breast cancer; however, this treatment has not demonstrated an improvement in survival in clinical trials (81). In addition, approximately 23% of breast cancers exhibit a loss of PTPN12 protein, which causes a loss of the ability to downregulate growth factor signal transduction and predicts poor prognosis (82).

The objective was to target mutated genes in the PI3K-AKT-mTOR signaling pathway, including the PI3K inhibitors alpelisib and inavolisib, and the AKT inhibitors ipatasertib and capivasertib. Although these drug studies have demonstrated a therapeutic effect on endocrine-resistant breast cancer, alpelisib is only suitable for patients with PIK3CA mutations and has not yet been approved in China. In contrast, capivasertib has not been approved for domestic or foreign use.

The BOLERO-2 and PrE 0102 studies demonstrated the clinical efficacy of a second-line combination regimen based on evolimus (83). A total of 724 patients were included in the international multicenter Phase III clinical study BOLERO-2, which opened a new treatment window for patients with endocrine-resistant breast cancer. The results demonstrated that the combination of everolimus and exemestane prolonged the median PFS in postmenopausal patients with late-stage HR-positive/HER2-negative breast cancer who relapsed or progressed after AI therapy; the HR = 0.45 (84). In BOLERO-2, the median PFS was longer in the everolimus plus exemestane group than in the exemestane alone group (7.4 months vs 2.0 months; HR = 0.52). This study provides a new strategy for postmenopausal patients with ER-positive/HER2-negative breast cancer (85). Based on the established efficacy of CDK4/6 inhibitors as second-line treatments, the TRINITI-1 study sought to evaluate the efficacy of a combination of exemestane, Ribociclib, and everolimus in patients with HR-positive/HER2-negative advanced breast cancer who progressed after CDK4/6 inhibitor treatment. The clinical benefit rate of the three-drug combination regimen at the end of 24 weeks was 41.1%, and the overall population median PFS was 5.7 months (86). The MIRACLE study included 199 domestic, multicenter patients with breast cancer. The results demonstrated that the ORR of the combined group treated with evolimus was 50.0% and 39.3%, respectively, compared to the ORR of the letrozole group. The median PFS was 19.4 months for the combined group and 12.9 months for the letrozole group, respectively (87). Table 2 lists inhibitors designed to target the P13K-AKT-mTOR pathway (88).

## 4.2 Notch

The Notch pathway is highly conserved and activated by receptor-mediated activation through signal-sending and signal-receiving cells (89). In the Notch signaling pathway, the cells that initiate the signaling process, referred to as "signaling-sending cells," express five ligands for the Notch receptor, whereas the cells that receive the signal, or "signaling-receiving cells," express four receptors for the Notch ligand in proximity to each other. The classic Notch signaling pathway is intimately associated with a multitude of biological functions in cancer cells (90). Upon ligand-mediated activation of the Notch receptor by a signaling cell, the extracellular domain of the Notch receptor (NotchEC) is endocytosed into the signaling cell, accompanied by the Notch ligand. The transmembrane domain (NotCHIC-TM) of the extracellular domain of the signaling recipient cell is cleaved by ADAM twice. Subsequently, the Notch extracellular domain is

TABLE 2 P13K-AKT-mTOR pathway potency (88).

| Inhibitor | Drug | | Target |
|---|---|---|---|
| Pan-class I PI3K inhibitors | | | |
| | Buparlisib | BKM120 | Pan-PI3K |
| | Pictilisib | GDC-0941 | Pan-PI3K |
| | Copanlisib | BAY 80-6946 | Pan-PI3K |
| | SAR245408 | XL147 | Pan-PI3K |
| | PX-866 | | Pan-PI3K |
| Isoform-specific PI3K inhibitors | | | |
| | Taselisib | GDC-0032 | p110α |
| | Alpelisib | BYL719 | p110α |
| | MLN1117 | | p110α |
| | BAY 1082439 | | p110α/β |
| | CH5132799 | | PI3Kα/γ |
| | GSK2636771 | | p110β |
| | AZD8186 | | p110β |
| | SAR260301 | | p110β |
| | Idelalisib | CAL-101 | p110δ |
| | IPI-145 | | p110δ |
| | AMG319 | | p110δ |
| Dual-specificity PI3K/mTOR inhibitors | | | |
| | BEZ235 | | PI3K/mTOR |
| | GDC-0980 | | PI3K/mTOR |
| | PF-05212384 | | PI3K/mTOR |
| | PF-04691502 | | PI3K/mTOR |
| | GSK-2126458 | | PI3K/mTOR |
| | SAR245409 | XL765 | PI3K/mTOR |
| mTOR inhibitors, rapalogs | | | |
| | Sirolimus | rapamycin | mTOR |
| | Nab-rapamycin | | mTOR |
| | Temsirolimus | | mTOR |
| | Everolimus | | mTOR |
| | Ridaforolimus | | mTOR |
| mTOR inhibitors, catalytic | | | |
| | OSI-027 | | mTOR |
| | AZD2014 | | mTOR |
| | MLN0128 | | mTOR |
| | PP242 | | mTOR |
| | ML-223 | | mTOR |

cleaved by the gamma secretase complex (GIS) to generate NotchIC. Subsequently, it combines with the transcription activators CSL and MAML1 to form the NotCHIC-MamL-CSL complex, initiating the transcription of Notch signaling target genes. Previous studies by Hao et al. demonstrated that Notch 1 can promote the expression of ERα target genes, including VEGFA, CD44, cyclinD1, C-myC, and PS2, in an E2-deficient culture medium (91). Notch3 plays a pivotal role in regulating ERα expression. Xiao-Wei Dou et al. demonstrated that Notch3 enhances ERα expression by binding to CSL elements within ERα promoters in cell lines. Furthermore, they observed a reduction in ERα gene and protein levels in McF-7 and T47D cells following Notch3 silencing (92). Notch signaling also plays a pivotal role in cancer stem cells. In a separate study, Sansone et al. demonstrated that inhibition of the IL6R/IL6-Notch3 pathway could restore ERα expression and render CD133hiCSCs dependent on ERα instead of the IL6/Notch3-Jagged1 pathway (93). Notch4 is inhibited when ERα activates target genes through classical e2-dependent pathways. Consequently, the Notch signaling pathway is activated when ER expression is downregulated or ER signaling pathway is inhibited (94). Furthermore, the Notch signaling pathway plays a pivotal role in tumor epithelial-mesenchymal transition (EMT). In an experiment conducted by Bui et al., high expression levels of mesenchymal marker proteins were observed in TAMR-MCF-7 cells, demonstrating that Notch4/STAT3 crosstalk plays an important role (95). Moreover, the Notch pathway is also associated with ESR1 mutations. Gelsomino et al. investigated the common ESR1 mutant types Y537S, Y537N, and D538G, and detected high expression of relevant molecules in the Notch signaling pathway in the mutants compared to the wild-type (96). These findings indicated that ESR1 mutations may contribute to drug resistance in cell lines by modulating the ER/Notch pathway.

## 4.3 NF-κB

Nuclear transcription factor kappa B (NF-κB) plays a pivotal role in endocrine drug resistance in breast cancer. Under normal conditions, NF-κB binds to its inhibitor IκBα to form homodimers or heterodimers. The classical activation pathway involves the action of inflammatory factors such as IL-1β and TNF-α, which initially activate TGF-β-activated kinase 1 (TAK1). This activates the IKK complex, which comprises IKKα (β) and NEMO. Subsequently, the serine residues of IκBα are phosphorylated, resulting in proteasome cleavage. The released NF-κB is then transferred from the cytoplasm to the nucleus and binds to its target genes, inducing transcription (97, 98).

Biswas et al. identified low NF-κB expression in HR+ breast cancer and subsequently demonstrated that the ER-dependent pathway inhibits NF-κB gene activation (99). This may indicate a comparable inverse correlation between ER and NF-κB expression in breast cancer cells, a relationship that has been well documented in the literature (100). Ruchi Nehra et al. observed that the

expression level of P65 was elevated in ER+ cell lines exhibiting resistance to TAM endocrine therapy, accompanied by an augmented transcriptional activity of NF-κB and AP-1 (97). Following the administration of an NF-κB inhibitor, the transcription of NF-κB was found to decrease, as was the proliferation of drug-resistant cell lines (97). Kubo et al. compared ER+ breast cancer patients before and after endocrine therapy with AI, and observed increased NF-κB expression and induced resistance to endocrine drugs in breast cancer cells with disease progression after treatment (101). In conclusion, these results demonstrate increased NF-κB expression in breast cancer cells exhibiting ER-positive recurrence and/or endocrine resistance. As previously discussed, NF-κB can influence the sensitivity of breast cancer cells to endocrine drugs by regulating ERα expression.

The Zeste Homolog 2 Enhancer (EZH2) can be activated by certain inflammatory factors within the tumor microenvironment in a manner dependent on NF-κB, and the expression of ER was significantly increased following the silencing of EZH2 (102). As previously stated, NF-κB expression is negatively correlated with ER expression in breast cancer cells. Wang et al. previously demonstrated that RelB, a member of the NF-κB family, inhibits the expression of ER (103). The forkhead box O3a (FOXO3a) transcription factor binds to the ER promoter to initiate ER transcription. Phosphorylation of FOXO3a by filamentous threonine protein kinase C (PKC) results in inactivation of FOXO3a protein, which regulates the activity of the c-Rel transcription factor (104). It has been demonstrated that ER and p65 exist in protein complexes in DNA. Furthermore, inhibition of the NF-κB pathway can block cytokine-dependent p65 recruitment and enhance ER recruitment (98). Both E2 and NF-κB play a role in promoter regulation, and crosstalk between them affects the ability of ERα to activate its target genes (98). This demonstrates how NF-κB affects ER binding to DNA and, thus, ER activity. NF-κB regulates ERα transcriptional activity through both classical and non-classical pathways. The classical NF-κB pathway is activated in a cytokine-dependent manner, as previously described. Cytokines such as TNF-α can induce S118 phosphorylation of the ERK-dependent AF1 fragment of ER, which directly activates the ERE. This enhances the binding of ER to co-stimulators, including SRC3 and CBP/P300. Consequently, the ER becomes more sensitive to E2 and less sensitive to TAM (98). Conversely, IKK-α, produced by the non-classical pathway, can recruit the co-stimulator A1B1/SRC3 through the phosphorylation of S118 to form a transcription complex with ER, IKKα, and A1B1/SRC3, mediating the transcription of E2 (105). Following the binding of ER to TAM, a conformational change occurs, enabling ER to bind to nuclear receptor corepressor 1 (NCoR1). This results in the inhibition of histone deacetylation (106), which is associated with target genes. However, this process can be inhibited by IL-1β (107). However, its precise mechanism of action remains unclear. The interdependence of the ER and NF-κB pathways can rapidly downregulate miR-181a/b in microRNAs (miRNAs), helping to generate amplification loops and upregulation of target genes. This represents another crosstalk between the ER and NF-κB (108), which reveals the complexity between them.

## 4.4 FGFR

Fibroblast growth factor receptors (FGFRs) are members of the RTK superfamily (109, 110), which includes FGFR1-4. These receptors contain tyrosine kinase and transmembrane and extracellular domains. FGFR5 (FGFRL1), which lacks an intracellular kinase domain, binds to FGFs and prevents their interaction with other FGFRs (110). Fibroblast growth factors (FGFs) are secreted by tumor cells or stroma and can be classified into different types based on their homology, which is not the focus of this discussion. Heparin sulfate proteoglycans (HSPGs) stabilize the binding of FGF ligands to FGFRs, induce self-dimerization following receptor activation, phosphorylate the intracellular tyrosine kinase region, and activate downstream signaling pathways, such as PLC-IP3-DG-Ca2+, Ras-MAPK, PI3K-AKT, and JAK-STAT (111, 112). FGFR alterations include point mutations and gene fusion (113, 114). The most common of these is FGFR1 gene amplification (115), which causes endocrine resistance as ligand-dependent and ligand-independent pathways in approximately 15% of ER+ metastatic breast cancers (115–117). However, tumor cells are sensitive only to highly amplified FGFR1/2, and the underlying mechanism has been confirmed in multiple studies (118, 119). Luigi Formisano et al. observed that *in vitro* simulated ER+/FGFR1-amplified breast cancer cell lines were given AI drugs to simulate estrogen deprivation (LTED). This resulted in an increase in FGFR1 amplification as well as an increase in FGF3/4/19 and CCND expression. Furthermore, FOXA1 was found to promote FGFR1 nuclear expression through FGFR1 recruitment, driving estrogen-independent ERα transcription and inducing endocrine drug resistance (120). Servetto et al. investigated the relationship between FGFR1 and nuclear expression and demonstrated that FGFR1 nuclear expression induces non-estrogen-dependent cell growth, whereas cell lines exhibit reduced sensitivity to tamoxifen and fulvestrant (121). Potential mechanisms include the promotion of transcription of anti-estrogen resistance-related genes, binding to RNA polymerase II, and occupation of transcriptional promoter sites (121). Mao et al. demonstrated that FGFR1 and FGFR2 overexpression in the presence of FGF2 activated the MAPK and PI3K/AKT pathways, leading to fulvestrant and CDK4/6i resistance. However, this process can be reversed (122). Formisano et al. conducted a study analyzed the mechanism of FGFR1 resistance to CDK4/6 inhibitors. Their findings indicated that cyclinD1-mediated FGFR signal transduction plays a pivotal role in cell resistance to CDK4/6 inhibitors. Furthermore, they demonstrated that FGFR1 inhibition restored cell sensitivity to drugs (123). Although FGFR2 alteration is relatively uncommon in breast cancer, it is involved in endocrine resistance of tumor cells. It has been demonstrated that the FGF7/FGFR2 pathway enhances PI3K/AKT-mediated phosphorylation of ERα, enhancing drug resistance to

SERM (124). Moreover, FGFR2 overactivation results in cross-resistance between the SERD and CDK inhibitors (122, 123).

## 4.5 IRE1-XBP1

The unfolded protein response (UPR) signaling pathway plays a pivotal role in maintaining the functionality of the ER. The UPR pathway increases the ER protein folding ability when there is damage to this ability; misfolded or unfolded proteins accumulate in the ER, stimulating the UPR-mediated activity of transcription factors. This reestablishes the ability of the ER to dispose of proteins. Alternatively, UPR may induce cytotoxic death (125, 126).

Where intracellular proteins must be produced in large quantities, the UPR signaling pathways are activated. For example, the estrogen pathway can induce the expression of target genes in breast cancer cells, resulting in the production of proteins that promote growth and proliferation (127). The activation of the stress sensor molecules IRE1, PERK, and ATF6, which are on the ER membrane, initiates activation of the UPR signaling pathway (128). Normally, the ER chaperone protein, GRP78, interacts with three sensors to inhibit its activity. In response to ENR stress, sensor molecules dissociate from chaperone proteins and are activated, initiating a signaling cascade that enhances the activity of relevant transcription factors (129). Activation of IRE1 results in mRNA-specific splicing of XBP1 to form XBP1-SMRNA, but not XBP1-UMRNA (130). Nevertheless, there is crosstalk between the IRE1-XBP1 pathway and estrogen, which can induce endocrine resistance in cancer cells. Inhibiting the UPR signaling pathway may help to reverse drug resistance. First, estrogen activates UPR signaling through the PLC-PIP2-IP3 pathway in an ENR stress-independent manner (128). ER can lead to the simultaneous upregulation of XBP1 and IRE1, which jointly promotes the production of XBP1-S (131). This results in the formation of a positive feedback loop between XBP1 and the ER. Concurrently, ER can form a complex with XBP1-S to enhance ligand-independent transcriptional activity (132). Both XBP1-S and XBP1-U have been shown to promote endocrine resistance in ER+ breast cancers. Increased expression of XBP1-SMRNA and protein was observed in endocrine-resistant breast cancer cells, which promoted SERM and SERD resistance (131). The current understanding of the mechanisms of drug resistance is as follows: (1) XBP1-S enhances the transcriptional activation of ER and NF-κB, promoting endocrine resistance through the NF-κB signaling pathway (133). (2) XBP1-S induces the production of NCOA3, whereas phosphorylated NCOA3 stimulates the expression of NF-κB and promotes endocrine resistance (134, 135). (3) XBP1-U can promote the degradation of transcription factors P53 and FOXO1 and enhance the activities of transcription factors NF-κB and ER (132, 133). (4) The ectopic expression of XBP1-S has been demonstrated to induce an increase in BCL2 protein expression levels and to promote the resistance of cells to endocrine stress (136).

# 5 Tumor microenvironment

In the early stages of tumor development, monocytes and macrophages are recruited into the tumor microenvironment. Tumor-associated macrophages (TAMs) are pivotal regulators of tumorigenesis and exhibit anti-inflammatory and other intricate regulatory functions that facilitate tumor growth in most cases (137). The abundance of these cells is closely related to several key processes, including tumor evasion, immune surveillance, neovascularization, invasion, metastasis, response to treatment, and poor prognosis (138–140). In human breast tumors, inflammatory mononuclear cells (IMCs) are recruited by binding chemokine (C-C motif) ligand 2 (CCL2), which is synthesized by tumor and stromal cells, to chemokine receptor 2 (CCR2), which promotes neovascularization and tumor cell infiltration (141). Tumor necrosis factor α (TNF-α) is an inflammatory mediator in the tumor microenvironment. It is primarily produced by the mononuclear macrophage system and plays a pivotal role in the inflammatory-tumor association mediated by the NF-κB pathway. In a co-culture of MCF-7 cells with macrophages, Castellaro et al. observed that although MCF-7 cells can induce TNF-α-treated macrophages (conditioned macrophages) to produce IL-6, IL-8, CCL5, TNF-α, and other inflammatory cytokines in the absence of E2 or in the presence of an ERα antagonist, endocrine resistance in breast cancer cells is promoted in a non-hormone-dependent manner via the TNF-α/IL-6 pathway (142). TNF-α induced a consistent increase in STAT3 expression in MCF-7 cells co-cultured with KG-1 compared to that in MCF-7 cells cultured alone. This effect was not inhibited (142). Simultaneous blocking of IL-6 and STAT3 resulted in a significant reduction in MCF-7 cell proliferation, suggesting that the IL-6/STAT3 pathway plays a key role (142).

Extracellular matrix (ECM), cancer-associated fibrocytes (CAFs) and cancer-associated adipocytes (CAAs) are all involved in the genesis and development of tumors (143, 144). CAFs in hormone receptor-positive breast cancer is closely related to drug resistance. In general, CAFs in breast cancer stroma stimulate tumor cell growth, promote angiogenesis, and induce immune regulation by producing multiple stimulatory factors (145). In hormone receptor-positive breast cancer, CAFs has been found to induce resistance to endocrine therapy by producing soluble factors, proteases, and β1 integrin (146). CD146 (MCAM) is a matrix surface marker (147). The study found that CD146-CAF inhibited ER expression in MCF-7 cells, reduced sensitivity to estrogen, and increased resistance to tamoxifen (148). However, the presence of CD146+CAF stimulated ER expression and maintained estrogen-dependent proliferation and sensitivity to tamoxifen (148). Bone morphogenetic proteins (BMPs) are essential for maintaining epithelial integrity and antagonizing epithelial to mesenchymal transition (149). GREMLIN1 (GREM1) is a secreted BMP antagonist that sequester BMP ligands and prevent their binding to receptors (150). Transforming growth factorβ(TGFβ) secreted by breast cancer cells, stimulated GREM1 expression in CAFs (151). GREM1 abrogated bone morphogenetic protein (BMP)/SMAD

signaling in breast cancer cells, and also promoted the fibrogenic activation of CAFs (151). These processes enhance the invasive ability of cancer cells, so the treatment of GREM1 targets may improve the prognosis of breast cancer patients with high GREM1 expression. CAAs are also the main cellular components of the breast cancer microenvironment. Changes in the expression and secretion profile of inflammatory mediators in CAAs, such as increased secretion of chemokines CCL2, CCL5, IL-6, TNF-α, VEGF, leptin, etc, will further promote the proliferation and invasion of tumor cells and the formation of new blood vessels (144). In addition, through the dynamic interaction between breast cancer cells and CAAs, CAAs are induced to initiate the high tumor-promoting ability of metabolic reprogramming to support tumor cell proliferation, a process involving almost all nutrients (144). Furthermore, studies have found that exosomes transfer carcinogenic miRNAs (such as miRNA-144, miRNA-126 and miRNA-155) from breast cancer cells to fat cells in the tumor microenvironment, leading to their transformation into CAAs (152). These mechanisms become an important driver of disease progression, and targeting cancer-associated fat cells could lead to the development of potential drug-assisted anti-tumor therapies. In fact, for individuals, especially obese breast cancer patients, this goal can be more easily and effectively achieved through reasonable diet and appropriate exercise.

Programmed death receptor 1 (PD-1) is an immune checkpoint protein expressed on activated T cells, primarily in non-lymphocyte tissues and some immune cells in the surrounding environment of breast cancer. It mediates the inhibition of tumor-infiltrating lymphocytes and reduces the killing ability of T cells to tumor cells by binding to the ligand PD-L1 (153). Previous studies have demonstrated that the mRNA and protein expression levels of PD-L1 are significantly elevated in ERα-positive breast cancer cell lines and that ESR1 is negatively correlated with PD-L1 mRNA expression (154). Consequently, ERα may act as a negative regulatory factor influencing the expression level of PD-L1. Clinical studies have analyzed the efficacy of the PD-L1 antagonist pembrolizumab in ER+/Her2- advanced breast cancer patients, with an ORR of 12% (95% confidence interval (CI), 2.5–31.2%) and clinical benefit rate (CBR) of 20% (95% CI, 7–41%) (155).

It has been demonstrated that mesenchymal action on tumor cells can protect against cancer cell death (156). Pontiggia et al. discovered that soluble factors derived from fibroblasts, including matrix metalloproteinases (MMPs) and growth factors, are involved in the paracrine induction of drug resistance in tamoxifen-sensitive breast cancer epithelial cells through the PI3K/AKT and EGFR pathways. This was demonstrated by studying cultured fibroblast LM05-F and epithelial cell LM05-E (146). (2) Fibronectin and β1 integrin induce drug resistance in TAM by activating the downstream MAPK/ERK1/2 and PI3K/AKT pathways. (3) The phosphorylation of ERα-specific serine in epithelial cells by stroma-derived soluble factors and fibronectin is associated with tamoxifen (TAM) resistance in breast cancer. Furthermore, Sampayo et al. demonstrated that fibronectin mediates the endocytosis of ERα in breast cancer cells, with a subset of these cells entering the nucleus and the remainder being dragged back to the cell surface by β1 integrin. This evidence supports the critical role of the β1 integrin/FN pathway in regulating ERα expression (157). Heather M. Brechbuhl et al. demonstrated that CD146-CAFs inhibited ERα expression in MCF-7 cells, whereas CD146+CAFs not only induced ERα expression but also restored the sensitivity of epithelial cells to TAM in conditioned medium (148). Fibroblast stromal cells from the bone marrow (hs5-CM) have been shown to mediate endocrine resistance in breast cancer cells by downregulating ER levels via the paracrine signaling pathway (158).

## 6 Antibody-drug conjugation

Antibody-drug conjugation (ADC) is a novel antitumor drug that has emerged in recent years. It involves joining a monoclonal antibody with a drug carrier through a linker. Monoclonal antibodies on ADC can bind to specific target antigens on the tumor surface, enter tumor cells through receptor-mediated endocytosis, form early endosomes, and rapidly release drugs. Furthermore, they can mature into late-stage endosome and lysosome fusion and release drug loading, ultimately leading to tumor cell death by inhibiting microscopic polymerization or DNA assembly (159) (Figure 3). ADC drugs have made remarkable clinical progress in the field of breast cancer. Common research targets include the trophoblast cell surface antigen 2 (Trop-2), HER2, HER3, poliovirus receptor 4, and receptor tyrosine kinase-like orphan hormone receptor 2 (Table 3).

Trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd) are ADCs that target HER2. Trastuzumab is the antibody component, and the drug antibody score is 3.5 for T-DM1 and 8 for T-DXd. In the Phase III clinical DESTINY-Breast04 study, 557 patients with HR-positive or HR-negative metastatic breast cancer with low HER2 expression who had previously received endocrine, first- or second-line chemotherapy were enrolled. Among patients with HR-positive disease, the T-DXd group was compared to the treatment of the physician's choice (TPC) group. The chemotherapy regimens used in the TPC group included alibrine, capecitabine, albumin-paclitaxel, gemcitabine, and paclitaxel. The results demonstrated that the median overall survival (OS) of the T-DXd group and the TPC group were 23.9 months and 17.5 months, respectively (HR= 0.64). Furthermore, the median PFS was 10.1 months and 5.4 months, respectively (HR = 0.51). The efficacy of T-DXd is satisfactory and its overall safety profile is manageable (160).

Gosatuzumab (SG), Dato-DXd, and SKB264 are ADCs target Trop-2. The Phase III TROPiCS-02 study included 543 patients with HR-positive/HER2-negative metastatic breast cancer. SG demonstrated a significant improvement in median PFS compared to TPC (5.5 vs. 4.0 months; HR = 0.66; $P = 0.0003$), as well as an advantage in median OS (14.4 months vs. 11.2 months; HR = 0.79; $P = 0.02$) (161, 162). The Phase III TROPION-Breast01 study demonstrated that the Dato-DXd group exhibited superior PFS in previously treated HR-positive/HER2-negative metastatic breast cancer patients compared to the chemotherapy group (6.9 months vs. 4.9 months; $P < 0.0001$) (163). A Phase II study of SKB264 also demonstrated favorable antitumor effects, with a median follow-up period of 8.2 months, an ORR of 36.8%, and a median PFS of 11.1 months (164).

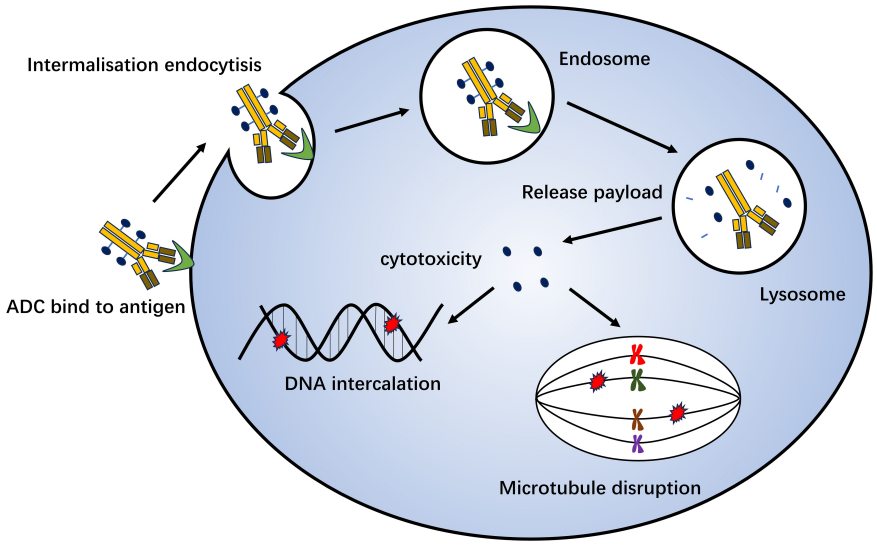



FIGURE 3
Mechanism of antibody-drug conjugation killing of tumor cells. Monoclonal antibodies on ADC can bind to specific target antigens on the tumor surface, enter tumor cells through receptor-mediated endocytosis, form early endosomes, and quickly release drugs. It can also mature into late-stage endosome and lysosome fusion and release drug loading, which ultimately leads to tumor cell death by inhibiting microscopic polymerization or DNA assembly.

TABLE 3 Antibody-drug conjugation in HR-positive/HER2-negative Advanced or Metastatic BC.

| Trial Name | Phase | Population | Treatment | Sample Size | Primary Outcome, HR (95% CI) |
|---|---|---|---|---|---|
| DESTINY-Breast04 (160) | III | HR+ disease considered endocrine refractory | T-DXd | | PFS 10.1 vs. 5.4 months |
| | | HER2-low(ICH 1+ vs 2+/ISH-), unresectable, and/or | vs. | 557 | (HR 0.51; 95% CI 0.40–0.64) |
| | | mBC treated with 1-2 prior lines of chemotherapy | TPC | | OS 23.9 vs. 17.5 months |
| | | in the metastatic setting | | | (HR 0.64; 95% CI 0.48–0.86) |
| TROPiCS-02 (161, 162) | III | Metastatic or locally recurrent inoperable HR+/HER2- | SG | | PFS 5.5 vs. 4.0 months |
| | | breast cancer that progressed after | vs. | 543 | (HR 0.66; 95% CI 0.53–0.83) |
| | | at least 1 endocrine therapy, taxane, and CDK4/6 inhibitor in any setting | TPC | | OS 14.4 vs. 11.2 months |
| | | at least 2, but no more than 4, lines of chemotherapy for metastatic disease | | | (HR 0.79; 95% CI 0.65–0.96) |
| TROPION-Breast01 (163) | III | Adult pts with inoperable or metastatic HR+/HER2-BC, | Dato-Dxd | | PFS 6.9 vs. 4.9 months |
| | | who had experienced progression on endocrine therapy | vs. | 732 | (HR 0.63; 95% CI 0.52–0.76) |
| | | and for whom ET was unsuitable, and who had received | ICC | | OS- |
| | | 1-2 prior lines of systemic chemotherapy | | | |
| SKB264 (MK-2870) (164) | II | pre-specified subpopulation of patients with HR +/HER2 mBC | SG | 54 | PFS 5.5 months (95%CI 3.6~7.6) |
| | | from the phase 1/2, single-arm trial (NCT01631552) | | | OS 12.0 months (95%CI 9.0~18.2) |

T-DXd-trastuzumab deruxtecan; SG-sacituzumab govitecan; TPC-treatment of physician's choice; ICC-investigator's choice of chemotherapy.

## 7 Biological metabolism

It has been demonstrated that abnormal endogenous lipid metabolism can cause increased cancer cell invasiveness and the development of drug resistance in tumors (165, 166). Fatty acid synthetase (FASN) is a key enzyme involved in lipid biosynthesis and the synthesis of long-chain fatty acids such as palmitate, which is subsequently involved in cell signal transduction (166, 167). FASN was initially identified as a highly expressed tumor marker in breast cancer (168). Studies have demonstrated that FASN plays an important role in the regulation of ERα expression and activity. Aleksandra Gruslova et al., building upon previous research, demonstrated that the inhibition of FASN in endocrine-resistant breast cancer cells induces endoplasmic reticulum stress (EnRs pathway), which mediates ERα degradation, resulting in a significant decrease in ERα levels in tumor cells ($P < 0.01$) and the inhibition of the growth of TAM-resistant breast cancer cells (169). In their experiments, Menendez et al. demonstrated that FASN controls the sensitivity of cells to E2-dependent ERα signals through the crosstalk of MAPK/ERα and AKT/ERα signals (165). Furthermore, it induces the expression of p21WAF1/CIP1, p27Kip1, and other cell cycle suppressor genes, inhibiting PI3K/AKT-mediated cell cycle progression and synergistically inhibiting E2-mediated cell survival (165). The etiology of breast cancer is multifactorial, with genetic susceptibility and environmental factors contributing to its pathogenesis.

## 8 Discussion

Treatment of hormone receptor-positive breast cancer has long been complicated by endocrine drug resistance. A considerable number of studies have identified numerous potential mechanisms and confirmed that the process of inducing drug resistance is complex. A multitude of studies on the molecular mechanisms have yielded new insights and novel therapeutic strategies that may overcome endocrine resistance.

Drugs are being developed to effectively block the transmission of estrogen signals and the activation of downstream molecules by regulating the expression and activation of ER and downstream signaling molecules. The previous treatment strategy was single estrogen antagonist therapy. Studies have demonstrated that Alterations in the ER genome play a pivotal role in the development of resistance to endocrine drugs. ESR mutants frequently exhibit drug resistance and distant metastases owing to their substantial aggressiveness. Single hormone receptor blockers have been ineffective in inhibiting tumor cell growth. In contrast, the combination of receptor blockade with downstream signaling molecule inhibitors has been shown to have antitumor effects. Combination therapy is often the recommended treatment for patients with hormone receptor-positive tumors. Endocrine resistance is not solely because of the "surface" molecular effect, but also encompasses the transmission of downstream signals. Of these, the RTK signaling pathway is of particular importance. Abnormal activation of this pathway leads to continuous activation of nuclear target genes and affects the expression level of ER. Consequently, activation of the RTK-mediated cell signaling pathway is largely associated with endocrine therapy resistance, which inhibits signal transduction and cell growth by inhibiting key targets. The intricate interrelationship between the ER signaling pathways and TKR, along with its downstream key PI3K-mTOR and RAS-ERK pathways, is a crucial aspect of endocrine resistance. In treatment-resistant endocrine-resistant breast cancer, ER can promote tumor growth and proliferation in a ligand-independent manner, replacing its classical activation pathway with a new mode influenced by other signal transduction pathways. Consequently, the development of drugs targeting downstream signaling molecules and a synergistic model combining them with endocrine therapy are anticipated to offer new hope for individuals with endocrine-resistant breast cancer.

The growth of tumor cells is also influenced by a multitude of factors, including intracellular and intracellular regulatory cytokines, immune molecules, tumor microenvironment, and stem cells. Further research into the immune microenvironment, oxidative stress space, and metabolome polymorphisms around tumor cells will help to elucidate novel mechanisms and linkages of drug resistance in tumors. Future research should aim to elucidate potential biomarkers in greater depth, identify more reliable targets, and develop more drugs for individuals resistant to frontline treatment. Individualized treatment was developed based on individual differences among the patients. A multitarget crossover model is expected to reverse endocrine resistance.

Previously, it was believed that breast cancer lacks immunogenicity. Over the years, immunotherapy has emerged as a new standard of care, demonstrating efficacy and therapeutic value in patients with tumors. CTLA-4, PD-1, and PD-L1 enhance the ability of immune cells to kill tumors by blocking immunoregulatory proteins that downregulate the immune system. Currently, medical evidence regarding the efficacy of immunotherapy for breast cancer is insufficient. Several tumor-infiltrating lymphocytes (TILs) and PD-L1 proteins may render TNBC sensitive to checkpoint inhibition (170). Nevertheless, the efficacy of immunotherapy in ER+ breast cancer remains unclear. Consequently, the combination of endocrine therapy and immunotherapy may represent a promising avenue for future research. Given the urgent need for further research into the role of immune checkpoints in endocrine resistance, it is imperative that clinical studies are conducted to determine the clinical benefits of combining endocrine and immunotherapies.

In recent years, macromolecular monoclonal antibodies, a novel class of targeted drugs represented by T-DXd, have emerged as a prominent area of research, ushering in the era of ADC drug therapy and offering expanded treatment options for advanced breast cancer patients who have progressed following CDK4/6 inhibitor treatment. The future of ADC drug research and development will continue to offer significant opportunities for improvement, including enhanced targeting, linker stability, and resistance to drug-induced toxicity.

## Author contributions

YG: Writing – original draft, Writing – review & editing. YY: Writing – original draft, Writing – review & editing. MZ: Writing –

original draft, Writing – review & editing. WY: Writing – original draft, Writing – review & editing. LK: Writing – review & editing.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The research is funded by Jilin Province Science and Technology Development Plan Project (No.20230402004GH).

## Acknowledgments

We would like to thank Editage (www.editage.cn) for English language editing.

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

2. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol*. (2013) 24:2206–23. doi: 10.1093/annonc/mdt303

3. Clark GM, Osborne CK, Mcguire WL. Correlations between estrogen receptor, progesterone receptor, and patient characteristics in human breast cancer. *J Clin Oncol*. (1984) 2:1102–9. doi: 10.1200/JCO.1984.2.10.1102

4. Lagranha CJ, Silva TLA, Silva SCA, Braz GRF, Da Silva AI, Fernandes MP, et al. Protective effects of estrogen against cardiovascular disease mediated via oxidative stress in the brain. *Life Sci*. (2018) 192:190–8. doi: 10.1016/j.lfs.2017.11.043

5. Senkus E, Cardoso F, Pagani O. Time for more optimism in metastatic breast cancer? *Cancer Treat Rev*. (2014) 40:220–8. doi: 10.1016/j.ctrv.2013.09.015

6. Reinert T, Barrios CH. Optimal management of hormone receptor positive metastatic breast cancer in 2016. *Ther Adv Med Oncol*. (2015) 7:304–20. doi: 10.1177/1758834015608993

7. Tryfonidis K, Zardavas D, Katzenellenbogen BS, Piccart M. Endocrine treatment in breast cancer: Cure, resistance and beyond. *Cancer Treat Rev*. (2016) 50:68–81. doi: 10.1016/j.ctrv.2016.08.008

8. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA, Proc Natl Acad Sci USA. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci U.S.A*. (1996) 93:5925–30. doi: 10.1073/pnas.93.12.5925

9. Zhou Y, Liu X. The role of estrogen receptor beta in breast cancer. *biomark Res*. (2020) 8:39. doi: 10.1186/s40364-020-00223-2

10. Ayaz G, Yasar P, Olgun CE, Karakaya B, Kars G, Razizadeh N, et al. Dynamic transcriptional events mediated by estrogen receptor alpha. *FBL*. (2019) 24:245–76. doi: 10.2741/4716

11. Ascenzi P, Bocedi A, Marino M. Structure-function relationship of estrogen receptor alpha and beta: impact on human health. *Mol Aspects Med*. (2006) 27:299–402. doi: 10.1016/j.mam.2006.07.001

12. Zwart W, De Leeuw R, Rondaij M, Neefjes J, Mancini MA, Michalides R. The hinge region of the human estrogen receptor determines functional synergy between AF-1 and AF-2 in the quantitative response to estradiol and tamoxifen. *J Cell Sci*. (2010) 123:1253–61. doi: 10.1242/jcs.061135

13. Souza PC, Barra GB, Velasco LF, Ribeiro IC, Simeoni LA, Togashi M, et al. Helix 12 dynamics and thyroid hormone receptor activity: experimental and molecular dynamics studies of Ile280 mutants. *J Mol Biol*. (2011) 412:882–93. doi: 10.1016/j.jmb.2011.04.014

14. Safarinejad MR, Shafiei N, Safarinejad S. Association of polymorphisms in the estrogen receptors alpha, and beta (ESR1, ESR2) with the occurrence of male infertility and semen parameters. *J Steroid Biochem Mol Biol*. (2010) 122:193–203. doi: 10.1016/j.jsbmb.2010.06.011

15. Barone I, Brusco L, Fuqua SA. Estrogen receptor mutations and changes in downstream gene expression and signaling. *Clin Cancer Res*. (2010) 16:2702–8. doi: 10.1158/1078-0432.CCR-09-1753

16. Toy W, Shen Y, Won H, Green B, Sakr RA, Will M, et al. ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. *Nat Genet*. (2013) 45:1439–45. doi: 10.1038/ng.2822

17. Li S, Shen D, Shao J, Crowder R, Liu W, Prat A, et al. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. *Cell Rep*. (2013) 4:1116–30. doi: 10.1016/j.celrep.2013.08.022

18. Robinson DR, Wu YM, Vats P, Su F, Lonigro RJ, Cao X, et al. Activating ESR1 mutations in hormone-resistant metastatic breast cancer. *Nat Genet*. (2013) 45:1446–51. doi: 10.1038/ng.2823

19. Pejerrey SM, Dustin D, Kim JA, Gu G, Rechoum Y, Fuqua S, et al. The impact of ESR1 mutations on the treatment of metastatic breast cancer. *Horm Cancer*. (2018) 9:215–28. doi: 10.1007/s12672-017-0306-5

20. Chandarlapaty S, Chen D, He W, Sung P, Samoila A, You D, et al. Prevalence of ESR1 mutations in cell-free DNA and outcomes in metastatic breast cancer: A secondary analysis of the BOLERO-2 clinical trial. *JAMA Oncol*. (2016) 2:1310–5. doi: 10.1001/jamaoncol.2016.1279

21. Fanning SW, Mayne CG, Dharmarajan V, Carlson KE, Martin TA, Novick SJ, et al. Estrogen receptor alpha somatic mutations Y537S and D538G confer breast cancer endocrine resistance by stabilizing the activating function-2 binding conformation. *Elife*. (2016) 5. doi: 10.7554/eLife.12792

22. Carausu M, Bidard FC, Callens C, Melaabi S, Jeannot E, Pierga JY, et al. ESR1 mutations: a new biomarker in breast cancer. *Expert Rev Mol Diagn*. (2019) 19:599–611. doi: 10.1080/14737159.2019.1631799

23. Merenbakh-Lamin K, Ben-Baruch N, Yeheskel A, Dvir A, Soussan-Gutman L, Jeselsohn R, et al. D538G mutation in estrogen receptor-α: A novel mechanism for acquired endocrine resistance in breast cancer. *Cancer Res*. (2013) 73:6856–64. doi: 10.1158/0008-5472.CAN-13-1197

24. Jeselsohn R, Bergholz JS, Pun M, Cornwell M, Liu W, Nardone A, et al. Allele-specific chromatin recruitment and therapeutic vulnerabilities of ESR1 activating mutations. *Cancer Cell*. (2018) 33:173–186.e5. doi: 10.1016/j.ccell.2018.01.004

25. Zinger L, Merenbakh-Lamin K, Klein A, Elazar A, Journo S, Boldes T, et al. Ligand-binding domain-activating mutations of ESR1 rewire cellular metabolism of breast cancer cells. *Clin Cancer Res*. (2019) 25:2900–14. doi: 10.1158/1078-0432.CCR-18-1505

26. O'leary B, Cutts RJ, Liu Y, Hrebien S, Huang X, Fenwick K, et al. The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. *Cancer Discovery*. (2018) 8:1390–403. doi: 10.1158/2159-8290.CD-18-0264

27. Martin LA, Ribas R, Simigdala N, Schuster E, Pancholi S, Tenev T, et al. Discovery of naturally occurring ESR1 mutations in breast cancer cell lines modelling endocrine resistance. *Nat Commun*. (2017) 8:1865. doi: 10.1038/s41467-017-01864-y

28. Spoerke JM, Gendreau S, Walter K, Qiu J, Wilson TR, Savage H, et al. Heterogeneity and clinical significance of ESR1 mutations in ER-positive metastatic breast cancer patients receiving fulvestrant. *Nat Commun*. (2016) 7:11579. doi: 10.1038/ncomms11579

29. Dustin D, Gu G, Fuqua SW. ESR1 mutations in breast cancer. *Cancer*. (2019) 125:3714–28. doi: 10.1002/cncr.v125.21

30. Toy W, Weir H, Razavi P, Lawson M, Goeppert AU, Mazzola AM, et al. Activating ESR1 mutations differentially affect the efficacy of ER antagonists. *Cancer Discovery*. (2017) 7:277–87. doi: 10.1158/2159-8290.CD-15-1523

31. Wang L, Sun J, Yin Y, Sun Y, Ma J, Zhou R, et al. Transcriptional coregualtor NUPR1 maintains tamoxifen resistance in breast cancer cells. *Cell Death Dis*. (2021) 12:149. doi: 10.1038/s41419-021-03442-z

32. Hartmaier RJ, Albacker LA, Chmielecki J, Bailey M, He J, Goldberg ME, et al. High-throughput genomic profiling of adult solid tumors reveals novel insights into cancer pathogenesis. *Cancer Res*. (2017) 77:2464–75. doi: 10.1158/0008-5472.CAN-16-2479

33. Giltnane JM, Hutchinson KE, Stricker TP, Formisano L, Young CD, Estrada MV, et al. Genomic profiling of ER(+) breast cancers after short-term estrogen suppression reveals alterations associated with endocrine resistance. *Sci Transl Med*. (2017) 9(402). doi: 10.1126/scitranslmed.aai7993

34. Hartmaier RJ, Trabucco SE, Priedigkeit N, Chung JH, Parachoniak CA, Vanden Borre P, et al. Recurrent hyperactive ESR1 fusion proteins in endocrine therapy-resistant breast cancer. *Ann Oncol*. (2018) 29:872–80. doi: 10.1093/annonc/mdy025

35. Turner NC, Bartlett CH, Cristofanilli M. Palbociclib in hormone-receptor-positive advanced breast cancer REPLY. *New Engl J Med*. (2015) 373:1672–3. doi: 10.1056/NEJMoa1505270

36. Piezzo M, Cocco S, Caputo R, Cianniello D, Gioia GD, Lauro VD, et al. Targeting cell cycle in breast cancer: CDK4/6 inhibitors. *Int J Mol Sci*. (2020) 21(18). doi: 10.3390/ijms21186479

37. Sherr CJ, Roberts JM. Living with or without cyclins and cyclin-dependent kinases. *Genes Dev*. (2004) 18:2699–711. doi: 10.1101/gad.1256504

38. Hwang HC, Clurman BE. Cyclin E in normal and neoplastic cell cycles. *Oncogene*. (2005) 24:2776–86. doi: 10.1038/sj.onc.1208613

39. Anders L, Ke N, Hydbring P, Choi YJ, Widlund HR, Chick JM, et al. A systematic screen for CDK4/6 substrates links FOXM1 phosphorylation to senescence suppression in cancer cells. *Cancer Cell*. (2011) 20:620–34. doi: 10.1016/j.ccr.2011.10.001

40. Sherr CJ, Beach D, Shapiro GI. Targeting CDK4 and CDK6: from discovery to therapy. *Cancer Discovery*. (2016) 6:353–67. doi: 10.1158/2159-8290.CD-15-0894

41. Malumbres M, Barbacid M. To cycle or not to cycle: a critical decision in cancer. *Nat Rev Cancer*. (2001) 1:222–31. doi: 10.1038/35106065

42. Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. *Nat Rev Drug Discovery*. (2015) 14:130–46. doi: 10.1038/nrd4504

43. O'leary B, Finn RS, Turner NC. Treating cancer with selective CDK4/6 inhibitors. *Nat Rev Clin Oncol*. (2016) 13:417–30. doi: 10.1038/nrclinonc.2016.26

44. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev*. (1999) 13:1501–12. doi: 10.1101/gad.13.12.1501

45. Lacy ER, Filippov I, Lewis WS, Otieno S, Xiao L, Weiss S, et al. p27 binds cyclin-CDK complexes through a sequential mechanism involving binding-induced protein folding. *Nat Struct Mol Biol*. (2004) 11:358–64. doi: 10.1038/nsmb746

46. Martín-Caballero J, Flores JM, García-Palencia P, Serrano M. Tumor susceptibility of p21(Waf1/Cip1)-deficient mice. *Cancer Res*. (2001) 61:6234–8.

47. Fero ML, Rivkin M, Tasch M, Porter P, Carow CE, Firpo E, et al. A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. *Cell*. (1996) 85:733–44. doi: 10.1016/S0092-8674(00)81239-8

48. Labaer J, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, et al. New functional activities for the p21 family of CDK inhibitors. *Genes Dev*. (1997) 11:847–62. doi: 10.1101/gad.11.7.847

49. Guiley KZ, Stevenson JW, Lou K, Barkovich KJ, Kumarasamy V, Wijeratne TU, et al. p27 allosterically activates cyclin-dependent kinase 4 and antagonizes palbociclib inhibition. *Science*. (2019) 366(6471). doi: 10.1126/science.aaw2106

50. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature*. (1993) 366:704–7. doi: 10.1038/366704a0

51. Álvarez-Fernández M, Malumbres M. Mechanisms of sensitivity and resistance to CDK4/6 inhibition. *Cancer Cell*. (2020) 37:514–29. doi: 10.1016/j.ccell.2020.03.010

52. Vanarsdale T, Boshoff C, Arndt KT, Abraham RT. Molecular pathways: targeting the cyclin D-CDK4/6 axis for cancer treatment. *Clin Cancer Res*. (2015) 21:2905–10. doi: 10.1158/1078-0432.CCR-14-0816

53. Hart CD, Migliaccio I, Malorni L, Guarducci C, Biganzoli L, Di Leo A. Challenges in the management of advanced, ER-positive, HER2-negative breast cancer. *Nat Rev Clin Oncol*. (2015) 12:541–52. doi: 10.1038/nrclinonc.2015.99

54. Yang C, Li Z, Bhatt T, Dickler M, Giri D, Scaltriti M, et al. Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. *Oncogene*. (2017) 36:2255–64. doi: 10.1038/onc.2016.379

55. Hydbring P, Malumbres M, Sicinski P. Non-canonical functions of cell cycle cyclins and cyclin-dependent kinases. *Nat Rev Mol Cell Biol*. (2016) 17:280–92. doi: 10.1038/nrm.2016.27

56. Pestell TG, Jiao X, Kumar M, Peck AR, Prisco M, Deng S, et al. Stromal cyclin D1 promotes heterotypic immune signaling and breast cancer growth. *Oncotarget*. (2017) 8:81754–75. doi: 10.18632/oncotarget.19953

57. Finn RS, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. *Lancet Oncol*. (2015) 16:25–35. doi: 10.1016/S1470-2045(14)71159-3

58. Li Z, Razavi P, Li Q, Toy W, Liu B, Ping C, et al. Loss of the FAT1 tumor suppressor promotes resistance to CDK4/6 inhibitors via the hippo pathway. *Cancer Cell*. (2018) 34:893–905.e8. doi: 10.1016/j.ccell.2018.11.006

59. Fribbens C, O'leary B, Kilburn L, Hrebien S, Garcia-Murillas I, Beaney M, et al. Plasma ESR1 mutations and the treatment of estrogen receptor-positive advanced breast cancer. *J Clin Oncol*. (2016) 34:2961–8. doi: 10.1200/JCO.2016.67.3061

60. Goel S, Decristo MJ, Watt AC, Brinjones H, Sceneay J, Li BB, et al. CDK4/6 inhibition triggers anti-tumour immunity. *Nature*. (2017) 548:471–5. doi: 10.1038/nature23465

61. Chaikovsky AC, Sage J. Beyond the cell cycle: enhancing the immune surveillance of tumors via CDK4/6 inhibition. *Mol Cancer Res*. (2018) 16:1454–7. doi: 10.1158/1541-7786.MCR-18-0201

62. Schaer DA, Beckmann RP, Dempsey JA, Huber L, Forest A, Amaladas N, et al. The CDK4/6 inhibitor abemaciclib induces a T cell inflamed tumor microenvironment and enhances the efficacy of PD-L1 checkpoint blockade. *Cell Rep*. (2018) 22:2978–94. doi: 10.1016/j.celrep.2018.02.053

63. Wander SA, Cohen O, Gong X, Johnson GN, Buendia-Buendia JE, Lloyd MR, et al. The genomic landscape of intrinsic and acquired resistance to cyclin-dependent kinase 4/6 inhibitors in patients with hormone receptor-positive metastatic breast cancer. *Cancer Discovery*. (2020) 10:1174–93. doi: 10.1158/2159-8290.CD-19-1390

64. Finn RS, Martin M, Rugo HS, Jones S, Im SA, Gelmon K, et al. Palbociclib and letrozole in advanced breast cancer. *N Engl J Med*. (2016) 375:1925–36. doi: 10.1056/NEJMoa1607303

65. Finn RS, Rugo HS, Dieras VC, Harbeck N, Im S-A, Gelmon KA, et al. Overall survival (OS) with first-line palbociclib plus letrozole (PAL+LET) versus placebo plus letrozole (PBO+LET) in women with estrogen receptor–positive/human epidermal growth factor receptor 2–negative advanced breast cancer (ER+/HER2– ABC): Analyses from PALOMA-2. *J Clin Oncol*. (2022) 40:LBA1003–LBA1003. doi: 10.1200/JCO.2022.40.17_suppl.LBA1003

66. Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Hart L, et al. Overall survival with ribociclib plus letrozole in advanced breast cancer. *N Engl J Med*. (2022) 386:942–50. doi: 10.1056/NEJMoa2114663

67. Johnston S, Martin M, Di Leo A, Im SA, Awada A, Forrester T, et al. MONARCH 3 final PFS: a randomized study of abemaciclib as initial therapy for advanced breast cancer. *NPJ Breast Cancer*. (2019) 5:5. doi: 10.1038/s41523-018-0097-z

68. Weigelt B, Downward J. Genomic determinants of PI3K pathway inhibitor response in cancer. *Front Oncol*. (2012) 2:109. doi: 10.3389/fonc.2012.00109

69. Dienstmann R, Rodon J, Serra V, Tabernero J. Picking the point of inhibition: a comparative review of PI3K/AKT/mTOR pathway inhibitors. *Mol Cancer Ther*. (2014) 13:1021–31. doi: 10.1158/1535-7163.MCT-13-0639

70. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet*. (2006) 7:606–19. doi: 10.1038/nrg1879

71. Dong C, Wu J, Chen Y, Nie J, Chen C. Activation of PI3K/AKT/mTOR pathway causes drug resistance in breast cancer. *Front Pharmacol*. (2021) 12:628690. doi: 10.3389/fphar.2021.628690

72. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, et al. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res*. (2008) 68:6084–91. doi: 10.1158/0008-5472.CAN-07-6854

73. Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, et al. The PIK3CA gene is mutated with high frequency in human breast cancers. *Cancer Biol Ther*. (2004) 3:772–5. doi: 10.4161/cbt.3.8.994

74. Araki K, Miyoshi Y. Mechanism of resistance to endocrine therapy in breast cancer: the important role of PI3K/Akt/mTOR in estrogen receptor-positive, HER2-negative breast cancer. *Breast Cancer*. (2018) 25:392–401. doi: 10.1007/s12282-017-0812-x

75. Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, et al. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature*. (2007) 448:439–44. doi: 10.1038/nature05933

76. Samuels Y, Velculescu VE. Oncogenic mutations of PIK3CA in human cancers. *Cell Cycle*. (2004) 3:1221–4. doi: 10.4161/cc.3.10.1164

77. Pérez-Tenorio G, Alkhori L, Olsson B, Waltersson MA, Nordenskjöld B, Rutqvist LE, et al. PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. *Clin Cancer Res*. (2007) 13:3577–84. doi: 10.1158/1078-0432.CCR-06-1609

78. Stommel JM, Kimmelman AC, Ying H, Nabioullin R, Ponugoti AH, Wiedemeyer R, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science*. (2007) 318:287–90. doi: 10.1126/science.1142946

79. Tokunaga E, Kimura Y, Mashino K, Oki E, Kataoka A, Ohno S, et al. Activation of PI3K/Akt signaling and hormone resistance in breast cancer. *Breast Cancer*. (2006) 13:137–44. doi: 10.2325/jbcs.13.137

80. Kim EK, Kim HA, Koh JS, Kim MS, Kim KI, Lee JI, et al. Phosphorylated S6K1 is a possible marker for endocrine therapy resistance in hormone receptor-positive breast cancer. *Breast Cancer Res Treat*. (2011) 126:93–9. doi: 10.1007/s10549-010-1315-z

81. Baselga J, Campone M, Piccart M, Burris HA 3rd, Rugo HS, Sahmoud T, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med*. (2012) 366:520–9. doi: 10.1056/NEJMoa1109653

82. Sun T, Aceto N, Meerbrey KL, Kessler JD, Zhou C, Migliaccio I, et al. Activation of multiple proto-oncogenic tyrosine kinases in breast cancer via loss of the PTPN12 phosphatase. *Cell*. (2011) 144:703–18. doi: 10.1016/j.cell.2011.02.003

83. Kornblum N, Zhao F, Manola J, Klein P, Ramaswamy B, Brufsky A, et al. Randomized phase II trial of fulvestrant plus everolimus or placebo in postmenopausal women with hormone receptor-positive, human epidermal growth factor receptor 2-negative metastatic breast cancer resistant to aromatase inhibitor therapy: results of prE0102. *J Clin Oncol*. (2018) 36:1556–63. doi: 10.1200/JCO.2017.76.9331

84. Piccart M, Hortobagyi GN, Campone M, Pritchard KI, Lebrun F, Ito Y, et al. Everolimus plus exemestane for hormone-receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: overall survival results from BOLERO-2†. *Ann Oncol*. (2014) 25:2357–62. doi: 10.1093/annonc/mdu456

85. Shao Z, Cai L, Wang S, Hu X, Shen K, Wang H, et al. 238P BOLERO-5: A phase II study of everolimus and exemestane combination in Chinese post-menopausal women with ER+/HER2- advanced breast cancer. *Ann Oncol*. (2021) 32:S463. doi: 10.1016/j.annonc.2021.08.521

86. Bardia A, Hurvitz SA, Demichele A, Clark AS, Zelnak A, Yardley DA, et al. Phase I/II trial of exemestane, ribociclib, and everolimus in women with HR+/HER2–advanced breast cancer after progression on CDK4/6 inhibitors (TRINITI-1). *Clin Cancer Res*. (2021) 27:4177–85. doi: 10.1158/1078-0432.CCR-20-2114

87. Fan Y, Sun T, Shao Z, Zhang Q, Ouyang Q, Tong Z, et al. Effectiveness of adding everolimus to the first-line treatment of advanced breast cancer in premenopausal women who experienced disease progression while receiving selective estrogen receptor modulators: A phase 2 randomized clinical trial. *JAMA Oncol*. (2021) 7:e213428–e213428. doi: 10.1001/jamaoncol.2021.3428

88. Lorusso PM. Inhibition of the PI3K/AKT/mTOR pathway in solid tumors. *J Clin Oncol*. (2016) 34:3803–15. doi: 10.1200/JCO.2014.59.0018

89. Gazave E, Lapébie P, Richards GS, Brunet F, Ereskovsky AV, Degnan BM, et al. Origin and evolution of the Notch signalling pathway: an overview from eukaryotic genomes. *BMC Evol Biol*. (2009) 9:249. doi: 10.1186/1471-2148-9-249

90. Meurette O, Mehlen P. Notch signaling in the tumor microenvironment. *Cancer Cell*. (2018) 34:536–48. doi: 10.1016/j.ccell.2018.07.009

91. Hao L, Rizzo P, Osipo C, Pannuti A, Wyatt D, Cheung LW, et al. Notch-1 activates estrogen receptor-alpha-dependent transcription via IKKalpha in breast cancer cells. *Oncogene*. (2010) 29:201–13. doi: 10.1038/onc.2009.323

92. Dou XW, Liang YK, Lin HY, Wei XL, Zhang YQ, Bai JW, et al. Notch3 maintains luminal phenotype and suppresses tumorigenesis and metastasis of breast cancer via trans-activating estrogen receptor-α. *Theranostics*. (2017) 7:4041–56. doi: 10.7150/thno.19989

93. Sansone P, Ceccarelli C, Berishaj M, Chang Q, Rajasekhar VK, Perna F, et al. Self-renewal of CD133(hi) cells by IL6/Notch3 signalling regulates endocrine resistance in metastatic breast cancer. *Nat Commun*. (2016) 7:10442. doi: 10.1038/ncomms10442

94. Rizzo P, Miao H, D'souza G, Osipo C, Song LL, Yun J, et al. Cross-talk between notch and the estrogen receptor in breast cancer suggests novel therapeutic approaches. *Cancer Res*. (2008) 68:5226–35. doi: 10.1158/0008-5472.CAN-07-5744

95. Bui QT, Im JH, Jeong SB, Kim YM, Lim SC, Kim B, et al. Essential role of Notch4/STAT3 signaling in epithelial-mesenchymal transition of tamoxifen-resistant human breast cancer. *Cancer Lett*. (2017) 390:115–25. doi: 10.1016/j.canlet.2017.01.014

96. Gelsomino L, Panza S, Giordano C, Barone I, Gu G, Spina E, et al. Mutations in the estrogen receptor alpha hormone binding domain promote stem cell phenotype through notch activation in breast cancer cell lines. *Cancer Lett*. (2018) 428:12–20. doi: 10.1016/j.canlet.2018.04.023

97. Zhang Q, Lenardo MJ, Baltimore D. 30 years of NF-κB: A blossoming of relevance to human pathobiology. *Cell*. (2017) 168:37–57. doi: 10.1016/j.cell.2016.12.012

98. Frasor J, El-Shennawy L, Stender JD, Kastrati I. NFκB affects estrogen receptor expression and activity in breast cancer through multiple mechanisms. *Mol Cell Endocrinol*. (2015) 418 Pt 3:235–9. doi: 10.1016/j.mce.2014.09.013

99. Biswas DK, Shi Q, Baily S, Strickland I, Ghosh S, Pardee AB, et al. NF-kappa B activation in human breast cancer specimens and its role in cell proliferation and apoptosis. *Proc Natl Acad Sci USA*. (2004) 101:10137–42. doi: 10.1073/pnas.0403621101

100. Van Laere SJ, van der Auwera I, Van Den Eynden GG, Elst HJ, Weyler J, Harris AL, et al. Nuclear factor-kappaB signature of inflammatory breast cancer by cDNA microarray validated by quantitative real-time reverse transcription-PCR, immunohistochemistry, and nuclear factor-kappaB DNA-binding. *Clin Cancer Res*. (2006) 12:3249–56. doi: 10.1158/1078-0432.CCR-05-2800

101. Kubo M, Kanaya N, Petrossian K, Ye J, Warden C, Liu Z, et al. Inhibition of the proliferation of acquired aromatase inhibitor-resistant breast cancer cells by histone deacetylase inhibitor LBH589 (panobinostat). *Breast Cancer Res Treat*. (2013) 137:93–107. doi: 10.1007/s10549-012-2332-x

102. Reijm EA, Jansen MP, Ruigrok-Ritstier K, Van Staveren IL, Look MP, Van Gelder ME, et al. Decreased expression of EZH2 is associated with upregulation of ER and favorable outcome to tamoxifen in advanced breast cancer. *Breast Cancer Res Treat*. (2011) 125:387–94. doi: 10.1007/s10549-010-0836-9

103. Wang X, Belguise K, Kersual N, Kirsch KH, Mineva ND, Galtier F, et al. Oestrogen signalling inhibits invasive phenotype by repressing RelB and its target BCL2. *Nat Cell Biol*. (2007) 9:470–8. doi: 10.1038/ncb1559

104. Belguise K, Sonenshein GE. PKCtheta promotes c-Rel-driven mammary tumorigenesis in mice and humans by repressing estrogen receptor alpha synthesis. *J Clin Invest*. (2007) 117:4009–21. doi: 10.1172/JCI32424

105. Park KJ, Krishnan V, O'malley BW, Yamamoto Y, Gaynor RB. Formation of an IKKalpha-dependent transcription complex is required for estrogen receptor-mediated gene activation. *Mol Cell*. (2005) 18:71–82. doi: 10.1016/j.molcel.2005.03.006

106. Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engström O, et al. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature*. (1997) 389:753–8. doi: 10.1038/39645

107. Zhu P, Baek SH, Bourk EM, Ohgi KA, Garcia-Bassets I, Sanjo H, et al. Macrophage/cancer cell interactions mediate hormone resistance by a nuclear receptor derepression pathway. *Cell*. (2006) 124:615–29. doi: 10.1016/j.cell.2005.12.032

108. Frasor J, Weaver A, Pradhan M, Dai Y, Miller LD, Lin CY, et al. Positive cross-talk between estrogen receptor and NF-kappaB in breast cancer. *Cancer Res*. (2009) 69:8918–25. doi: 10.1158/0008-5472.CAN-09-2608

109. Babina IS, Turner NC. Advances and challenges in targeting FGFR signalling in cancer. *Nat Rev Cancer*. (2017) 17:318–32. doi: 10.1038/nrc.2017.8

110. Servetto A, Formisano L, Arteaga CL. FGFR signaling and endocrine resistance in breast cancer: Challenges for the clinical development of FGFR inhibitors. *Biochim Biophys Acta Rev Cancer*. (2021) 1876:188595. doi: 10.1016/j.bbcan.2021.188595

111. Ornitz DM, Itoh N. The Fibroblast Growth Factor signaling pathway. *Wiley Interdiscip Rev Dev Biol*. (2015) 4:215–66. doi: 10.1002/wdev.2015.4.issue-3

112. Santolla MF, Maggiolini M. The FGF/FGFR system in breast cancer: oncogenic features and therapeutic perspectives. *Cancers (Basel)*. (2020) 12(10). doi: 10.3390/cancers12103029

113. Wu YM, Su F, Kalyana-Sundaram S, Khazanov N, Ateeq B, Cao X, et al. Identification of targetable FGFR gene fusions in diverse cancers. *Cancer Discovery*. (2013) 3:636–47. doi: 10.1158/2159-8290.CD-13-0050

114. Forbes SA, Beare D, Gunasekaran P, Leung K, Bindal N, Boutselakis H, et al. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. *Nucleic Acids Res*. (2015) 43:D805–11. doi: 10.1093/nar/gku1075

115. Reis-Filho JS, Simpson PT, Turner NC, Lambros MB, Jones C, Mackay A, et al. FGFR1 emerges as a potential therapeutic target for lobular breast carcinomas. *Clin Cancer Res*. (2006) 12:6652–62. doi: 10.1158/1078-0432.CCR-06-1164

116. Turner N, Pearson A, Sharpe R, Lambros M, Geyer F, Lopez-Garcia MA, et al. FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. *Cancer Res*. (2010) 70:2085–94. doi: 10.1158/0008-5472.CAN-09-3746

117. Mcleskey SW, Zhang L, El-Ashry D, Trock BJ, Lopez CA, Kharbanda S, et al. Tamoxifen-resistant fibroblast growth factor-transfected MCF-7 cells are cross-resistant *in vivo* to the antiestrogen ICI 182,780 and two aromatase inhibitors. *Clin Cancer Res*. (1998) 4:697–711.

118. Pearson A, Smyth E, Babina IS, Herrera-Abreu MT, Tarazona N, Peckitt C, et al. High-level clonal FGFR amplification and response to FGFR inhibition in a translational clinical trial. *Cancer Discovery*. (2016) 6:838–51. doi: 10.1158/2159-8290.CD-15-1246

119. Wynes MW, Hinz TK, Gao D, Martini M, Marek LA, Ware KE, et al. FGFR1 mRNA and protein expression, not gene copy number, predict FGFR TKI sensitivity across all lung cancer histologies. *Clin Cancer Res*. (2014) 20:3299–309. doi: 10.1158/1078-0432.CCR-13-3060

120. Formisano L, Stauffer KM, Young CD, Bhola NE, Guerrero-Zotano AL, Jansen VM, et al. Association of FGFR1 with ERα Maintains ligand-independent ER transcription and mediates resistance to estrogen deprivation in ER(+) breast cancer. *Clin Cancer Res*. (2017) 23:6138–50. doi: 10.1158/1078-0432.CCR-17-1232

121. Servetto A, Kollipara R, Formisano L, Lin CC, Lee KM, Sudhan DR, et al. Nuclear FGFR1 regulates gene transcription and promotes antiestrogen resistance in ER(+) breast cancer. *Clin Cancer Res*. (2021) 27:4379–96. doi: 10.1158/1078-0432.CCR-20-3905

122. Mao P, Cohen O, Kowalski KJ, Kusiel JG, Buendia-Buendia JE, Cuoco MS, et al. Acquired FGFR and FGF alterations confer resistance to estrogen receptor (ER) targeted therapy in ER(+) metastatic breast cancer. *Clin Cancer Res*. (2020) 26:5974–89. doi: 10.1158/1078-0432.CCR-19-3958

123. Formisano L, Lu Y, Servetto A, Hanker AB, Jansen VM, Bauer JA, et al. Aberrant FGFR signaling mediates resistance to CDK4/6 inhibitors in ER+ breast cancer. *Nat Commun*. (2019) 10:1373. doi: 10.1038/s41467-019-09068-2

124. Turczyk L, Kitowska K, Mieszkowska M, Mieczkowski K, Czaplinska D, Piasecka D, et al. FGFR2-driven signaling counteracts tamoxifen effect on ERα-positive breast cancer cells. *Neoplasia*. (2017) 19:791–804. doi: 10.1016/j.neo.2017.07.006

125. Hetz C, Papa FR. The unfolded protein response and cell fate control. *Mol Cell*. (2018) 69:169–81. doi: 10.1016/j.molcel.2017.06.017

126. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat Rev Mol Cell Biol*. (2012) 13:89–102. doi: 10.1038/nrm3270

127. Andruska N, Zheng X, Yang X, Helferich WG, Shapiro DJ. Anticipatory estrogen activation of the unfolded protein response is linked to cell proliferation and poor survival in estrogen receptor α-positive breast cancer. *Oncogene*. (2015) 34:3760–9. doi: 10.1038/onc.2014.292

128. Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. *Nat Rev Mol Cell Biol*. (2020) 21:421–38. doi: 10.1038/s41580-020-0250-z

129. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science*. (2011) 334:1081–6. doi: 10.1126/science.1209038

130. Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, et al. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature*. (2002) 415:92–6. doi: 10.1038/415092a

131. Davies MP, Barraclough DL, Stewart C, Joyce KA, Eccles RM, Barraclough R, et al. Expression and splicing of the unfolded protein response gene XBP-1 are significantly associated with clinical outcome of endocrine-treated breast cancer. *Int J Cancer*. (2008) 123:85–8. doi: 10.1002/ijc.v123:1

132. Ding L, Yan J, Zhu J, Zhong H, Lu Q, Wang Z, et al. Ligand-independent activation of estrogen receptor alpha by XBP-1. *Nucleic Acids Res*. (2003) 31:5266–74. doi: 10.1093/nar/gkg731

133. Hu R, Warri A, Jin L, Zwart A, Riggins RB, Fang HB, et al. NF-κB signaling is required for XBP1 (unspliced and spliced)-mediated effects on antiestrogen responsiveness and cell fate decisions in breast cancer. *Mol Cell Biol*. (2015) 35:379–90. doi: 10.1128/MCB.00847-14

134. Gupta A, Hossain MM, Miller N, Kerin M, Callagy G, Gupta S. NCOA3 coactivator is a transcriptional target of XBP1 and regulates PERK-eIF2α-ATF4 signalling in breast cancer. *Oncogene*. (2016) 35:5860–71. doi: 10.1038/onc.2016.121

135. Xu J, Wu RC, O'malley BW. Normal and cancer-related functions of the p160 steroid receptor co-activator (SRC) family. *Nat Rev Cancer*. (2009) 9:615–30. doi: 10.1038/nrc2695

136. Gomez BP, Riggins RB, Shajahan AN, Klimach U, Wang A, Crawford AC, et al. Human X-box binding protein-1 confers both estrogen independence and antiestrogen resistance in breast cancer cell lines. *FASEB J*. (2007) 21:4013–27. doi: 10.1096/fj.06-7990com

137. Ostuni R, Kratochvill F, Murray PJ, Natoli G. Macrophages and cancer: from mechanisms to therapeutic implications. *Trends Immunol*. (2015) 36:229–39. doi: 10.1016/j.it.2015.02.004

138. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell*. (2010) 141:39–51. doi: 10.1016/j.cell.2010.03.014

139. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell*. (2006) 124:263–6. doi: 10.1016/j.cell.2006.01.007

140. Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. *Immunity*. (2014) 41:49–61. doi: 10.1016/j.immuni.2014.06.010

141. Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, et al. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature*. (2011) 475:222–5. doi: 10.1038/nature10138

142. Castellaro AM, Rodriguez-Baili MC, Di Tada CE, Gil GA. Tumor-associated macrophages induce endocrine therapy resistance in ER+ Breast cancer cells. *Cancers (Basel)*. (2019) 11(2). doi: 10.3390/cancers11020189

143. Hui L, Chen Y. Tumor microenvironment: Sanctuary of the devil. *Cancer Lett*. (2015) 368:7–13. doi: 10.1016/j.canlet.2015.07.039

144. Wu Q, Li B, Li Z, Li J, Sun S, Sun S. Cancer-associated adipocytes: key players in breast cancer progression. *J Hematol Oncol*. (2019) 12:95. doi: 10.1186/s13045-019-0778-6

145. Kalluri R, Zeisberg M. Fibroblasts in cancer. *Nat Rev Cancer*. (2006) 6:392–401. doi: 10.1038/nrc1877

146. Pontiggia O, Sampayo R, Raffo D, Motter A, Xu R, Bissell MJ, et al. The tumor microenvironment modulates tamoxifen resistance in breast cancer: a role for soluble stromal factors and fibronectin through β1 integrin. *Breast Cancer Res Treat*. (2012) 133:459–71. doi: 10.1007/s10549-011-1766-x

147. Bianco P, Cao X, Frenette PS, Mao JJ, Robey PG, Simmons PJ, et al. The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. *Nat Med*. (2013) 19:35–42. doi: 10.1038/nm.3028

148. Brechbuhl HM, Finlay-Schultz J, Yamamoto TM, Gillen AE, Cittelly DM, Tan AC, et al. Fibroblast subtypes regulate responsiveness of luminal breast cancer to estrogen. *Clin Cancer Res*. (2017) 23:1710–21. doi: 10.1158/1078-0432.CCR-15-2851

149. Davis H, Raja E, Miyazono K, Tsubakihara Y, Moustakas A. Mechanisms of action of bone morphogenetic proteins in cancer. *Cytokine Growth Factor Rev*. (2016) 27:81–92. doi: 10.1016/j.cytogfr.2015.11.009

150. Todd GM, Gao Z, Hyvönen M, Brazil DP, Ten Dijke P. Secreted BMP antagonists and their role in cancer and bone metastases. *Bone*. (2020) 137:115455. doi: 10.1016/j.bone.2020.115455

151. Ren J, Smid M, Iaria J, Salvatori DCF, Van Dam H, Zhu HJ, et al. Cancer-associated fibroblast-derived Gremlin 1 promotes breast cancer progression. *Breast Cancer Res*. (2019) 21:109. doi: 10.1186/s13058-019-1194-0

152. Wu Q, Li J, Li Z, Sun S, Zhu S, Wang L, et al. Exosomes from the tumour-adipocyte interplay stimulate beige/brown differentiation and reprogram metabolism in stromal adipocytes to promote tumour progression. *J Exp Clin Cancer Res*. (2019) 38:223. doi: 10.1186/s13046-019-1210-3

153. Iwai Y, Hamanishi J, Chamoto K, Honjo T. Cancer immunotherapies targeting the PD-1 signaling pathway. *J BioMed Sci*. (2017) 24:26. doi: 10.1186/s12929-017-0329-9

154. Liu L, Shen Y, Zhu X, Lv R, Li S, Zhang Z, et al. ERα is a negative regulator of PD-L1 gene transcription in breast cancer. *Biochem Biophys Res Commun*. (2018) 505:157–61. doi: 10.1016/j.bbrc.2018.09.005

155. Rugo HS, Delord JP, Im SA, Ott PA, Piha-Paul SA, Bedard PL, et al. Safety and antitumor activity of pembrolizumab in patients with estrogen receptor-positive/human epidermal growth factor receptor 2-negative advanced breast cancer. *Clin Cancer Res*. (2018) 24:2804–11. doi: 10.1158/1078-0432.CCR-17-3452

156. Bissell MJ, Radisky D. Putting tumours in context. *Nat Rev Cancer*. (2001) 1:46–54. doi: 10.1038/35094059

157. Sampayo RG, Toscani AM, Rubashkin MG, Thi K, Masullo LA, Violi IL, et al. Fibronectin rescues estrogen receptor α from lysosomal degradation in breast cancer cells. *J Cell Biol*. (2018) 217:2777–98. doi: 10.1083/jcb.201703037

158. Huang J, Woods P, Normolle D, Goff JP, Benos PV, Stehle CJ, et al. Downregulation of estrogen receptor and modulation of growth of breast cancer cell lines mediated by paracrine stromal cell signals. *Breast Cancer Res Treat*. (2017) 161:229–43. doi: 10.1007/s10549-016-4052-0

159. Chau CH, Steeg PS, Figg WD. Antibody-drug conjugates for cancer. *Lancet*. (2019) 394:793–804. doi: 10.1016/S0140-6736(19)31774-X

160. Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, et al. Trastuzumab deruxtecan (T-DXd) versus treatment of physician's choice (TPC) in patients (pts) with HER2-low unresectable and/or metastatic breast cancer (mBC): Results of DESTINY-Breast04, a randomized, phase 3 study. *J Clin Oncol*. (2022) 40:LBA3–3. doi: 10.1200/JCO.2022.40.17_suppl.LBA3

161. Rugo HS, Bardia A, Marmé F, Cortés J, Schmid P, Loirat D, et al. LBA76 Overall survival (OS) results from the phase III TROPiCS-02 study of sacituzumab govitecan (SG) vs treatment of physician's choice (TPC) in patients (pts) with HR+/HER2- metastatic breast cancer (mBC). *Ann Oncol*. (2022) 33:S1386. doi: 10.1016/j.annonc.2022.08.012

162. Rugo HS, Bardia A, Marmé F, Cortés J, Schmid P, Loirat D, et al. Overall survival with sacituzumab govitecan in hormone receptor-positive and human epidermal growth factor receptor 2-negative metastatic breast cancer (TROPiCS-02): a randomised, open-label, multicentre, phase 3 trial. *Lancet*. (2023) 402:1423–33. doi: 10.1016/S0140-6736(23)01245-X

163. Bardia A, Jhaveri K, Im SA, Simon SP, De Laurentiis M, Wang S, et al. LBA11 Datopotamab deruxtecan (Dato-DXd) vs chemotherapy in previously-treated inoperable or metastatic hormone receptor-positive, HER2-negative (HR+/HER2&x2013); breast cancer (BC): Primary results from the randomised phase III TROPION-Breast01 trial. *Ann Oncol*. (2023) 34:S1264–5. doi: 10.1016/j.annonc.2023.10.015

164. Kalinsky K, Diamond JR, Vahdat LT, Tolaney SM, Juric D, O'shaughnessy J, et al. Sacituzumab govitecan in previously treated hormone receptor-positive/HER2-negative metastatic breast cancer: final results from a phase I/II, single-arm, basket trial. *Ann Oncol*. (2020) 31:1709–18. doi: 10.1016/j.annonc.2020.09.004

165. Menendez JA, Lupu R. Fatty acid synthase regulates estrogen receptor-α signaling in breast cancer cells. *Oncogenesis*. (2017) 6:e299. doi: 10.1038/oncsis.2017.4

166. Jones SF, Infante JR. Molecular pathways: fatty acid synthase. *Clin Cancer Res*. (2015) 21:5434–8. doi: 10.1158/1078-0432.CCR-15-0126

167. Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat Rev Cancer*. (2007) 7:763–77. doi: 10.1038/nrc2222

168. Kuhajda FP, Jenner K, Wood FD, Hennigar RA, Jacobs LB, Dick JD, et al. Fatty acid synthesis: a potential selective target for antineoplastic therapy. *Proc Natl Acad Sci USA*. (1994) 91:6379–83. doi: 10.1073/pnas.91.14.6379

169. Gruslova A, Mcclellan B, Balinda HU, Viswanadhapalli S, Alers V, Sareddy GR, et al. FASN inhibition as a potential treatment for endocrine-resistant breast cancer. *Breast Cancer Res Treat*. (2021) 187:375–86. doi: 10.1007/s10549-021-06231-6

170. Hu X, Huang W, Fan M. Emerging therapies for breast cancer. *J Hematol Oncol*. (2017) 10:98. doi: 10.1186/s13045-017-0466-3

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 15 November 2024
DOI 10.3389/fendo.2024.1397839



OPEN ACCESS

EDITED BY
Zili Zhang,
Nanjing University of Chinese Medicine, China

REVIEWED BY
Antonio d'Amati,
University of Bari Aldo Moro, Bari, Italy
Reetobrata Basu,
Ohio University, United States

*CORRESPONDENCE
Paramita Baruah
paramita.baruah@nhs.net

RECEIVED 08 March 2024
ACCEPTED 29 August 2024
PUBLISHED 15 November 2024

CITATION
Baruah P, Marshall JL, Nefla M, Pucino V, Adams H, Turner JD, Gilbert S, Powell E, Neag G, Monksfield P, Irving RM, Croft AP, Dumitriu IE and Buckley CD (2024) Functional analysis of fibroblasts and macrophages in head and neck paragangliomas.
*Front. Endocrinol.* 15:1397839.
doi: 10.3389/fendo.2024.1397839



# Functional analysis of fibroblasts and macrophages in head and neck paragangliomas

Paramita Baruah[1,2,3]*, Jennifer L. Marshall[3], Meriam Nefla[3], Valentina Pucino[3], Holly Adams[3], Jason D. Turner[3], Sebastian Gilbert[4], Emily Powell[3], Georgiana Neag[3], Peter Monksfield[2], Richard M. Irving[2], Adam P. Croft[3], Ingrid E. Dumitriu[5] and Christopher D. Buckley[3]

[1]Department of Ear Nose and Throat (ENT), University of Leicester National Health Service (NHS) Trust, Birmingham, United Kingdom, [2]Department of Ear Nose and Throat (ENT), Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom, [3]Institute of Inflammation and Ageing, University of Birmingham, Birmingham, United Kingdom, [4]Birmingham Tissue Analytics, University of Birmingham, Birmingham, United Kingdom, [5]Cardiovascular Research Institute, University of Birmingham, Birmingham, United Kingdom

**Background and aim:** Head and neck paragangliomas (HNPGN) are tumours that carry significant morbidity The role of the stroma in the pathogenesis of HNPGN is not completely understood. This study explores the profile of fibroblasts and macrophages in HNPGN.

**Methods:** Ten patients undergoing HNPGN surgery were recruited. CD68 and CD163 immunohistochemistry was performed for macrophage analysis; CD90 and podoplanin (PDPN) expression was examined to identify fibroblasts. RT-qPCR was performed on HNPGN tissue for macrophage- and fibroblast-associated molecules. Fibroblast cultures were established from HNPGN were analysed by RT-qPCR and flowcytometry. Confocal microscopy for MCT1 and MCT4 was performed in HNPGN.

**Results:** CD68 and CD163 expressing macrophages were noted in HNPGN. CD90 and PDPN expressing cells were present in HNPGN. RT-qPCR analysis showed expression of phenotypic and functional macrophage- and fibroblast-associated molecules in HNPGN. RT-qPCR analysis of fibroblasts cultured from HNPGN confirmed the expression of several molecules including PDPN at comparable levels to healthy tissue fibroblasts. Expression of FAP, MCT-1, insulin receptor (CD220) and insulin growth factor receptor-2 (CD222) was noted on HNPGN derived fibroblasts on flowcytometry. MCT1 and MCT4 were expressed in HNPGN tumour cells and stromal macrophages *in-situ*.

**Conclusion:** Fibroblasts and macrophages are present in the HNPGN tumour microenvironment, and several macrophage and fibroblast functional markers

are expressed in HNPGN. Macrophages in HNPGN tissue express metabolic markers MCT1 and MCT4. Further analysis of the fibroblast and macrophage function in HNPGN will improve our understanding of their potential roles in tumour pathogenesis.

KEYWORDS

fibroblasts, macrophages, head and neck paraganglioma, CD90, CD163, MCT1, MCT4

# Introduction

Head and neck paragangliomas (HNPGNs) are neoplasms of the autonomic system arising from neural crest derived cells of the parasympathetic paraganglia of the skull base and neck (1). Also called glomus tumours, HNPGNs can arise in relation to the jugular vein (glomus tympanicum and glomus jugulare), cranial nerves (e.g. vagal paraganglioma) and the carotid artery (carotid body tumour). HNPGNs are usually slow growing tumours but their location at the base of the skull and propensity for intracranial expansion cause considerable morbidity (stroke, cranial nerve palsies with speech and swallowing impairment, facial palsy, hearing loss), sometimes with fatal outcomes. Growing HNPGNs are traditionally managed via surgery and/or radiotherapy, which carry significant morbidity and mortality due to proximity to the great vessels of the neck and several cranial nerves. It is therefore imperative to develop newer treatments to improve outcomes in patients with HNPGNs. In addition to the challenges described above in treating HNPGNs, their clinical behaviour is difficult to assess and predict. The benign versus malignant nature of HNPGNs remains a clinical conundrum as it cannot be determined based solely on histology or current imaging methods. Over 15% of HNPGNs are malignant but are discovered late in the course of the disease when patient develops metastases. Further diagnostic challenges arise when a patient presents with multiple paragangliomas simultaneously and it becomes difficult to determine if these are individual primary tumours or metastatic foci. The presence of regional metastases is associated with less than 60% survival rates and distant metastases further worsen the prognosis. The etiopathogenesis of HNPGNs is not completely understood. They can be sporadic but up to 30-50% of HNPGNs are hereditary and associate with germline mutations in various genes. Of note, about 50% of these mutations involve the succinate dehydrogenase (SDH) gene, which codes an enzyme integral to the Krebs cycle (1). Very little is known of the cellular and molecular mechanisms, and in particular the role of the stromal microenvironment in regulating HNPGN pathogenesis. A better understanding of the biology of the HNPGN tumour microenvironment is likely to reveal markers of growth and malignant behaviour and thereby enable early detection, targeted therapies and prevention of recurrences and metastases.

Tumour stroma has been shown to play a profound role in regulating tumour progression by supporting angiogenesis, tumour cell proliferation, invasion, metastasis and mechanisms of resistance to treatment (2). The role of the stromal microenvironment in HNPGN pathogenesis and, specifically, their functional profile has not been addressed so far. Tissue resident fibroblasts and macrophages are important components of tumour stroma. We have previously shown that VEGF, a pro-angiogenic factor, localises mainly in the stroma of HPV-positive head and neck cancers (3) and that HPV-positive cancer head and neck cancer cells can up-regulate programmed death ligands PD-L1 and PD-L2 on fibroblasts (4). We have also shown that fibroblasts derived from vestibular schwannomas exhibit a pro-tumorigenic profile (5). Tissue resident macrophages are known to undergo metabolic reprogramming in an inflammatory environment via the succinate dehydrogenase (SDH) pathway (6). This is of particular relevance in hereditary HNPGNs that display SDH mutations as alterations in cellular metabolism could drive HNPGN initiation and progression. There is evidence that cancer associated fibroblasts and macrophages undergo metabolic reprogramming, which supports tumour progression (7). Stromal cells may thus have hitherto unknown roles in the progression of HNPGN. In this study we show expression of fibroblast markers CD90 and PDPN in HNPGN tissue *in-situ* as well as presence of CD163 expressing macrophages. We isolate fibroblasts from HNPGN and confirmed the expression of CD90 *in vitro*. We further identified the expression of monocarboxylate transporters MCT1 in HNPGN derived fibroblasts by flowcytometry and the expression of MCT1 and MCT4 in tumour cells and macrophages *in situ* in HNPGN tissue. Further dissection of the phenotypic and functional profile of fibroblasts and macrophages in HNPGN will help decipher the tumour microenvironment in HNPGN with the aim of uncovering new risk stratification and therapeutic targets.

# Materials and methods

## Patient demographics

Ethical approval for the study was obtained from the University of Birmingham research ethics committee (Human Biomaterials Resource Centre HBRC 17-295) and the tissue samples were released via HBRC. Patients were recruited into the study

following informed consent. HNPGN tissue was collected from ten patients undergoing tumour excision. Healthy tissue from the ear (mastoid mucosa and ear canal skin) was collected from 6 patients to culture fibroblasts. The diagnosis of HNPGN in the patients was made on clinical grounds and confirmed by histopathology in the Department of Pathology, University of Birmingham NHS Trust. The age range of the patients at surgery was 26-72 years with a mean of 43.2 years; 3 of the tissue samples originated from male and 7 from female patients. Clinical data obtained included patient age at operation, gender, SDH mutation status staging of the disease and clinical outcomes and is summarized in Supplementary Table S1.

## Human tissue processing and histology

Tumour tissue samples were frozen in Tissue-Tek OCT medium or formalin fixed and paraffin embedded (FFPE). For immunohistochemistry, antigen retrieval was performed at pH9. Sections were stained using polyclonal sheep anti-human CD90 (AF2067, R&D) and rat anti-human podoplanin (PDPN) (Clone NZ-1.3, eBioscience), Mouse anti-CD163 (clone 10D6, Leica) or Mouse anti-CD68 (Leica, clone 514H12) for one hour at room temperature. For the DABs and Red staining, Leica Bond Polymer Refine Detection systems were used. Nuclei were counterstained with Hematoxylin QS (Vector Laboratories). Images were acquired using the Zeiss Axio Scan and analysed with QuPath software (v0.4.3). Visiopharm quantitative analysis was performed to threshold the DAB and FsstRed on images, after thresholding for Haematoxylin. Marker positive area (in $\mu m^2$) and nuclei area (in $\mu m^2$) were measured, and the ratio calculated for each sample studied. Co-relation analyses were performed based on tumour stage/gender and mutation status.

For the MCT1/MCT4 and CD90/CD68 staining, after the antigen retrieval step (45 minutes) and block of non-specific binding (1 hour), paraffin-embedded tissue sections were incubated at 4°C for 1 hour with the following antibodies: rabbit polyclonal anti-MCT1(1:300, Bethy), mouse monoclonal anti-MCT4 (1:100, Santa Cruz), polyclonal sheep anti-CD90 (1:100, R&D), biotin anti-CD68 (1:100, Novus). Streptavidin Alexa Fluor™ 594, donkey anti-sheep IgG Alexa Fluor™ 546, donkey anti-Rabbit IgG AlexaFluor™ 488, donkey anti-Mouse IgG Alexa Fluor™ 647 were used as secondary antibodies (1:300). Hoechst was used for staining nuclei. Slides were mounted with Prolong Gold Antifade reagent (Invitrogen). Images were acquired on a confocal microscope (Zeiss LSM 780) and analysed using Zen software.

## Human fibroblast cell culture

Primary human fibroblasts were isolated as described (8, 9) and cultured in RPMI (Sigma-Aldrich) with heat inactivated 10% foetal calf serum (FCS; Labtech International, Sussex, UK), L-glutamine, Sodium Orthopyruvate (Sigma Aldrich), antibiotics (penicillin and streptomycin), and MEM non-essential amino acids (Sigma Aldrich). Passage 1 fibroblast lines at were used for the RT-qPCR experiments. Flowcytometry was performed on fibroblasts at passages 2-5.

## Quantitative reverse transcription PCR

RT-qPCR was carried out using customised macrophage and fibroblast panels (Applied Biosystems). RNA was isolated from frozen tissue or frozen fibroblasts at passage 1 using the RNAeasy RNA isolation kit (Qiagen) according to the manufacturer's instructions. cDNA synthesis was performed on all samples (500 ng of RNA was transcribed) using SensiFAST cDNA Synthesis Kit (Bioline) on a Mastercycler (Eppendorf) thermal cycler PCR machine. Reverse transcription with quantitative PCR (RT –qPCR) was performed using a Taqman Gene Expression array and Taqman universal Mastermix on the ABI 7900 real-time PCR detection system (both Applied Biosystems) and using the TaqMan Array Microfluidic Card. Expression levels were normalized to an internal housekeeping gene (GAPDH) and a relative amount of expression for genes of interest was calculated form the delta CT to the housekeeping gene ($2^{-}\Delta CT$) The primers used in the arrays (Fibroblast panel) were: EGF-Hs01099999_m1, FGF2-Hs00266645_m1, TP53BP2-Hs00610488_m1, HTR2A-Hs00167241_m1, TNS3-Hs00224228_m1, EPAS1-Hs01026149_m1, HIF1A-Hs00153153_m1, PADI4-Hs00202612_m1, MYC-Hs00153408_m1, RAF1-Hs00234119_m1, SYVN1-Hs00381211_m1, INHBA-Hs00170103_m1, CXCL12-Hs00171022_m1, GAPDH-Hs99999905_m1, B2M-Hs00187842_m1, VCAM1-Hs01003372_m1, CD248-Hs00535586_s1, PDPN-Hs00366766_m1, LGALS1-Hs00355202_m1, LGALS9-Hs00371321_m1, LGALS3-Hs00173587_m1, LGALS12-Hs00263821_m1, CXCL16-Hs00222859_m1, 18S-Hs99999901_s1, MMP1-Hs00899658_m1, MMP2-Hs01548727_m1, MMP3-Hs00968308_m1, MMP9-Hs00234579_m1, MMP13-Hs00233992_m1, CTSL1-Hs00377632_m1, CTSB-Hs00947433_m1, SUMO1-Hs02339312_g1, S100A4-Hs00243201_m1, DKK1-Hs00183740_m1, NAMPT-Hs00237184_m1, MMP14-Hs00237119_m1, CTSK-Hs00166156_m1, IGF2-Hs00171254_m1, ACTA2-Hs00909449_m1, TLR2-Hs01014511_m1, TLR3-Hs00152933_m1, TLR4-Hs00152939_m1, TNFSF11-Hs00243522_m1, LGALS3BP-Hs00174774_m1, SLIT3-Hs00171524_m1, TRERF1-Hs00363301_m1, SHOX-Hs00230846_m1, PIAS1-Hs00184008_m1, EGF-Hs01099999_m1. For the macrophage RT-qPCR panel the primers used in the arrays were for the following genes of interest: STAT-1, ALOX15, INHBA, CCL2, CCL5, IL8, CxCL10, CD64, SPl1, CD32, IL6, IL10, TNF, IL1b, RANK, MRC1, PTPRC, EpCAM, ACP5, CTSK, CD68, RPL13A, MERTK, HLADRA, CD163, CD14, FN1, Thy1(CD90), PDPN, CD80, CD16b, MCT4, MCT3, Cav1, HIF1a, CSF1R, CSF2RA and IDO.

## Flow-cytometry

For detection of CD90 and PDPN, cultured fibroblasts were detached by incubation with Trypsin/EDTA (Sigma-Aldrich) at 37°C, followed by washes in culture medium. Cells were washed several times in PBS with 2% FCS and stained with the following antibodies: anti-CD90 (PerCP-Cy5.5 conjugated (eBio5E10), eBioscience), PDPN (PE conjugated (NZ-1.3), eBioscience) and anti-FAP (Fibroblast Activation Protein) (sheep anti-human, R&D). Staining was also performed with MCT1 (Rabbit polyclonal, Bethyl), MCT4 (D-1, Santa Cruz), PE anti-human CD220 (clone REA260, Miltenyi Biotec) and PE anti-human CD222 (clone REA187, Miltenyi Biotec). Fixable Viability Stain 575V (BD Biosciences) was used to exclude dead cells from the analysis. Samples were acquired on a Beckman Coulter CytoFLEX flow cytometer and data analysis was performed using FlowJo software version 10.

## Statistical analysis

Statistical analysis was performed using GraphPad Prism software version 10.0.3. RT-qPCR data on fibroblasts derived from HNPGN and normal tissue were compared using two tailed Mann-Whitney test. Correlation analysis of expression level with tumour stage were performed with the Spearman's test. Analysis of expression level with gender or mutation status was performed with Mann-Whitney test. Probability values (p) of less than 0.05 were considered statistically significant.

# Results

## Fibroblasts and macrophages in HNPGN tissue on immunohistochemistry

We first evaluated the presence of macrophages and fibroblasts in HNPGN tissue from ten patients using immunohistochemistry (IHC). CD68 and CD163 staining was performed as they are canonical macrophage markers- CD68 is a pan macrophage marker and CD163 is a M2 macrophage marker (10). CD68 expression was noted in HNPGN indicating macrophage infiltration (Figure 1A, middle panel). Furthermore, CD163 expression was noted in all ten macrophage samples (Figure 1; Supplementary Figure S1). Double staining with CD68 and CD163 on immunohistochemistry was performed and co-localisation noted indicating M2 polarisation of macrophages in HNPGN (Figure 1B). We next examined the expression of CD90 and podoplanin (PDPN), both of which are expressed by fibroblasts (11). CD90 expression was noted in elongated nucleated cells in stromal areas of the HNPGN in keeping with fibroblasts. Similarly, PDPN expression was noted in cells in the stromal region (Figure 2). The two markers were however not found to co-localise. A quantitative analysis of expression of CD163, CD90 and PDPN was performed and was analysed for correlation with stage, gender and mutational status. No significant correlation was found with the tumour stage and expression of CD163, CD90 and PDPN in this cohort (CD90, r=0.04, p=0.9; PDPN, r = -0.4, p=0.19; CD163, r=0.27, p=0.43). Further comparison of the expression of

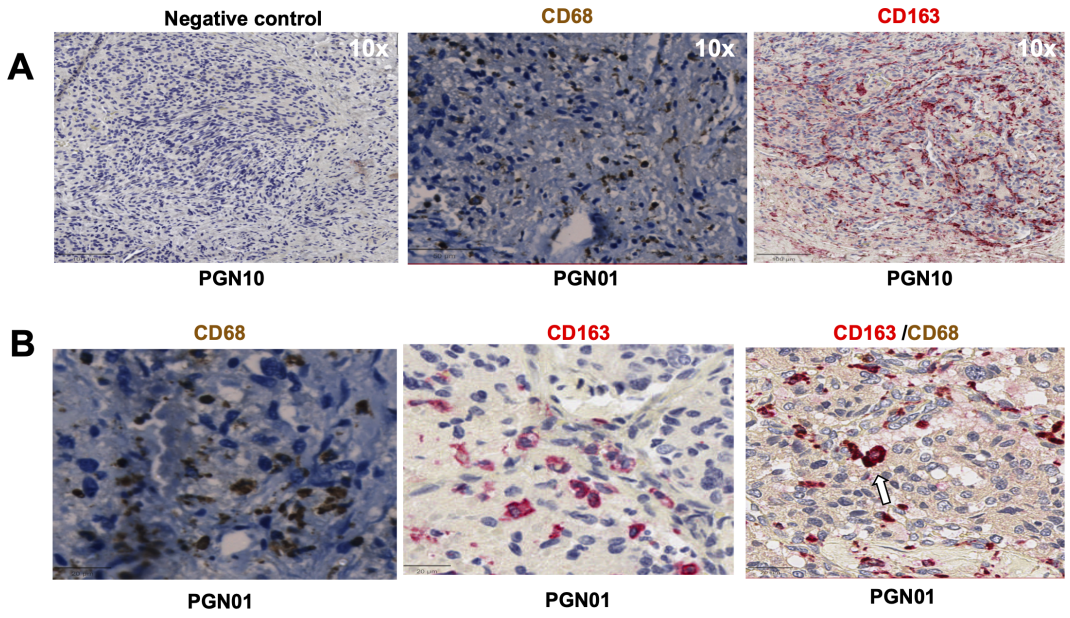



FIGURE 1
Expression of CD68, CD163 in head and neck paraganglioma (HNPGN) tissue on immunohistochemistry. Immunohistochemistry images of HNPGN tissue (representative of n = 10 HNPGN patient samples). **(A)** Control antibody staining (left panel, negative control, patient sample PGN10), CD68 staining (middle panel, patient sample PGN01) and CD163 staining (right panel, patient sample PGN10). CD68 is in dark brown, CD163 is in red and nuclei are blue. **(B)** Double staining with CD68 (dark brown) and CD163 (pink). CD68/CD163 double co-expressing macrophages (arrow). Images are at a magnification of 20x.

these markers versus gender and mutation status did not show significant differences (Supplementary Figure S4).

## Fibroblast and macrophage related markers in HNPGN tissue on RT-qPCR

Having confirmed the presence of macrophages and fibroblasts in HNPGN tissue we performed RT-qPCR on fresh frozen HNPGN tissue to study a panel of fibroblast-associated and macrophage-associated markers (details in Materials and Methods). PDPN (Supplementary Figure S5, upper panel) and CD90 (Thy 1) (Supplementary Figure S5, lower panel) were also found to be expressed in HNPGN tissue on RT-qPCR. Similarly, macrophage markers CD68 and CD163 were expressed in HNPGN tissue on RT-qPCR (Supplementary Figure S5, lower panel). In addition, several molecules belonging to the Galectin family (LGALS1, LGALS3, LGALS9, LGALS3BP), Toll-like receptors (TLR2/TLR4) and matrix metalloproteinases (MMP2/MMP9/MMP14) were expressed in HNPGN tissue (Supplementary Figure S5, upper panel). HNPGN associates with SDH mutations which could result in metabolic changes within the HNPGN microenvironment. In keeping with this, markers of cellular metabolism such as MCT4 and HIF-1α were found to be

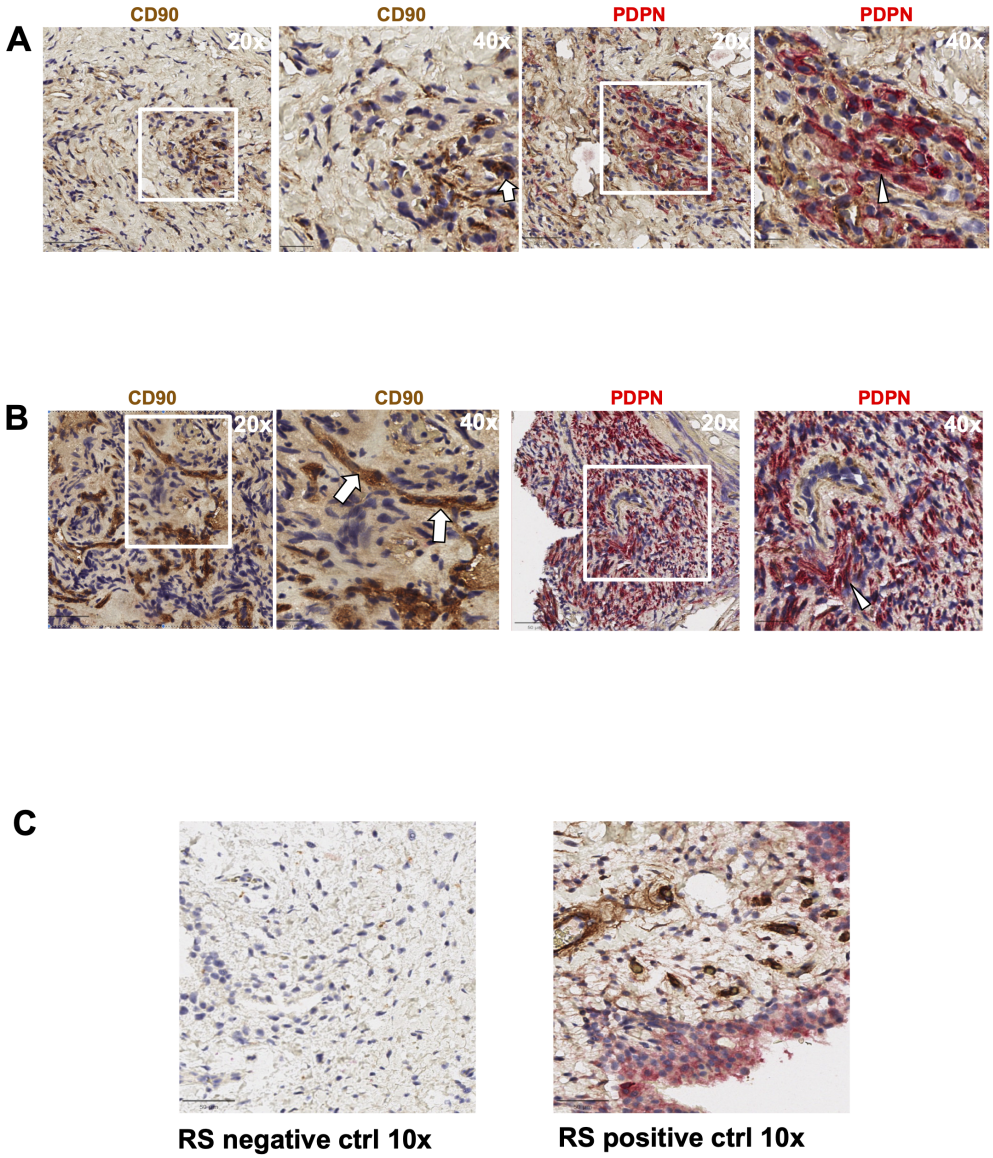



FIGURE 2
Expression of PDPN and CD90 in head and neck paraganglioma (HNPGN) tissue on immunohistochemistry. Immunohistochemistry images of HNPGN tissue. CD90 is in brown and podoplanin (PDPN) is in pink, nuclei are blue. **(A)** PDPN and CD90 expression in HNPGN sample from patient PGN010. Left panel is at 20x magnification while the right panel show 40x magnification of the tissue area in the white box inset. **(B)** PDPN and CD90 staining as above in HNPGN tissue from patient PGN09. Arrows point to CD90 stained cells and arrowheads to the PDPN stained cells **(C)** Control staining (left panel) and positive staining (right panel) with CD90 (brown) and PDPN (pink) is displayed in tissue from synovium in rheumatoid arthritis (RA).

expressed in HNPGN tissue on RT-qPCR (Supplementary Figure S5, lower panel).

## Fibroblast related markers in HNPGN derived fibroblasts on RT-qPCR

We next compared fibroblasts derived from HNPGN to healthy tissue (mastoid mucosa and/or ear canal skin) fibroblasts to verify the expression of fibroblast- associated molecules that were identified in the RT-qPCR in HNPGN tissue. For this we first cultured fibroblasts from HNPGN tissue (described in Materials and Methods). Fibroblast cultures were successfully established from all the ten HNPGN samples (Supplementary Figure S6A left panel). Fibroblast cultures were also successfully established from mastoid mucosa and/or ear canal skin (healthy tissue) from six patients (Supplementary Figure S6A right panel). RT-qPCR analysis was undertaken on the cultured HNPGN fibroblasts and healthy tissue fibroblasts with the panel of fibroblast markers performed previously on the HNPGN tissue. Results from paired samples (HNPGN and normal tissue fibroblasts from the same patient) of tumour and healthy tissue fibroblasts are presented (Supplementary Figure S6A). Levels of LGALS1 and MMP14 showed a higher expression trend in HNPGN fibroblasts but did not reach significance. Other molecules such as LGALS9, LGALS3BP, MMP2 etc were similar in the two groups.

## Fibroblast associated markers in HNPGN derived fibroblasts on flow-cytometry

We next evaluated expression of fibroblast markers on the cultured cells using flowcytometry. In keeping with our immunohistochemistry findings, cultured fibroblasts from

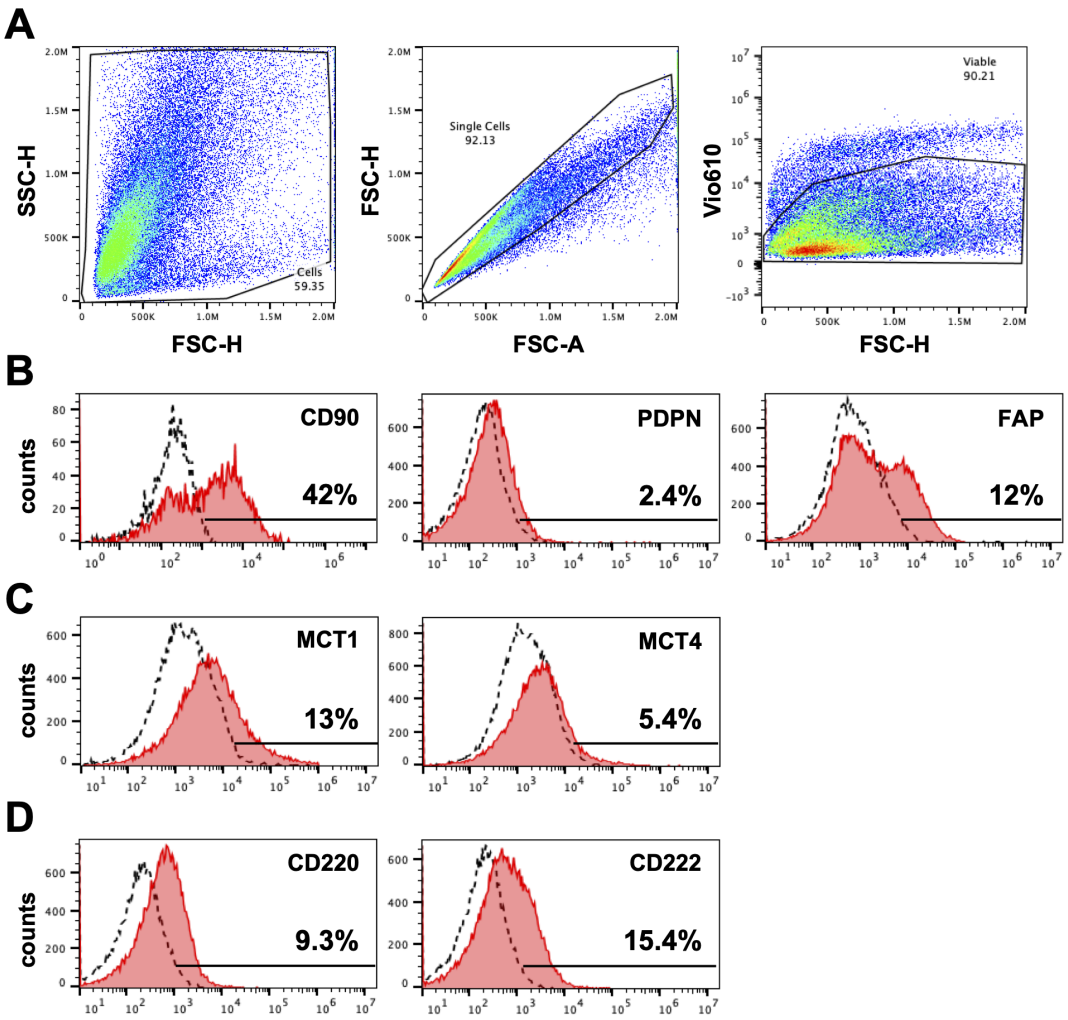



FIGURE 3
Expression of markers on fibroblasts derived from head and neck paraganglioma (HNPGN). Expression on fibroblast associated markers were examined by fibroblasts cultured from HNPGN using flow cytometry. **(A)** The sequential gating strategy is illustrated based on forward scatter and side scatter (left panel), exclusion of doublets (middle panel) and exclusion of dead cells (Fixable viability stain 575V negative population, right panel). **(B)** Illustrative histogram plots depicting the expression of CD90, PDPN and FAP (pink histograms). Dashed histograms show staining with isotype control antibodies. **(C)** Illustrative histogram plots showing the expression of MCT1 and MCT4 (pink histograms). **(D)** Illustrative histogram plots showing expression of CD220 AND CD222 (pink histograms). Dashed histograms depict staining with isotype control antibodies. Representative of fibroblasts from three different HNPGN samples.

HNPGN were found to express CD90 (fibroblast marker) on flowcytometry (Figure 3) Very low PDPN expression was noted in the cultured fibroblasts (Figure 3). FAP (Fibroblast activation protein) expression was observed in a fraction of the cultured fibroblasts. As HNPGN is frequently associated with SDH mutations and we found MCT4 expression in HNPGN tissue on RTPCR, we also examined the cultured fibroblasts for expression of members of the metabolic pathway. Approximately 13% of the cultured fibroblast population expressed metabolic marker monocarboxylate transporter-1 (MCT1) while monocarboxylate transporter-4 (MCT4) expression was lower. Similarly, about 10% of cultured fibroblasts expressed insulin receptor (CD220) and about 15% expressed insulin-like growth factor 2 receptor (CD222) (Figure 3).

## Expression of metabolic markers MCT1 and MCT4 in HNPGN tissue

MCT1 and MCT4 are plasma membrane transporters involved in the transport of lactate and pyruvate (12). We found expression of MCT4 in HNPGN tissue on RT-qPCR (Supplementary Figure S2). In addition, we found MCT1 expression in fibroblasts derived from HNPGN on flowcytometry (Figure 3). We therefore examined the presence of MCT1 and MCT4 in HNPGN tissue using immunofluorescence. Three samples from HNPGN patients with germline SDH mutations and 3 samples from HNPGN patients without SDH mutations were analysed. Both MCT1 and MCT4 were noted in all the six HNPGN tissue (Figures 4, 5). MCT1 and MCT4 was found to be present in both tumour and stromal regions.

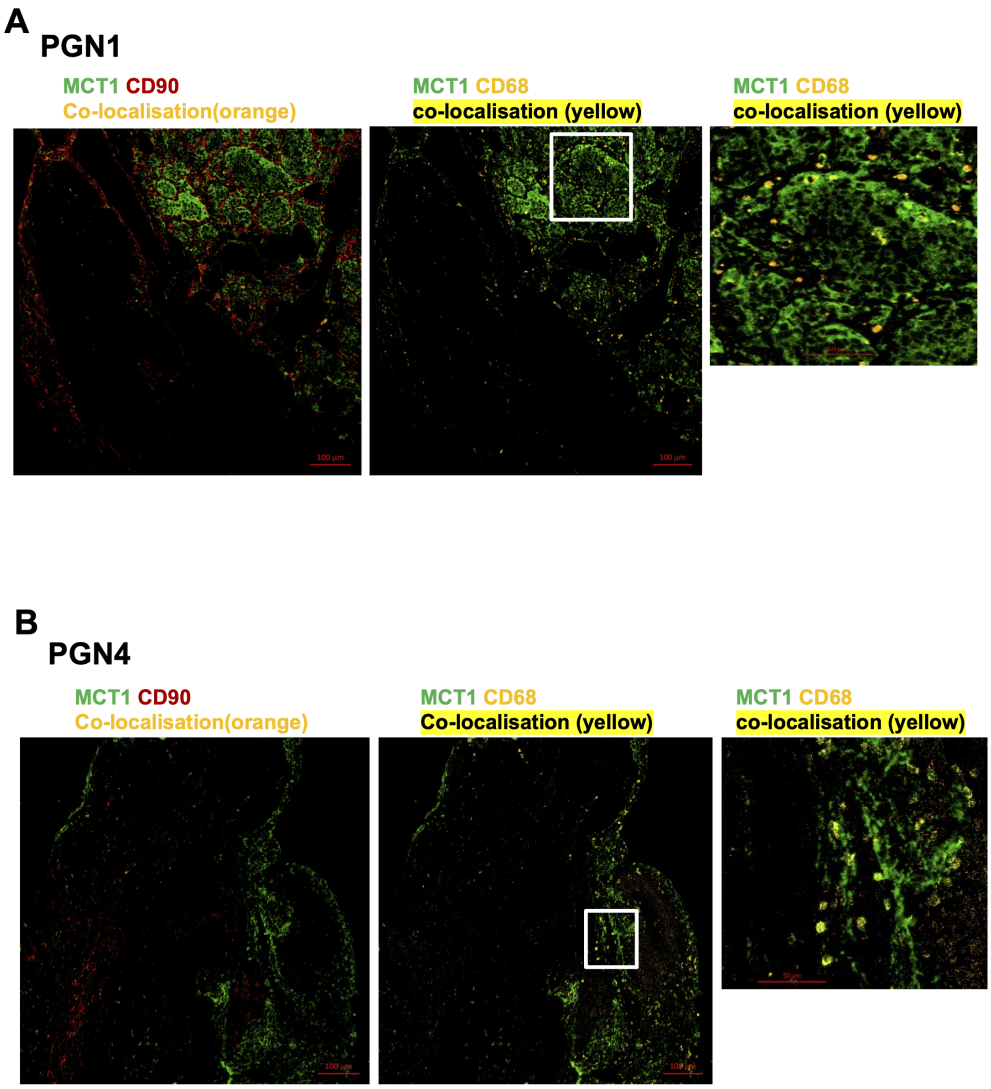



FIGURE 4
Expression of Monocarboxylate transporter 1 (MCT1) in head and neck paraganglioma (HNPGN). HNPGN tissue was stained with fluorochrome-labelled antibodies to assess lactate transporter MCT expression. **(A)** Representative immunofluorescence staining of MCT1 (green) expression in HNPGN tissue from patient PGN01 (no SDH mutation). Fibroblasts are in red (CD90) and macrophages in orange (CD68). MCT1 co-localisation with CD68 is in yellow. Right side small panel is a magnified image of the tissue area in the white box inset showing CD68 and MCT1 co-expressing macrophages in yellow. (n=3 HNPGN tissue). **(B)** Representative immunofluorescence staining of MCT1 (green) expression in HNPGN tissue from patient PGN04 (with SDH mutation). Staining as in **(A)**. (n=3 HNPGN tissue).

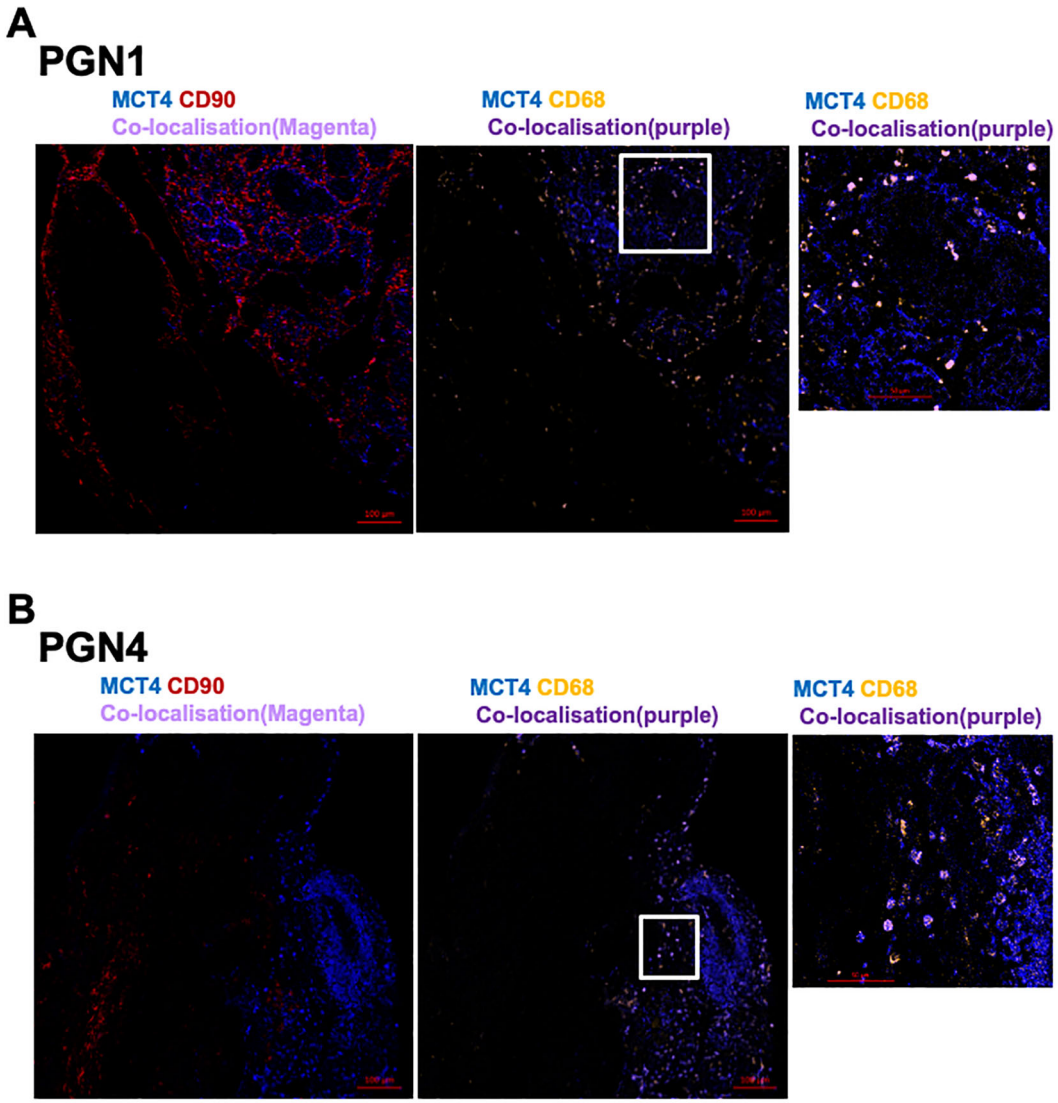



FIGURE 5
Expression of Monocarboxylate transporter 4 (MCT4) in head and neck paraganglioma (HNPGN). HNPGN tissue was stained with fluorochrome-labelled antibodies to assess lactate transporter MCT4 expression. **(A)** Representative immunofluorescence staining of MCT4 (blue) expression in HNPGN tissue from patient PGN01 (no SDH mutation). Fibroblasts are in red (CD90) and macrophages in orange (CD68). MCT4 co-localisation with CD68 is in purple. Right side small panel is magnified image of the tissue area in the white box inset showing CD68 and MCT4 co-expressing macrophages (in purple). (n=3 HNPGN tissue). **(B)** Representative immunofluorescence staining of MCT4 (blue) expression in HNPGN tissue from patient PGN04 (with SDH mutation). Staining as in **(A)** (n=3 HNPGN tissue).

No colocalization was observed between MCT1 and MCT4 indicating that the two transporters are expressed in different cell subsets in HNPGN. Further co-localization analysis was performed with concomitant staining with CD68 (macrophage marker) and CD90 (fibroblast marker). Good colocalization of MCT4 with CD68 was observed in all six tissue samples stained indicating that macrophages expressed MCT4 in HNPGN tissue *in situ* (Figure 5). MCT1 colocalization with macrophages was observed in two of the three patients with SDH mutations (Figure 4B, right panels and data not shown) while none of the samples from patients without SDH mutations showed MCT1 expression in macrophages. In keeping with the low expression of MCT1 and MCT4 on the cultured fibroblasts, limited co-localisation was observed between MCT1/MCT4 and CD90 *in-situ*.

## Discussion

This study reports on macrophage and fibroblast profile in the stromal microenvironment in HNPGN. Here we demonstrate several fibroblast related molecules such as podoplanin and CD90 in HNPGN tissue. We also successfully cultured CD90 expressing fibroblasts from HNPGN. In addition, we demonstrate the presence of M2 macrophages and show expression of metabolic markers MCT1 and MCT4 within macrophages and in tumour cells in HNPGN. To the best of our knowledge this is the first report examining the expression profile of functional and metabolic markers on fibroblasts and macrophages in HNPGN.

CD90, a molecule expressed in fibroblasts, was found both in HNPGN tissue and in the fibroblasts cultured from HNPGN. CD90

or Thy 1 is a small membrane glycophosphatidylinositol (GPI) anchored protein (13) that is involved in cell-cell and cell-matrix interactions, with specific roles in fibroblast proliferation and migration in wound healing, inflammation and fibrosis (14). It is also important to highlight that fibroblasts are a heterogenous cell type and that there is no marker exclusive to fibroblasts. Other than fibroblasts, CD90 is expressed in several cell types including T cells, neurons and pericytes (15, 16). Our immunohistochemistry results as well as the flowcytometry expression of CD90 on cultured HNPGN fibroblasts suggest that CD90 is a good marker to identify fibroblasts in HNPGN; however, its precise functional role in fibroblasts in HNPGN remains to be elucidated. In addition, we found expression of podoplanin (PDPN), another putative fibroblast marker in HNPGN tissue on immunohistochemistry. PDPN is a mucin-type transmembrane glycoprotein of 38-kDa molecular weight and can be demonstrated in a variety of normal cells, e.g. peritoneal mesothelial cells, follicular dendritic cells in lymphoid tissue (17) and is known to be expressed in various tumours (e.g. germ cell tumours and squamous cell carcinoma) (18). Of note PDPN positive fibroblasts are associated with poorer prognosis in some tumours such as lung cancers (19). It is interesting that on immunohistochemistry we found CD90 and PDPN were both expressed in non-lymphatic areas but did not co-localise in HNPGN microenvironment. This suggests that CD90 and PDPN are expressed on different cellular subsets, perhaps different fibroblast subsets in HNPGN. A report in literature suggests that PDPN may be expressed on macrophages and could promote tumour invasion (20). While CD90 expression was noted in cultured fibroblasts, PDPN expression was low in cultured fibroblasts on flowcytometry, which could potentially mean that cultured fibroblasts lose PDPN expression. Fibroblasts derived from HNPGN were also found to express FAP. While cancer associated fibroblasts express many markers, 90% of epithelial tumours show increased expression of FAP in the stroma (21) and this is associated with increased local tumour invasion, increased risk of lymph node metastasis and decreased survival (22). PDPN and FAP expression may have relevance in HNPGN behaviour and will be explored in further large-scale studies. We also found expression of insulin receptor (IR) and insulin growth factor 2-receptor (IGF2-R) on fibroblasts cultured from HNPGN. IR is known to be overexpressed in breast cancer (23), while IGF2-R is correlated to poor prognosis in patients with triple negative breast cancer (24) but their roles in HNPGN is unknown. Of note, overexpression of the related insulin-like growth factor 1 receptor (IGF-1R) has been associated with malignancy in familial pheochromocytomas and paragangliomas (25). However, the specific roles of insulin and insulin growth factors receptors in fibroblasts in HNPGN have not been studied so far.

The presence of CD163 and CD68 positive macrophages in pheochromocytoma and extra adrenal abdominal paragangliomas has been reported previously (26). A recent study has examined immune cell infiltrate in pheochromocytoma and peripheral paragangliomas and reported the presence of macrophages, with the density of the macrophage infiltrate being linked to the aggressive nature of the tumour (27). The latter study however had a small number of HNPGN (2 out of 65 tumours studied) and further subsite delineation of the HNPGN was not described. We show the presence of consistent macrophage infiltration on immunohistochemistry in a larger cohort of HNPGN, and these macrophages displayed a predominantly M2 phenotype as evidenced by CD163 expression. In our cohort of 10 patients, we did not see any correlation between CD163 or CD90 expression and tumour stage, gender or SDH mutational status, however this will need further examination in a larger cohort of patients. In addition, the functional status of the macrophages and fibroblasts may also influence the behaviour of HNPGN and will need future work. A recent paper has examined 32 pheochromocytoma/paraganglioma (PC/PG, including 5 HNPGN) using single cell RNA sequencing and reports that stromal and immune infiltrate can contribute to 0.5 to 76.7% of the cellular composition. Of these, macrophages formed the largest constituent of the immune infiltrate and macrophage markers were found to be expressed at a higher level in PC/PG with SDH and von-Hippel Lindau (VHL) mutations (28).

Our findings and that of the studies above are of great interest as tumour associated macrophages are linked to poor prognosis in several cancers and are actively being explored as targets of cancer immunotherapy (29). Metabolic changes occur in the cancer associated macrophages as well as cancer associated fibroblasts (30, 31). We have examined the functional and metabolic profile of the macrophages and fibroblasts in HNPGN and show that MCT1 and MCT4 are expressed in both the tumour and stromal components of HNPGN. Macrophages in particular express MCT4 in HNPGN, whilst MCT1 expression was also observed in macrophages in HNPGN carrying SDH mutations. Lactate is a major source of carbon for the tricarboxylic acid (TCA) cycle in healthy and cancerous tissue (32). MCT1 is ubiquitously expressed and regulates lactate-$H^+$ import. MCT4 is strongly expressed by hypoxic and glycolytic tissue and is mainly responsible for lactate export (33). Several studies indicate that both these molecules are amenable to drug induced alterations and therefore could represent new therapeutic targets in cancer and inflammation (12, 34). The clinical significance of MCT expression has been investigated in several tumours and high MCT1 and MCT4 expression is associated with poor prognosis (34). Macrophages express MCT1 through which they can uptake lactate, in an autocrine or paracrine way, which in turn promotes their differentiation into a regulatory anti-inflammatory phenotype (M2 phenotype). MCT4 expression in macrophages is required for glycolytic reprogramming and inflammatory responses (35). While MCT1 expression was noted on a small proportion of cultured HNPGN fibroblasts, neither MCT1 nor MCT4 co-localised with CD90 *in situ* suggesting that in HNPGN, MCT1/MCT4 driven metabolic changes may be more pronounced in macrophages than in fibroblasts. Our findings that MCT1 and MCT4 are expressed in HNPGN stroma *in situ* suggests a potential role of stromal metabolism in the pathogenesis of these tumours. We highlight however that while our study has described important features of macrophages and fibroblasts in HNPGN with their functional attributes, detailed analysis of the functional relevance of these findings will need to be addressed in future larger cohort studies to establish how these cells influence tumour pathogenesis.

In summary, our results show that fibroblasts and macrophages are an important component of HNPGN tumour microenvironment and express several markers involved in metabolic and cellular interactions. This could shape the tumour stroma interaction in HNPGN and thereby impact on tumour growth. Further dissection of the function of macrophages and fibroblasts in HNPGN could reveal novel strategies to improve patient risk stratification and management by targeting its stromal microenvironment.

## 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

Ethical approval for the study was obtained from the University of Birmingham research ethics committee (Human Biomaterials Resource Centre HBRC 17-295) and the tissue samples were released via HBRC. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

PB: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Validation, Writing – original draft, Writing – review & editing. JM: Formal analysis, Investigation, Methodology, Writing – review & editing. MN: Formal analysis, Investigation, Writing – review & editing. VP: Formal analysis, Resources, Writing – review & editing. HA: Investigation, Writing – review & editing. JT: Investigation, Writing – review & editing. SG: Formal analysis, Writing – review & editing. EP: Investigation, Writing – review & editing. GN: Investigation, Resources, Writing – review & editing. PM: Resources, Writing – review & editing. RI: Resources, Writing – review & editing. AC: Funding acquisition, Resources, Writing – review & editing. ID: Formal analysis, Funding acquisition, Investigation, Resources, Writing – review & editing. CB: Funding acquisition, Resources, Supervision, Writing – review & editing.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The work was funded by the British Society of Otology Grant to PB (Grant number BSO2023-02) and ENTUK grants to PB ENTUK Foundation Research Grant 2021 & 2022 to study fibroblasts, macrophages and metabolism in head and neck paragangliomas. Work in the Rheumatology Research Group laboratory at Center of Inflammation and Ageing, University of Birmingham is funded by Arthritis Research UK to CB. Work in the Cardiovascular Research group of IED is funded by BHF. Funding was provided by NIHR and Wellcome Trust to AC.

## Acknowledgments

We are very grateful to all the patients for their participation in the study. We are grateful to Triin Major and Nayandeep Kaur (HBRC Birmingham histology services) for the CD163, CD68, CD90 and PDPN immunohistochemistry. The authors would like to acknowledge the Flow Cytometry Core facility at the University of Birmingham for support with flow cytometry experiments.

## Conflict of interest

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

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1397839/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Expression of CD163 in head and neck paraganglioma (HNPGN) tissue on immunohistochemistry. Immunohistochemistry images of HNPGN tissue (representative of n = 10 HNPGN patient samples) showing CD163 expression in red. The images are labelled PGN02, PGN03 etc denoting origin from different patients. Panels are at 20x magnification.

SUPPLEMENTARY FIGURE 2
Expression of CD90 in head and neck paraganglioma (HNPGN) tissue on immunohistochemistry. Immunohistochemistry images of HNPGN tissue (representative of n = 10 HNPGN patient samples) showing CD90 expression in brown. The images are labelled PGN02, PGN03 etc denoting origin from different patients.

SUPPLEMENTARY FIGURE 3
Expression of PDPN in head and neck paraganglioma (HNPGN) tissue on immunohistochemistry. Immunohistochemistry images of HNPGN tissue (representative of n = 10 HNPGN patient samples) showing PDPN expression in red. The images are labelled PGN02, PGN03 etc denoting origin from different patients.

SUPPLEMENTARY FIGURE 4
Comparison of CD163, CD90 and PDPN quantitative expression. Graphs depict CD90, PDPN and CD163 quantitative expression (each data point represents positive nuclei/area of tissue for individual samples) in the HNPGN samples based on mutation status (upper panels) and gender (lower panels). Supplementary Figure 4 Analysis of fibroblast and macrophage associated molecules in head and neck paraganglioma (HNPGN). **(A)** Expression of a panel of fibroblasts genes on RT-qPCR in fresh frozen tissue from six human HNPGN samples. Each data point is one patient sample and shows the relative amounts for each gene using GAPDH as the housekeeping gene. **(B)** Expression of a panel of fibroblasts genes on RT-qPCR in fresh frozen tissue from six human HNPGN samples. Each data point is one patient sample and shows the relative amounts for each gene using GAPDH as the housekeeping gene.

SUPPLEMENTARY FIGURE 5
Fibroblasts derived from head and neck paraganglioma (HNPGN) and normal skin/mucosa. **(A)** Phase contrast photomicrography images of fibroblasts cultured from fresh HNPGN tissue (representative of n=10 HNPGN patient samples) and normal mastoid mucosa/ear canal skin tissue (representative of n=6 patient samples). **(B)** RT-qPCR was used to quantify the expression of macrophage associated molecules on the HNPGN and normal fibroblasts (representative of n=3-4 paired samples). Panels show expression of genes of the galectin (LGALS1, LGALS9, LGALS3, LGALS3BP) family, CTSB, CTSL1, matrix metalloproteinases (MMP2, MMP14), CxCL16, MYC, EPAS1, SUMO1, TNS3, PIAS, and HTR2. Each data point is one sample and shows the relative amounts for each gene using GAPDH as the housekeeping gene.

# References


1. Offergeld C, Brase C, Yaremchuk S, Mader I, Rischke HC, Glasker S, et al. Head and neck paragangliomas: clinical and molecular genetic classification. *Clinics (Sao Paulo)*. (2012) 67(Suppl 1):, 19–28.

2. Tlsty TD, Coussens LM. Tumor stroma and regulation of cancer development. *Annu Rev Pathol*. (2006) 1:119–50. doi: 10.1146/annurev.pathol.1.110304.100224

3. Baruah P, Lee M, Wilson PO, Odutoye T, Williamson P, Hyde N, et al. Impact of p16 status on pro- and anti-angiogenesis factors in head and neck cancers. *Br J Cancer*. (2015) 113:653–9. doi: 10.1038/bjc.2015.251

4. Baruah P, Bullenkamp J, Wilson POG, Lee M, Kaski JC, Dumitriu IE. TLR9 mediated tumor-stroma interactions in human papilloma virus (HPV)-positive head and neck squamous cell carcinoma up-regulate PD-L1 and PD-L2. *Front Immunol*. (2019) 10:1644. doi: 10.3389/fimmu.2019.01644

5. Baruah P, Marshall J, Jones PN, Major T, Pucino V, O'Neil JD, et al. Fibroblasts derived from vestibular schwannoma express protumorogenic markers. *Otol Neurotol*. (2023). doi: 10.1097/MAO.0000000000004011

6. Mills EL, Kelly B, Logan A, Costa ASH, Varma M, Bryant CE, et al. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. *Cell*. (2016) 167:457–470 e413. doi: 10.1016/j.cell.2016.08.064

7. Fiaschi T, Chiarugi P. Oxidative stress, tumor microenvironment, and metabolic reprogramming: a diabolic liaison. *Int J Cell Biol*. (2012) 2012:762825. doi: 10.1155/2012/762825

8. Hardy RS, Filer A, Cooper MS, Parsonage G, Raza K, Hardie DL, et al. Differential expression, function and response to inflammatory stimuli of 11beta-hydroxysteroid dehydrogenase type 1 in human fibroblasts: a mechanism for tissue-specific regulation of inflammation. *Arthritis Res Ther*. (2006) 8:R108. doi: 10.1186/ar1993

9. Croft AP, Campos J, Jansen K, Turner JD, Marshall J, Attar M, et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature*. (2019) 570:246–51. doi: 10.1038/s41586-019-1263-7

10. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol*. (2003) 3:23–35. doi: 10.1038/nri978

11. Buechler MB, Turley SJ. A short field guide to fibroblast function in immunity. *Semin Immunol*. (2018) 35:48–58. doi: 10.1016/j.smim.2017.11.001

12. Pucino V, Nefla M, Gauthier V, Alsaleh G, Clayton SA, Marshall J, et al. Differential effect of lactate on synovial fibroblast and macrophage effector functions. *Front Immunol*. (2023) 14:1183825. doi: 10.3389/fimmu.2023.1183825

13. Pont S. Thy-1: a lymphoid cell subset marker capable of delivering an activation signal to mouse T lymphocytes. *Biochimie*. (1987) 69:315–20. doi: 10.1016/0300-9084(87)90022-8

14. Leyton L, Hagood JS. Thy-1 modulates neurological cell-cell and cell-matrix interactions through multiple molecular interactions. *Adv Neurobiol*. (2014) 8:3–20. doi: 10.1007/978-1-4614-8090-7_1

15. Rege TA, Hagood JS. Thy-1 as a regulator of cell-cell and cell-matrix interactions in axon regeneration, apoptosis, adhesion, migration, cancer, and fibrosis. *FASEB J*. (2006) 20:1045–54. doi: 10.1096/fj.05-5460rev

16. Inoue A, Tanaka J, Takahashi H, Kohno S, Ohue S, Umakoshi A, et al. Blood vessels expressing CD90 in human and rat brain tumors. *Neuropathology*. (2016) 36:168–80. doi: 10.1111/neup.12244

17. Kalof AN, Cooper K. D2-40 immunohistochemistry–so far! *Adv Anat Pathol*. (2009) 16:62–4. doi: 10.1097/PAP.0b013e3181915e94

18. Sonne SB, Herlihy AS, Hoei-Hansen CE, Nielsen JE, Almstrup K, Skakkebaek NE, et al. Identity of M2A (D2-40) antigen and gp36 (Aggrus, T1A-2, podoplanin) in human developing testis, testicular carcinoma in *situ* and germ-cell tumours. *Virchows Arch*. (2006) 449:200–6. doi: 10.1007/s00428-006-0223-4

19. Suzuki H, Kaneko MK, Kato Y. Roles of podoplanin in Malignant progression of tumor. *Cells*. (2022) 11. doi: 10.3390/cells11030575

20. Bieniasz-Krzywiec P, Martin-Perez R, Ehling M, Garcia-Caballero M, Pinioti S, Pretto S, et al. Podoplanin-expressing macrophages promote lymphangiogenesis and lymphoinvasion in breast cancer. *Cell Metab*. (2019) 30:917–936 e910. doi: 10.1016/j.cmet.2019.07.015

21. Scanlan MJ, Raj BK, Calvo B, Garin-Chesa P, Sanz-Moncasi MP, Healey JH, et al. Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. *Proc Natl Acad Sci USA*. (1994) 91:5657–61. doi: 10.1073/pnas.91.12.5657

22. Liu F, Qi L, Liu B, Liu J, Zhang H, Che D, et al. Fibroblast activation protein overexpression and clinical implications in solid tumors: a meta-analysis. *PloS One*. (2015) 10:e0116683. doi: 10.1371/journal.pone.0116683

23. Papa V, Pezzino V, Costantino A, Belfiore A, Giuffrida D, Frittitta L, et al. Elevated insulin receptor content in human breast cancer. *J Clin Invest*. (1990) 86:1503–10. doi: 10.1172/JCI114868

24. Zhong Y, Ren X, Cao X, Xu Y, Song Y, Zhou Y, et al. Insulin-like growth factor 2 receptor is a key immune-related gene that is correlated with a poor prognosis in patients with triple-negative breast cancer: A bioinformatics analysis. *Front Oncol*. (2022) 12:871786. doi: 10.3389/fonc.2022.871786

25. Fernandez MC, Martin A, Venara M, Calcagno Mde L, Sanso G, Quintana S, et al. Overexpression of the insulin-like growth factor 1 receptor (IGF-1R) is associated with Malignancy in familial pheochromocytomas and paragangliomas. *Clin Endocrinol (Oxf)*. (2013) 79:623–30. doi: 10.1111/cen.12205

26. Farhat NA, Powers JF, Shepard-Barry A, Dahia P, Pacak K, Tischler AS. A previously unrecognized monocytic component of pheochromocytoma and paraganglioma. *Endocr Pathol*. (2019) 30:90–5. doi: 10.1007/s12022-019-9575-6

27. Tufton N, Hearnden RJ, Berney DM, Drake WM, Parvanta L, Chapple JP, et al. The immune cell infiltrate in the tumour microenvironment of phaeochromocytomas and paragangliomas. *Endocr Relat Cancer*. (2022) 29:589–98. doi: 10.1530/ERC-22-0020

28. Zethoven M, Martelotto L, Pattison A, Bowen B, Balachander S, Flynn A, et al. Single-nuclei and bulk-tissue gene-expression analysis of pheochromocytoma and paraganglioma links disease subtypes with tumor microenvironment. *Nat Commun*. (2022) 13:6262. doi: 10.1038/s41467-022-34011-3

29. Mantovani A, Allavena P, Marchesi F, Garlanda C. Macrophages as tools and targets in cancer therapy. *Nat Rev Drug Discovery*. (2022) 21:799–820. doi: 10.1038/s41573-022-00520-5

30. Xiao L, Wang Q, Peng H. Tumor-associated macrophages: new insights on their metabolic regulation and their influence in cancer immunotherapy. *Front Immunol*. (2023) 14:1157291. doi: 10.3389/fimmu.2023.1157291

31. Sazeides C LA. Metabolic relationship between cancer-associated fibroblasts and cancer cells. *Heterogeneity Cancer Metab*. (2021). doi: 10.1007/978-3-030-65768-0_14

32. Faubert B, Li KY, Cai L, Hensley CT, Kim J, Zacharias LG, et al. Lactate metabolism in human lung tumors. *Cell*. (2017) 171:358–371 e359. doi: 10.1016/j.cell.2017.09.019

33. Pucino V, Cucchi D, Mauro C. Lactate transporters as therapeutic targets in cancer and inflammatory diseases. *Expert Opin Ther Targets*. (2018) 22:735–43. doi: 10.1080/14728222.2018.1511706

34. Payen VL, Mina E, Van Hee VF, Porporato PE, Sonveaux P. Monocarboxylate transporters in cancer. *Mol Metab*. (2020) 33:48–66. doi: 10.1016/j.molmet.2019.07.006

35. Tan Z, Xie N, Banerjee S, Cui H, Fu M, Thannickal VJ, et al. The monocarboxylate transporter 4 is required for glycolytic reprogramming and inflammatory response in macrophages. *J Biol Chem*. (2015) 290:46–55. doi: 10.1074/jbc.M114.603589

# Frontiers in Endocrinology

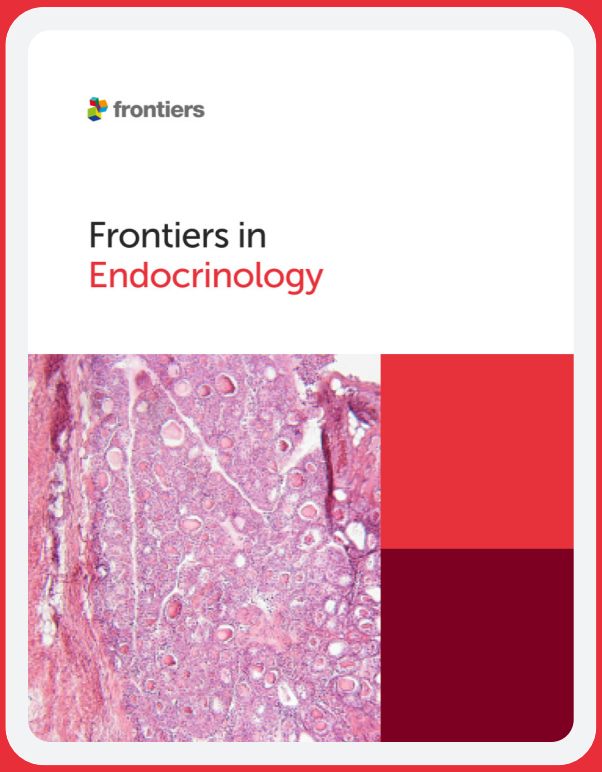
**Explores the endocrine system to find new therapies for key health issues**

The second most-cited endocrinology and metabolism journal, which advances our understanding of the endocrine system. It uncovers new therapies for prevalent health issues such as obesity, diabetes, reproduction, and aging.

---

## 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