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Community series in immunotherapy and small molecule inhibitors as combinational cancer therapeutics, volume II

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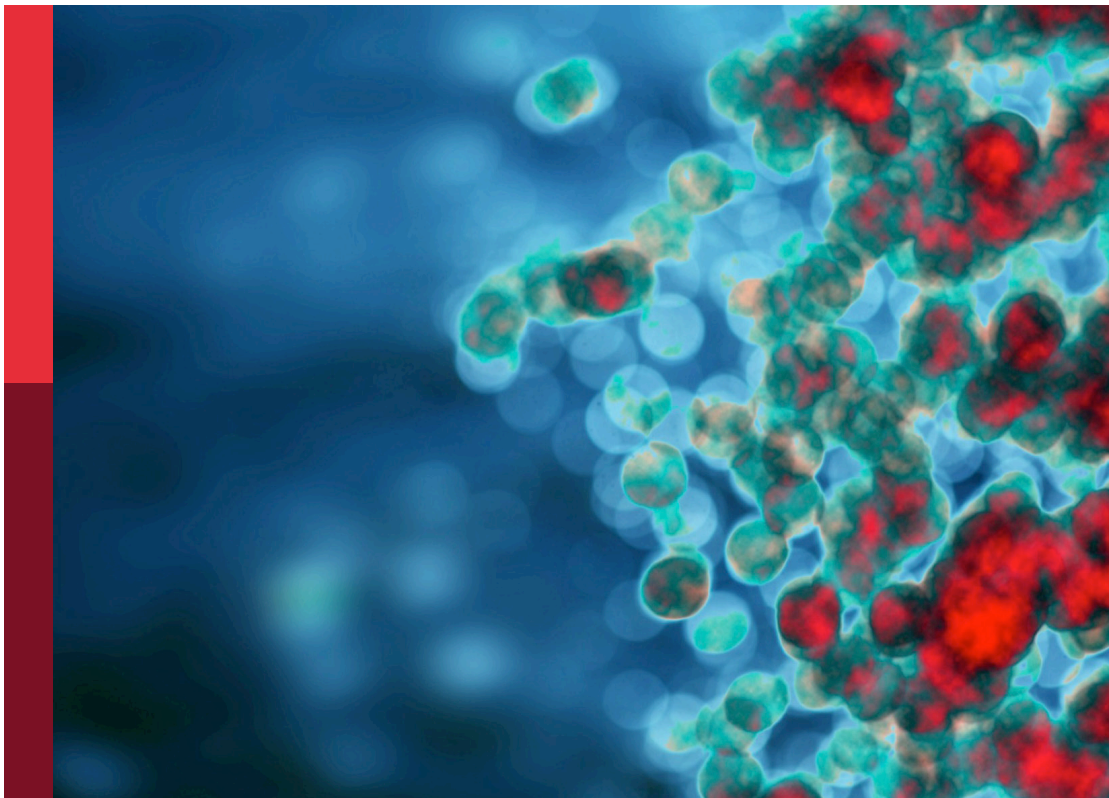

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Community series in immunotherapy and small molecule inhibitors as combinational cancer therapeutics, volume II

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Editorial: Community series in immunotherapy and small molecule inhibitors as combinational cancer therapeutics, volume II

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Editorial on the Research Topic
Community series in immunotherapy and small molecule inhibitors as combinational cancer therapeutics, volume II

The traditional methods of cancer treatments, such as surgery, chemotherapy, and radiotherapy, are not sufficient, and we need to focus on a new approach where these modalities are strategically combined with immunotherapy to unleash the full efficacy of the anti-tumor immune response. This transformation is shifting us from conventional treatments toward a future of a highly personalized and synergistic therapeutic era. The recent publications in the Research Topic "*Community Series in Immunotherapy and Small Molecule Inhibitors as Combinational Cancer Therapeutics: Volume II*" published in Frontiers in Immunology collectively provide a comprehensive overview of this evolution, elucidating the biological mechanisms, confirming clinical efficacy across major cancer types, and outlining the tools and novel targets that will define the future prospects in oncology care.

1 Radiotherapy—potential as systemic immune regulator

Development and innovation of novel therapies for advanced cancer are based on conventional treatments such as radiotherapies and chemotherapies, which lead to a diverse array of immune responses. Wang et al. showed evidence that radiotherapy (RT) can function as an *in situ* vaccine. It induces immunogenic cell death (ICD), releasing tumor antigens and damage-associated molecular patterns (DAMPs) that may initiate the activation of immune cells such as dendritic cells. Crucially, by causing DNA damage, RT activates the cGAS (Cyclic GMP-AMP synthase)-STING (Stimulator of interferon genes)

pathway, leading to type I interferon production and inducing a robust T-cell response. This transformation of the tumor immune microenvironment (TIME) from an immunosuppressive state to an immunologically active state narrates the physiological phenomenon for the remarkable systemic tumor response, where localized irradiation results in the regression of metastatic lesions outside the radiation field. Whereas the same biological processes can induce immunosuppression, RT can upregulate checkpoint proteins like PD-L1, promote the expansion of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), and cause systemic lymphopenia, thereby counteracting its own immunostimulatory effects. This ensures that RT is not a passive partner but an active immune modulator. The clinical challenge, therefore, is to strategically harness its immunostimulatory potential while reducing its suppressive effect. This can effectively be achieved via a combination with immune checkpoint inhibitors (ICIs).

2 Efficacy of combination therapeutics in cancer treatments

An extensive study on advanced Non-Small Cell Lung Cancer (NSCLC) by Wang et al. showed that pembrolizumab with radiotherapy treatment enhanced the patient conditions and led to improved progression-free survival (PFS) and overall survival (OS), which is favorable compared to pembrolizumab alone, with notably enhanced distant tumor response rates.

This finding is strongly supported by the real-world study of Zheng et al., who developed a predictive nomogram for stage IV NSCLC. Data from 462 patients were collected in this clinical study exhibited the plan of the treatment was a key determinant of survival. Their findings provide evidence that chemotherapy in synergy with chemotherapy exhibited better outcomes than single therapeutic treatment. Further evidence of the success of the combinational therapeutics is exhibited by the meta-analysis studied by Sisodiya et al. for breast cancer. In their systematic review, they included 55 clinical trials that demonstrated that combination immunotherapies significantly improved both OS and PFS in all trial phases (I-IV) when compared with single therapy. The outcome from these clinical trials suggests that combinational therapies, which can include two or more treatment regimens such as RT, immune molecules, chemotherapy, etc., exhibited significant survival outcomes.

3 Testing novel immune molecules to enhance combinatorial therapeutics

The combinatorial therapeutics have had a significant effect on solid tumor treatments. A meta-analysis of phase III clinical trials conducted by Zhang et al. analysed the role of ICIs as first-line standard therapy for recurrent or advanced cervical cancer. The overall outcome of the study exhibited improvements in both progression-free survival (HR 0.67) and overall survival (HR 0.66) with ICI-based treatments compared to single therapeutic treatments. The positive outcome was observed in patients with higher expression of PD-L1 in tumors and those with histology of squamous carcinoma. The combination of ICIs with conventional therapies, however, was associated with a slight increase in adverse events (AVs) relative to standard therapy alone. These findings emphasize the importance of careful patient monitoring during combination therapy. This also sheds light on the need for a thorough assessment of toxicity risks before adopting such treatment strategies in clinical practice.

In a retrospective study, Wang et al. investigated the efficacy of combining the anti-angiogenic agent anlotinib with immune checkpoint inhibitors (ICIs) and platinum-based chemotherapy to improve outcomes in patients with non-small cell lung cancer (NSCLC). The triple combination therapy (AIC: anlotinib, ICI, and chemotherapy) achieved a median progression-free survival of 7.76 months, which was significantly longer (by 2.33 months) than that observed with the combination of ICIs and chemotherapy alone. These findings proved the significant role of adding anti-angiogenic agents to combination treatment regimens. Notably, even the two-drug combination of anlotinib and chemotherapy demonstrated superior progression-free survival compared with the ICI–chemotherapy regimen. These findings strongly suggest that for later-line NSCLC patients, the addition of an anti-angiogenic agent is critical to delaying disease progression. Furthermore, the authors reported that the overall risks and toxicities were tolerable and could be controlled. Although the study included a small sample size with single-center collection, the study showed the potential of triple therapy as an effective treatment option for NSCLC patients who have not responded to standard conventional treatments. Further randomized controlled trials are warranted to validate these findings and confirm the efficacy and safety of this therapeutic approach.

Li et al. carried out a retrospective study and compared the effectiveness of targeted immunotherapy vs targeted therapy alone in the third-line or beyond setting for microsatellite stable (MSS) metastatic colorectal cancer (mCRC) patients (n=71) to help identify the beneficial population of combined targeted-immunotherapy. Out of a total of 71 subjects, 31 received targeted therapies alone (TT group), and 40 received combinations of targeted therapy and immunotherapy (TI group). The outcome of the study was that combination therapy improved response rates (20% vs. 3.2%) and controlled disease (82.5% vs. 58.1%), with longer median progression-free survival (4.6 vs. 4.1 months). The most significant outcome of the combinational targeted immunotherapy was observed in patients with lung metastasis alone. These findings suggest that targeted immunotherapy combinations can enhance efficacy in selected MSS mCRC patients. Further studies with larger patient cohorts are still necessary to strengthen reliability and validity.

In another retrospective study involving 71 patients, Zhao et al. investigated whether baseline lymphocyte counts could help identify which hepatocellular carcinoma (HCC) patients would benefit from targeted combination immune therapy. The study showed that both progression-free survival (PFS) and overall

survival (OS) improved (p = 0.058 and p = 0.077, respectively) in patients receiving combination therapy with tyrosine kinase inhibitors (TKIs) and PD-1 inhibitors. Notably, patients with a high peripheral blood lymphocyte count (PBLC) exhibited better OS and PFS as compared to the cancer patients with low absolute PBLC. These results highlight that PBLC could be a routine blood measure that can be used as a potential biomarker to identify HCC patients most likely to benefit from TKI and PD-1–based combination therapy. Implementing lymphocyte count as a stratification or decision-making tool could optimize precision therapy and minimize unnecessary toxicity and cost.

In a review published by Liu et al., the authors provided a wide overview of immunotherapeutic strategies for hepatocellular carcinoma (HCC), emphasizing various combination approaches. The authors discuss the clinical outcome of ICIs monotherapy and essential mechanisms by which ICIs activate immune cells and lead to the shift of immunosuppression in the tumor microenvironment towards immune activation. PD1 blockers such as nivolumab and pembrolizumab were found to be safe in the treatment of cancer patients. Both nivolumab and pembrolizumab exhibited lower efficacy for the HCC's immunosuppressive tumor microenvironment, yielding objective response rates (ORRs) typically below 20%.

Consequently, therapeutic strategies have shifted toward combination regimens that synergistically enhance antitumor immunity and are now considered the standard of care. A clinical trial study published in 2018 showed that a combination of atezolizumab and bevacizumab introduced into unresectable HCC patients (n=104) resulted in a manageable safety profile with a PFS of 12.4 months, a median survival time of 17.1 months, an ORR of 36%, and a DCR of 71%. The landmark IMbrave150 trial established atezolizumab plus bevacizumab ("T+A") as a first-line regimen, demonstrating a significant overall survival (OS) advantage over sorafenib. Additional trials, such as CARES-310 (camrelizumab plus apatinib) and HIMALAYA (durvalumab plus tremelimumab), also showed encouraging efficacy, with the latter achieving an ORR of 20.1%, median PFS of 3.8 months, and median OS of 16.4 months in unresectable HCC. Several studies have been conducted based on transcatheter arterial chemoembolization (TACE) in combination with ICIs for the treatment of unresectable advanced HCC patients. In addition, there is also the phase II study of TACE in combination with nivolumab for intermediate-stage HCC (IMMUTACE) and the phase III LEAP-012 (NCT04246177) study of TACE in combination with lenvatinib and pembrolizumab for intermediate-stage HCC, which also exhibited better results. However, this rapidly expanding combinational therapeutics involves significant challenges, including the need to identify optimal biomarkers for patient selection, manage unique immune-related adverse events, overcome primary and acquired resistance, and define the most effective sequences and combinations within an increasingly complex treatment landscape. The future of HCC therapy lies in deepening our understanding of the tumor-immune environment to guide these sophisticated, personalized combination approaches.

A meta-analysis study by Zhao et al. evaluates the safety and efficacy of combining concurrent chemoradiotherapy (CCRT) with ICIs in locally advanced cervical cancer (LACC). The combined data suggest that together, CCRT and ICIs may improve objective response rates (ORR) compared to CCRT alone, with an improved disease-free survival trend. Whilst these findings are promising, the evidence remains limited, and hence, long-term outcomes and overall safety require further investigation. This study emphasizes the potential of combining immunotherapy with standard LACC treatment to enhance therapeutic efficacy.

A systematic review and meta-analysis study evaluated the efficacy and safety of anlotinib in advanced digestive system neoplasms (DSNs). In total, 20 clinical trials, which included 1,613 patients, exhibited anlotinib combined with conventional cancer treatments significantly improved short-term outcomes. Overall patient survival time increased by 6 months. This study exhibited that the combinational therapy resulted in a higher incidence of adverse events, including hypertension, proteinuria, fatigue, and gastrointestinal disturbances. There were no treatment-related deaths. Subgroup analysis indicated a relatively less effect in advanced gastric cancer. These findings demonstrate anlotinib with other combinational interventions proved as promising therapeutics in DSN treatment (Zhou et al.). Furthermore, a more careful risk-benefit assessment is needed, and further studies must define long-term efficacy and optimal patient selection.

Nandi and Sharma showed the latest research relevant for the future directions of immunotherapy research and clinical trials: (a) destroying treatment-resistant cell populations through dendritic cell vaccines or CAR-T cells targeting Cancer Stem Cells (CSC) markers (e.g., CD44, EpCAM) is a promising strategy to prevent metastasis; (b) the presence, type, and functional state of tumor infiltrating lymphocytes (TILs) are important as prognostic and predictive biomarkers, and adoptive cell therapy using expanded TILs represents a highly personalized and potent treatment regime; (c) the gut and tumor microbiota are now recognized as potent regulators of immunotherapy response, and interventions like fecal microbiota transplantation (FMT) and specific probiotic/prebiotic regimens are being actively investigated to overcome primary and acquire resistance.

4 The imperative for personalization: the role of predictive modeling

With combinatorial therapeutics expanding to include immunotherapy with radiotherapy, chemotherapy, and other targeted agents, the clinical treatment decision-making process will become faster and more robust. The question is no longer merely whether to combine, but which agents to combine, for which patient, and in what sequence. The outcome of these strategies provides the transition from a one-size-fits-all approach to a deeply personalized treatment strategy. The work of Zheng et al. is a direct response to this need, developing a predictive nomogram for stage

IV NSCLC that integrates patient-specific data to forecast individual survival probability. Such tools represent a favorable new era in clinical oncology.

Predictive models are essential for several reasons. First, they move clinical practice beyond population-level evidence, which is highly important for establishing efficacy. Heterogeneity of treatment effects suggests that individual patient responses to the treatment may vary. The therapy offers a modest survival benefit for one patient could be entirely ineffective for another. By including variables such as tumor genomics (e.g., PD-L1 status and mutational burden), clinical parameters (e.g., lactate dehydrogenase levels and sites of metastasis), host factors (e.g., baseline lymphocyte count as highlighted by Zhao et al. in HCC), and specific treatment conditions, these models can classify patients into subgroups most likely to derive benefit.

Second, these models are crucial for risk mitigation. As starkly illustrated by the case report of sintilimab-induced agranulocytosis by Qin et al., the potent activation of the immune system by ICIs carries the risk of severe and unpredictable toxicities. Predictive modeling is not solely about predicting efficacy; it is equally about identifying patients at high risk for immune-related adverse events (irAEs). A model that could flag a patient's predisposition to hematological toxicity, for instance, would allow for enhanced monitoring and preemptive management, thereby improving safety.

The predicted future of these treatment tools relies on the development of a dynamic treatment plan designed by AI, using clinical data derived from the electronic health records of a diverse range of patients, multi-omics profiling, and even digital biomarkers. This continuous learning AI-designed treatment plan will enable the best use of available therapy and eventually create a "digital treatment planner" that can simulate the possible therapeutic outcome and side effects of various combinations of drug treatment for a particular patient. The data-driven treatment plan can ensure individual-specific cancer care and thereby maximize the therapeutic potential of a combination of drugs with minimal side effects.

5 Possible adverse effects of ICI

Qin et al. reported immune-related adverse events (irAEs) caused by a cancer immunotherapy drug, sintilimab (anti-PD-1 Ab). Sintilimab induced agranulocytosis in a patient with non-small cell lung cancer, which highlights the unpredictable side effects and limitations of ICI cancer therapy. Although ICI treatment is effective in cancer treatment, its mechanism of activating T-cells is primarily related to over-response of the immune system, leading to side effects like autoreactive immune responses, which can cause a life-threatening condition with severely low levels of white blood cells called neutrophils. Distinguishing the side effects of chemotherapy from irAEs is challenging and time-consuming. To treat the sintilimab-induced agranulocytosis, a high dose of corticosteroid was administered, which is not usually included in standard cancer care. The irAEs pose a significant clinical management challenge as they counterbalance the therapeutic benefits of ICIs.

6 Conclusion and future perspectives

The collective evidence confirms a major shift in oncology based on synergistic combinational therapies. We are moving decisively from the era of sequential, non-specific cytotoxic treatments to a synergistic era defined by rationally designed combination therapies that strategically harness and augment the host's immune system. The combination of immunotherapy along with chemotherapy, radiotherapy, targeted agents, and/or localized treatments has become an effective clinical treatment strategy in treating various cancers, including NSCLC, breast cancer, HCC, and cervical cancer.

This new frontier, however, is accompanied by numerous challenges that need to be addressed for tailoring proper cancer treatment. As our therapeutic regimen expands, the principal challenge is the lack of robust, predictive biomarkers to guide selection among numerous combination options. The promising findings regarding baseline lymphocyte counts in HCC and PD-L1 status in cervical cancer are initial steps; the future demands the discovery and validation of multi-analyte signatures that can predict both efficacy and toxicity for specific drug combinations.

Optimizing treatment sequencing and timing has become crucial. The superior efficacy of neoadjuvant immunotherapy to some extent highlighted the importance of the treatment schedule. Choosing concurrent or sequential delivery in an optimal order of radiotherapy, chemotherapy, and immunotherapy is critical for maximizing synergistic potential and minimizing antagonistic effects.

To achieve the greater clinical benefit of innovative therapies, the management of irAEs is essential; this can be achieved by developing standardized, preventive management protocols and predictive models for irAEs.

Finally, the Research Topic of "easy access for everyone" must be focused on. The affordability of the multi-drug combination treatment is a significant barrier to widespread clinical use. Drug price control, by the combined efforts of researchers, clinicians, and policymakers, is essential to prevent disparity in cancer care. Looking forward, the future era of combinational therapeutics will exploit artificial intelligence and multi-omics data to create dynamic and individualized "digital treatment planners" based on the clinical effectiveness of the drugs with respect to the patient-specific factors.

In conclusion, combinational treatment designed with a multi-target approach on the tumor-immune ecosystem will be the future of standard cancer treatment. This promising therapeutic approach has the potential to significantly improve the quality of life and survivability of cancer patients, effectively transforming cancer into a more manageable disease.

Author contributions

MK: Conceptualization, Data curation, Resources, Supervision, Writing – original draft, Writing – review & editing. ST: Conceptualization, Data curation, Resources, Supervision, Writing – original draft, Writing – review & editing. SR: Writing – original draft, Writing – review & editing.

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Immune modulatory roles of radioimmunotherapy: biological principles and clinical prospects

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Radiation therapy (RT) not only can directly kill tumor cells by causing DNA double-strand break, but also exerts anti-tumor effects through modulating local and systemic immune responses. The immunomodulatory effects of RT are generally considered as a double-edged sword. On the one hand, RT effectively enhances the immunogenicity of tumor cells, triggers type I interferon response, induces immunogenic cell death to activate immune cell function, increases the release of proinflammatory factors, and reshapes the tumor immune microenvironment, thereby positively promoting anti-tumor immune responses. On the other hand, RT stimulates tumor cells to express immunosuppressive cytokines, upregulates the function of inhibitory immune cells, leads to lymphocytopenia and depletion of immune effector cells, and thus negatively suppresses immune responses. Nonetheless, it is notable that RT has promising abscopal effects and may achieve potent synergistic effects, especially when combined with immunotherapy in the daily clinical practice. This systematic review will provide a comprehensive profile of the latest research progress with respect to the immunomodulatory effects of RT, as well as the abscopal effect of radioimmunotherapy combinations, from the perspective of biological basis and clinical practice.

KEYWORDS

radiotherapy, immune modulation, immune checkpoint inhibitors, abscopal effect, review

Introduction

Cancer remains the leading disease burden worldwide (1–3). Radiation therapy (RT) plays an important role in the treatment of cancers and is an effective local treatment method. Traditionally, it is wide acknowledged that RT leads to DNA double stand breaks (DSBs) and thereby kills tumor cells (4). In recent years, multiple studies have suggested that RT could exert anti-tumor immune effects by regulating local and systemic immune responses (5). Currently, with the development of immune checkpoint inhibitors (ICIs), the immune modulatory effect of RT and the synergistic effect of radioimmunotherapy combinations have attracted extensive attention and discussions (6, 7). However, the immune modulatory effect of RT has a double-sided nature: it can enhance the host's anti-tumor immune response, but it may also produce immune suppression effects under certain conditions (8). The key molecular mechanisms of RT promoting or inhibiting adaptive and innate anti-tumor immune responses not only have triggered numerous exploration and investigations, but also remain the research hotspot now and in the future (9).

In addition, in the clinical practice of combining RT with ICI treatments, it has been observed that effective anti-tumor immune responses can occur at distant lesions outside the irradiation field, known as the "abscopal effect", further emphasizing the immune modulatory and synergistic effects of RT (10–13). Therefore, the combinatorial use of RT and ICIs may produce complex interactions. This review focuses on the latest research progress on the immune modulatory effects of RT and systematically summarizes the theoretical basis and clinical evidence for the synergistic effects of radioimmunotherapy, aiming to elucidate the biological mechanisms and practical principles when combining RT with ICIs and provide reference for improving the comprehensive cancer treatment.

Abbreviations: APCs, Antigen-presenting cells; CCL, Chemokine ligand; cGAS, Cyclic GMP-AMP synthase; CTLA-4, Cytotoxic T lymphocyte-associated antigen-4; CTLs, Cytotoxic T lymphocytes; DAMPs, Damage-associated molecular patterns; DCs, Dendritic cells; DSBs, Double strand breaks; dsDNA, Double-stranded DNA; GM-CSF, Granulocyte macrophage-colony stimulating factor; Th, Helper T cells; ICIs, Immune checkpoint inhibitors; ICD, Immunogenic cell death; IDO, Indoleamine 2,3-dioxygenase; iNOS, Inducible nitric oxide synthase; IL-10, Interleukin-10; MHC, Major histocompatibility complex; mtDNA, Mitochondrial DNA; MDSCs, Myeloid-derived suppressor cells; NK, Natural killer; PD-L1, Programmed cell death ligand-1; PD-1, Programmed cell death protein-1; RT, Radiation therapy; ROS, Reactive oxygen species; Tregs, Regulatory T cells; STAT1, Signal transducer and activator of transcription 1; STING, Stimulator of interferon genes; SBRT, Stereotactic body radiotherapy; TAAs, Tumor-associated antigens; TAMs, Tumor-associated macrophages; TIME, Tumor immune microenvironment; TILs, Tumor infiltrating lymphocytes; TNF-α, Tumor necrosis factor-alpha; TGF-β, Transforming growth factor-beta.

Immune-activating effect of radiation therapy

Induce immunogenic cell death to promote T cell immune response

The key molecular mechanism that ionizing radiation promotes anti-tumor immune responses is mainly by inducing the immunogenic cell death (ICD), which leads to the release of specific antigens from tumor cells and the stimulation of clone expansion in tumor-specific T lymphocyte subsets (14, 15). Antigen-presenting cells (APCs) capture specific antigens and present them in conjunction with major histocompatibility complex (MHC) to activate helper T cells (Th), which can include cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells to exert anti-tumor immune effects and eliminate tumor cells (16, 17). Overall, ICD induced by RT can effectively stimulate T lymphocyte recruitment and differentiation to recognize and kill tumor cells (18, 19).

Prior studies have suggested that RT can induce oxidative stress sources, such as reactive oxygen species (ROS), leading to endoplasmic reticulum stress responses and mediating ICD (20, 21). This process is accompanied by an increase in antigen release and damage-associated molecular patterns (DAMPs), which participate in the activation of immune response signaling pathways and facilitate anti-tumor immune responses (22). DAMPs are one of the most crucial molecular steps during the radiation-induced ICD. DAMPs include cell surface expression of calreticulin and heat shock proteins, release of high mobility group box 1 protein, and active secretion of adenosine triphosphate (23). In addition, DAMPs can upregulate the expression of tumor-associated antigens (TAAs), that is, primarily neoantigens that are immunogenic mutations induced by ionizing radiation. With the release of inflammatory cytokines, DAMPs can also enhance the function of cytotoxic $CD8^+$ T cells (15, 24). Recent research has also shown that RT can further reshape the T cell receptor repertoire of tumor-infiltrating lymphocytes (TILs) (25, 26).

Activate cGAS-STING pathway to induce type I interferon response

Stimulator of interferon genes (STING) is an endoplasmic reticulum membrane protein that regulates innate immune signaling (27). Cyclic GMP-AMP synthase (cGAS) is a nucleotidyltransferase that senses cytoplasmic DNA and activates the STING-TBK1-IRF-3 signaling axis, thereby producing type I interferon signaling (28). The cGAS-STING pathway is crucial to innate immune responses, anti-viral immune responses, and tumor adaptive immunity (24). Another pivotal mechanism by which RT promotes anti-tumor immune effects is activating the cGAS-STING pathway, subsequently triggering type I interferon cascade reactions, and recruiting APCs to capture and cross-present TAAs to deploy cytotoxic $CD8^+$ T-cell functions (24, 28). Specifically, RT promotes the release of double-stranded DNA (dsDNA) in the cell nucleus, increases the permeability of the

outer mitochondrial membrane, and triggers the exposure of mitochondrial DNA (mtDNA) in the cytoplasm (29). Both dsDNA and mtDNA are effective mediators for initiating the cGAS-STING pathway and the transcription of type I interferons (30, 31). The type I interferon signal further activates dendritic cells (DCs). After being matured, DCs present antigens to T cells. Tumor antigen-specific T cell effector functions is therewith activated, the number of effector lymphocytes increases, and macrophage activity is also promoted, resulting in the amplification of adaptive anti-tumor immune responses (31).

Enhance MHC-I expression and increase the visibility of antigen

MHC-I molecules bind to endogenous antigen peptides produced within cells and are capable of displaying and conveying antigenic information on the cell surface (32). By binding to $CD8^+$ T cells, MHC-I molecules enable the recognition and effective killing of pathological cells that synthesize abnormal proteins, such as tumor cells that express mutated proteins (33, 34). MHC-I tumor antigens play an important role in anti-tumor immune responses. However, during the development of malignant tumors, tumor cells often lack or have low expression of MHC-I molecules to evade the recognition, immune surveillance, and attack by T lymphocytes (33, 34). Therefore, tumor cells could achieve immune escape by losing MHC-I antigen expression, which not only damages the anti-tumor effect of innate immune responses, but also weakens the therapeutic effect produced by some immune checkpoint inhibitors that can reactivate $CD8^+$ T cells to exert anti-tumor effects (35). Many recent studies have indicated that RT can significantly increase the expression of MHC-I on the surface of tumor cells and promote the generation of TAAs (36, 37). This can expand the antigen pool that can be presented by APCs, improve the ability of CTL to recognize tumor cells, increase the visual imprint of the host immune system on tumor cells, effectively reduce tumor escape, and enhance anti-tumor immune responses (34).

Release proinflammatory cytokines to activate tumor microenvironment

In addition to directly killing tumor cells, RT regulates tumor immune microenvironment (TIME) and transforms it from an immunosuppressive "cold" to immune-activated "hot" tumors. RT can stimulate the release of many pro-inflammatory chemokines, including CXCL9, CXCL10, CXCL11, and CXCL16, from tumor cells and stromal cells, which promote the immune infiltration and increase the cell abundance of DCs, macrophages, and T lymphocytes, thereby effectively activating TIME (38, 39). Recent research has demonstrated that conventional fractionated RT with 2 Gy per fraction could reprogram the phenotype of tumor-associated macrophages (TAMs), making them more prone to promote immune antigenicity and increase their anti-tumor immunity (40). In general, TAMs have shown to inhibit T lymphocytes and accelerate tumor metastases, whereas after polarization they could exhibit anti-tumor effects. RT can promote the polarization of M2-like macrophages towards inducible nitric oxide synthase (iNOS)-positive M1-like polarized macrophages. Though M2-like macrophages express CD206 and Arg-1 and release anti-inflammatory cytokines, M1 iNOS-positive macrophages can induce Th1 chemokine expression, release a variety of inflammatory cytokines, recruit $CD8^+$ and $CD4^+$ T cells, and promote T cell-mediated anti-tumor responses (41, 42). Hence, the theoretical principle of RT driving stress signals to reshape TIME mainly lies in the fact that RT can increase various immune regulatory proteins, adhesion molecules, cytokines, and pro-oxidants, positively activating TIME and anti-tumor immune responses.

Upregulate the expression of death receptor on tumor cell surface

FAS, a member of the death receptor family and expressed on the cell surface, is essential to initiate programmed cell death signaling (43). The combination of FAS and its specific ligand FAS-L can enable the recruitment of the death-inducing signaling complex and proteolytic activation of effector caspases 3, 6 and 7 that mediate apoptosis, resulting in cytotoxic signals and effectively promoting the local and systemic anti-tumor immune response (43, 44). Studies have shown that RT can activate the endogenous apoptotic signaling pathway, upregulate the expression of FAS apoptotic receptors on the surface of tumor cells, mediate the effective binding of CTLs and FAS on tumor cells, and promote tumor cell apoptosis (45). Therefore, the upregulation of FAS expression is one of the critical mechanisms by which RT increases the susceptibility of tumor cells to immune response-mediated cell death (43). In conclusion, local RT can exert immune-activating effect through various ways, which has obvious advantages and wider clinical application prospect. Specific mechanisms are summarized in Figure 1.

Immunosuppressive effect of radiation therapy

Induce chronic type I interferon and interferon-stimulated gene expression

RT can cause accumulation of dsDNA in tumor cells, which activates the cGAS/STING pathway and promotes the transcription of type I interferon genes (46). STING can activate different interferon-stimulated genes through its downstream signaling pathway. However, in some cases, interferon signaling may also have negative effects. For example, repeated irradiation of tumor cells could induce chronic type I interferon and interferon-stimulated gene expression, which could make effector T cells to express more inhibitory factors and exhaust T cells, leading to treatment resistance and tumor immune escape via multiple inhibitory pathways (47). Studies have illustrated that prolonged interferon signaling was synergistically associated with programmed cell death ligand-1 (PD-L1)-dependent and programmed cell death protein-1 (PD-1)-independent ICI resistance, as well as resistance to radioimmunotherapy (48).

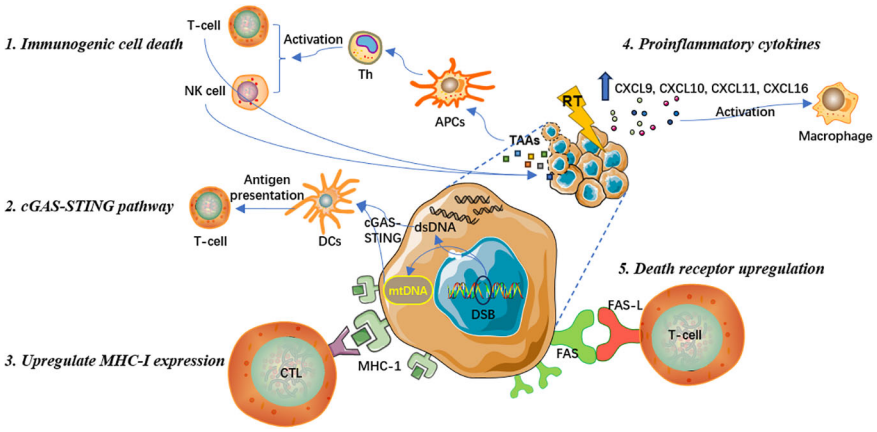

FIGURE 1
Mechanisms of the immune-activating effect of radiotherapy.

Continued interferon signal transduction enables tumor cells to acquire signal transducer and activator of transcription 1 (STAT1)-related epigenomic changes and increase the expression of interferon-stimulated genes and various T cell inhibitory receptor ligands (48, 49). Moreover, both type I and type II interferons can induce the above mechanisms of tumor resistance to treatments.

Upregulate expression of PD-L1 and IDO on tumor cell surface

It is generally accepted that RT could activate the cGAS-STING signaling pathway and thus promote the transcription of interferon-stimulated genes. Nevertheless, interferon-gamma and type I interferon could also upregulate the expression of PD-L1 on the surface of tumor cells, which could increase the immune escape of tumor cells and further induce T lymphocyte exhaustion, weakening the anti-tumor immune response (50). In addition, research indicated that RT not only upregulated the expression of PD-L1 on tumor cells, but also could regulate the expression of multiple immune checkpoint ligands on the surface of immune cells in the tumor microenvironment, producing suppressive tumor immune effects (51, 52). Furthermore, indoleamine 2,3-dioxygenase (IDO), a crucial enzyme involved in the tumor proliferation and immune suppression, could be upregulated by interferon-gamma and type I interferon as an immune inhibitory factor (53–55). Previous studies demonstrated that IDO could result in T cell exhaustion and further upregulate the expression of inhibitory receptors and ligands (55). Meanwhile, the overexpression of IDO on the surface of DCs was associated with decreased T lymphocyte proliferation and poor clinical prognosis in multiple cancer types (55, 56).

Promote and enhance the function of inhibitory immune cells

The STING signaling pathway activated by RT can further enhance the recruitment of regulatory T cells (Tregs) and facilitate the development of myeloid-derived suppressor cells (MDSCs), consequently eliminating the tumor immunogenicity, counteracting the immunostimulatory properties of radiation, and causing immunosuppression (24, 52, 57). Both Tregs and MDSCs exert immunosuppressive effects in immunological responses to cancers and other diseases through various pathways and mechanisms (57, 58). MDSCs express Arg-1 and iNOS, produce ROS, and downregulate anti-tumor immune activity via the release of different chemicals and factors *in vivo* (59–61). Local irradiation of tumor lesions could increase the production of chemokine ligand (CCL)2 and CCL5, which are associated with the recruitment of Tregs and monocytes (62, 63). Recruited monocytes activate Tregs through the tumor necrosis factor-alpha (TNF-α) mediated pathway, which suppresses anti-tumor immune responses and further reduces therapeutic efficacy (64). Besides, by secreting interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), and other cytokines, Tregs can not only enhance the immunosuppressive function of MDSCs, but also inhibit the immune function of effector T cells (65–68).

Cause lymphopenia and depletion of immune effector cells

Lymphopenia is one of the most common adverse events during and after RT in a daily basis, and is deemed to be associated with poorer survival prognosis for cancer patients (69, 70). Given that hematopoietic stem cells are sensitive to ionizing radiation, even low-dose irradiation may cause temporary bone marrow dysfunction, while high-dose RT may result in irreversible damage to bone marrow hematopoietic function and mesenchymal stromal cells (71–73). In real-world clinical settings, patients are often given a certain dose of irradiation which can achieve the purpose of killing tumor cells, whereas some patients could experience severe bone marrow dysfunction, resulting in a significant decrease in lymphocyte count and accordingly decreased anti-tumor immune response (74). Chen et al(75) found that lymphopenia post-RT could affect the occurrence of abscopal responses and thus negatively influence prognosis in patients treated with RT and immunotherapy.

Similarly, monocytes in the peripheral blood circulation are highly sensitive to ionizing radiation. Repeated conventional fractionated RT for 5 consecutive days per week may cause potential cell toxicity damage, deplete immune effector cells that migrate to the peripheral circulation, accelerate aging-related clonal hematopoiesis, and eventually lead to immunosuppressive effects (76). Another potential mechanism for radiation-induced lymphocyte reduction is the irradiation of lymphoid organs. Due to the extreme sensitivity of immature T cells to RT, even low-dose irradiation of lymphoid organs could contribute to rapid p53-mediated apoptosis, which is related to reduced lymphocyte count, increased T cell apoptosis activity, as well as poorer prognosis (62). Hence, lymphopenia, cytotoxic effects on leukocytes, and depletion of immune effector cells are also important reasons for the immunosuppressive effects caused by RT. In brief, RT could also play a negative role in modulating the systemic immune system, which is worthy of further elaboration in future research. Detailed mechanisms of the immunosuppressive effect are presented in Figure 2.

Abscopal effect of radiation combined with immunotherapy

Clinical application and prospect of abscopal effect

About 60 years ago, radiation oncologists discovered the "abscopal effect" of RT, that is, the effective treatment response of tumor shrinkage was observed at a distant site out of the radiation field (77). Although there were merely 47 literatures regarding the abscopal effect reported between 1960 and 2018, this number has rapidly surged after the advent of immunotherapy, presumably because the combination of RT and ICIs could effectively promote anti-tumor effects of the immune system (78). In 2012, Postow et al(79) first reported the abscopal effect of RT in combination with immunotherapy in a case report: a patient with melanoma who received local RT on oligometastatic sits and ipilimumab, a cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) inhibitor, exhibited regression of distant lesions outside the radiation field. Subsequently, mounting evidence has reported the abscopal effect of combining RT with ICIs, and indicated the increased infiltration of immune cells and the enhancement of anti-tumor immune response outside the radiation field. In 2015, Golden et al(80) conducted a proof-of-principle clinical trial in which the immunogenicity of granulocyte macrophage-colony stimulating factor (GM-CSF) was regulated by irradiation, and the effect of RT was validated in clinic for the first time. This study adopted a Simon two-stage design and included a total of 41 patients. In the phase I stage with 10 subjects, abscopal effects were observed in 4 patients. In the phase II stage, 31 additional patients were included, and 11 of the cumulative 41 patients (26.8%) developed abscopal effects. Overall, this research is the first clinical evidence that the combination of RT and immunotherapy can induce the abscopal effect in solid metastatic tumors, and distant remission of metastatic sites can predict better survival outcomes (80).

In 2018, Formenti et al(81) found that in advanced non-small-cell lung cancer (NSCLC) patients with resistance to chemotherapy, RT combined with CTLA-4 inhibitors effectively induced systemic T lymphocyte anti-tumor responses. In this study, CTLA-4 inhibitor alone or in combination with chemotherapy had unsatisfactory efficacy, whereas CTLA-4 inhibitor plus RT showed significant anti-tumor effects (81). Exploratory analysis of the peripheral blood specimens from subjects indicated that the increase of serum interferon β and the early dynamic change of T cell cloning after RT were potent predictors of efficacy (81). Moreover, one patient with complete response revealed a large expansion of $CD8^+$ T cells and the recognition of neoantigens encoded by genes upregulated after RT (81). Hence, the mechanisms of the abscopal effect explained in this study were as follows: After exposure to the systemic immune system of the immunogenic mutation induced by RT, tumor cells in the irradiated field were attacked by circulating immune cells and thus demonstrated distant anti-tumor responses. At present, the exact mechanism and principle of the abscopal effect of RT combined with ICIs observed in clinic remain unclear and warrant further investigations (82).

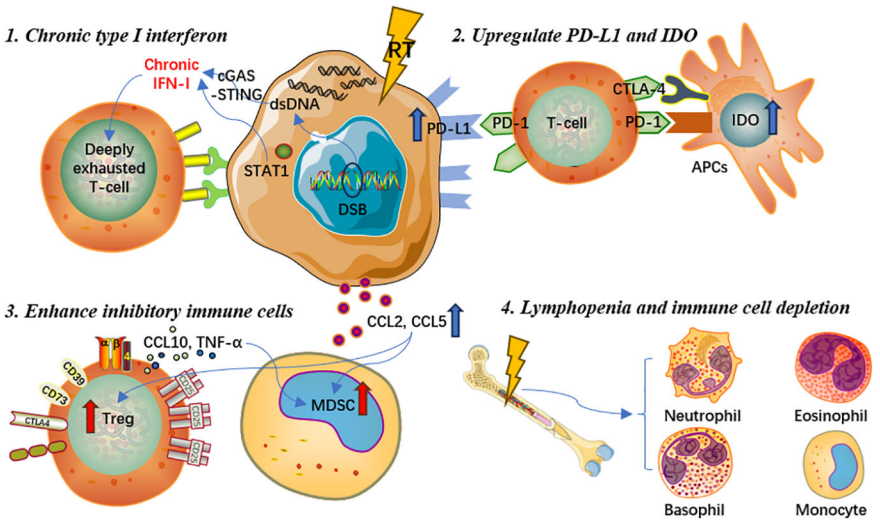

FIGURE 2
Mechanisms of the immunosuppressive effect of radiotherapy.

In recent year, the abscopal effect of RT in combination with immunotherapy has attracted increasing attention from the public. In the secondary analysis of the KEYNOTE-001 trial (83), patients treated with pembrolizumab and RT exhibited significantly longer progression-free survival (PFS; median 4·4 vs 2·1 months; hazard ratio [HR] 0·56; P=0·019) and overall survival (OS; median 10·7 vs 5·3 months; HR 0·58; P=0·026) than patients without previous RT. These data suggest that RT combined with pembrolizumab treatment could bring the synergistic survival benefits to patients with advanced NSCLC (83). In the randomized phase II PEMBRO-RT study (84), compared with pembrolizumab alone, stereotactic body radiotherapy (SBRT) prior to pembrolizumab brought a doubling of overall response rate (36% vs 18%; P=0·070) and a significantly prolonged PFS (median 6.6 vs 1.9 months; HR 0·58; P=0·026). Subgroup analyses further showed the largest benefit from the addition of RT in patients with PD-L1-negative tumors, implying that RT may activate non-inflamed NSCLC toward a more inflamed tumor microenvironment (84). Additionally, a pooled analysis of the PRMBRO-RT (phase II) and MDACC (phase I/II) trials demonstrated significantly improved PFS (median 9·0 vs 4·4 months; HR 0·67; P=0·045) and OS (median 19·2 vs 8·7 months; HR 0·67; P<0·001) with pembrolizumab plus RT than pembrolizumab alone in patients with metastatic NSCLC (85). Meanwhile, both the best out-of-field (abscopal) response rate (41.7% vs 19.7%; P=0·004) and best abscopal disease control rate (65.3% vs 43.4%; P=0·007) was significantly greater with pembrolizumab plus RT versus with pembrolizumab alone, highlighting the significantly increased antitumoral responses and augmented survival benefit noted in the combination treatment (85). In hepatocellular carcinoma, SBRT and ICI combinations were also found potentially effective in inducing the immunomodulatory effects as an"*in situ* vaccine" to increase T-cell receptor diversity and further result in out-of-field abscopal antitumor effects (86).

Limitations of abscopal effect

In clinical practice, there are many factors affecting the abscopal effect of RT combined with ICIs, including radiation dose and segmentation, irradiation sites, general condition of patients, disease stage, tumor characteristics, the sequence of RT and ICIs, and the selection of different ICI agents (7, 82). While radiation can activate the immune system, the optimal dose and timing of RT for the maximal abscopal effect is not fully understood (87). In terms of the radiation dose and segmentation, prior research implied that the positive activating effects of RT on immune responses may be "dose-dependent" within a certain range, and higher single dose RT of ≥15 Gy (12-18 Gy) could lead to increased immunosuppressive effects, such as the accumulation of $CD4^+$ $FoxP3^+$ Treg or Trex1 induction to attenuate tumor immunogenicity (88–90). Nevertheless, other studies suggested different RT doses and segmentations played various immunomodulatory role (87). Some scholars considered low-dose RT, which is commonly used for patients with metastatic diseases as palliative care (91, 92), can better induce anti-tumor immune activation at the molecular level, reshape TIME, and improve the infiltration and function of effector immune cells in distant tumor foci (9, 93–95). Therefore, anti-tumor responses outside the radiation field strengthened by low-dose RT were termed the "RadScopal effect" by them (9, 96). Positive and negative responses of radioimmunotherapy-induced abscopal effect are summarized in Table 1.

TABLE 1 Clinical evidence for radioimmunotherapy-induced abscopal response.

Study	Study Type	Type of Cancer	Treatment	Abscopal Response
Postow et al (2012) (79)	Case report	Melanoma	SBRT (28.5 Gy/3 fractions/9.5 Gy) + Ipilimumab	Positive
Golden et al (2015) (80)	Proof-of-principle trial	Metastatic solid tumors	RT (35 Gy/10 fractions/3.5 Gy) + GM-CSF	Positive in 11/ 41 patients (26.8%); Negative in 73.2%
Formenti et al (2018) (81)	Two-satge phase I/II	Metastatic NSCLC	SBRT (30 Gy/5 fractions/6 Gy in phase I, 28.5 Gy/3 fractions/9.5 Gy in phase II) + Ipilimumab	Positive in 12/39 patients (31%); Negative in 69%
Shaverdian et al (2017)/KEYNOTE-001 (83)	Phase I	Metastatic NSCLC	Previous RT + Pembrolizumab	Positive (mPFS 4·4 ms, mOS 10.7 ms)
Theelen et al(2019)/ PEMBRO-RT (84)	Phase II	Metastatic NSCLC	Privious SBRT (24 Gy/3 fractions/8 Gy) + Pembrolizumab	Positive (12-week ORR 36%, mPFS 6.6 ms, mOS 15.9 ms)
Theelen et al (2021) (85)	Pooled analysis of phase II (PEMBRO-RT) and phase I/ II (MDACC)	Metastatic NSCLC	PEMBRO-RT: Privious SBRT (24 Gy/3 fractions/ 8 Gy) + Pembrolizumab MDACC: Concurrent RT (50 Gy/4 fractions/12.5 Gy or 45 Gy/15 fractions/3 Gy) + Pembrolizumab	Positive (best ARR 41.7%, best ACR 65.3%, mPFS 9.0 ms, mOS 19.2 ms)
Menon et al (2019) (95)	Post-hoc analysis of two phase I/II and one phase II	Metastatic tumors	LDRT (1-20 Gy total) + Ipilimumab or Pembrolizumab or other immunotherapy	Postive in 22/38 patients (58%); Negative in 42%

SBRT, stereotactic body radiotherapy; Gy, gray; RT, radiation therapy; GM-CSF, granulocyte macrophage-colony stimulating factor; NSCLC, non-small-cell lung cancer; mPFS, median progression-free survival; mOS, median overall survival; ms, months; ORR, overall response rate; ARR, abscopal response rate; ACR, abscopal disease control rate; LDRT, low-dose radiation therapy.nical evidence for radioimmunotherapy-induced abscopal response.

Taken together, the immunomodulatory effect of RT is two-sided. On the one hand, it can enhance anti-tumor immune effect through various mechanisms; on the other hand, it may have immunosuppressive effect in certain cases. The key principles of RT to promote local and systemic anti-tumor immune responses include: inducing ICD to facilitate T lymphocyte proliferation; activating cGAS-STING pathway to promote type I interferon response; upregulating the expression of MHC-I on the surface of tumor cells; and enhancing the immunogenicity and antigen visibility of tumor cells; stimulating the release of various proinflammatory cytokines in tumor cells and stromal cells to reshape TIME; increasing immune checkpoint and FAS expression on tumor cell surface to enhance the anti-tumor immune effect. On the contrary, the negative immunosuppressive mechanism mainly includes: RT induced chronic type I interferon and interferon-stimulated gene expression; upregulating PD-L1 and IDO expression on tumor surface; promoting the inhibitory immune cell functions; causing lymphocytopenia and depletion of immune effector cells. At the same time, the abscopal effect of RT and the radscopal effect of low-dose RT combined with ICIs, which constitute an important basis for the synergistic effect, brought substantial therapeutic benefits during the clinical practice. Currently, the best combination modality of RT plus ICIs remains uncertain and warrants further in-depth research and more exploration in the future, which is expected to significantly improve the survival prognosis of cancer patients, promote the scientific progress of comprehensive treatments, and facilitate the development of accurate cancer personalization.

Author contributions

XW: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft. YuW: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft. YZ: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft. HS: Conceptualization, Investigation, Project administration, Writing – original draft. KL: Conceptualization, Investigation, Project administration, Writing – original draft. FW: Conceptualization, Investigation, Project administration, Writing – original draft. YueW: Conceptualization, Investigation, Project administration, Writing – original draft. HC: Conceptualization, Investigation, Project administration, Writing – original draft. YS: Conceptualization, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing. RW: Conceptualization, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

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Case report: A rare case of anti-PD-1 sintilimab-induced agranulocytosis/severe neutropenia in non-small cell lung cancer and literature review

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Immune checkpoint inhibitors (ICIs) demonstrate unique advantages in the treatment of lung cancer and are widely used in the era of immunotherapy. However, ICIs can cause adverse reactions. Hematological toxicities induced by immunotherapy are relatively rare. Agranulocytosis, a rare hematologic adverse event associated with immune checkpoint inhibitors, has received limited attention in terms of treatment and patient demographics. Herein, we report the case of a 68-year-old male with non-small cell lung cancer(NSCLC) who received two cycles of programmed cell death-1 (PD-1) antibody sintilimab immunotherapy combined with albumin-bound paclitaxel and carboplatin chemotherapy and one cycle of sintilimab monotherapy. He was diagnosed with grade 4 neutropenia and sepsis (with symptoms of fever and chills) after the first two cycles of treatment. Teicoplanin was promptly initiated as antimicrobial therapy. The patient presented with sudden high fever and developed agranulocytosis on the day of the third cycle of treatment initiation, characterized by an absolute neutrophil count of 0.0×10^9/L. The patient was treated with granulocyte colony-stimulating factor but did not show improvement. He was then treated with corticosteroids, and absolute neutrophil counts gradually returned to normal levels. To the best of our knowledge, this is the first reported case of sintilimab-induced agranulocytosis in a patient with NSCLC. Sintilimab-induced severe neutropenia or agranulocytosis is a rare side effect that should be distinguished from chemotherapy-induced neutropenia and treated promptly with appropriate therapies; otherwise, the condition may worsen.

KEYWORDS

immune checkpoint inhibitors, PD-1-immune related adverse effects, sintilimab, agranulocytosis, neutropenia, non-small cell lung cancer

1 Introduction

In recent years, significant advancements have been made in cancer immunotherapy, particularly with the advent of widely used immune checkpoint inhibitors (ICIs), such as programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitors and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) inhibitors, which can prolong patient survival (1). PD-1 and PD-L1 are inhibitory co-stimulatory molecules that serve as negative immune regulatory factors, playing a pivotal role in adaptive cellular immunity. By selectively binding to the receptor molecule PD-1 on T cells, tumor-expressed PD-L1 is involved in modulating T cell activation and differentiation while also impeding the anti-tumor immune response mediated by T cells (1). Blocking the PD-1/PD-L1 signaling pathway with drugs or monoclonal antibodies has emerged as a novel cancer immunotherapy strategy, demonstrating efficacy in treating various types of cancers, including malignant melanoma, non-small cell lung cancer, renal cell carcinoma, squamous cell carcinoma and gastric cancer (1, 2). Despite the effectiveness of these therapies, the potential for immune-related adverse events (irAEs) cannot be ignored. The main irAEs associated with ICIs include skin, gastrointestinal, pulmonary, hepatic, and endocrine toxicities. Haematological immune-related adverse events (hem-irAEs), including pancytopenia and hemophagocytic lymphohistiocytosis, have rarely been reported. These irAEs affect the process and efficacy of immunotherapy and some can be fatal. A meta-analysis of 9,324 patients showed that 0.94% of patients treated with ICIs experienced neutropenia (3). Sintilimab, a monoclonal antibody against the PD-1 receptor, is increasingly used in patients with previously treated advanced non-small cell lung cancer (NSCLC). To date, hem-irAEs have not been extensively characterized, and there are no reports of neutropenia caused by sintilimab administration nor, standardized treatment and care protocols. Therefore, it is important for healthcare staff to be aware of these fatal irAEs and develop useful strategies to treat them. Here, we report a rare case of severe neutropenia/agranulocytosis after receiving immunotherapy plus chemotherapy and describe the process of differentiation between immune-related and chemotherapy-related neutropenia.

2 Case presentation

A 68-year-old man was referred to our hospital complaining of a recurrent fever for a month and a 3-day-long chest pain on October 5, 2021. He was previously diagnosed with right lung squamous cell carcinoma (cT3N3M0, stage IIIC) and had a history of deep venous thrombosis for the past five years without previous related treatment. Histopathological analysis suggested non-keratinizing squamous cell carcinoma, with immunohistochemical staining showing EMA(+), CK5/6(+), P40(+), P63(+), TTF-1(-), CK7(-), Napsin A(-), and Ki67 (+, 40%) (Figure 1). PD-L1 expression in the tumor was negative. The patient had been a smoker for 40 years.

Treatment with sintilimab (200 mg) plus paclitaxel liposomes (240 mg) and carboplatin (0.45 g) was initiated on August 27, 2021, at a local hospital. Five days after the first cycle of drug infusion, the patient reported recurrent fever with peak temperature of 40.5°C, and a blood test revealed grade 3 neutropenia with a white blood cell count(WBC) of 1.7×10^9/L and an absolute neutrophil count(ANC) of 0.64×10^9/L. Despite receiving granulocyte-colony stimulating factor (G-CSF) and cephalosporin, the patient experienced no significant improvement in symptoms. Peripheral blood culture results revealed infection with human herpesvirus 5 (HHV-5), Staphylococcus aureus, and Corynebacterium equi, which were considered indicative of sepsis. After the administration of teicoplanin, the fever subsided, and his WBC and ANC returned to normal values (5.04×10^9/L and 3.32×10^9/L, respectively).

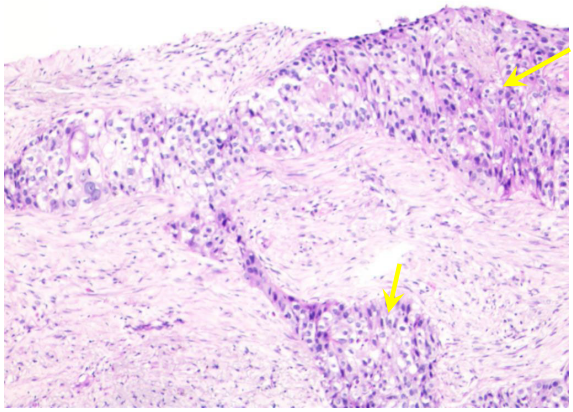

FIGURE 1
Histopathological characteristics of percutaneous lung biopsy. Microscopic examination reveals irregular nest-like structures of cancer cells with round nuclei, visible small nucleoli, abundant translucent and red-stained cytoplasm, accompanied by areas of necrosis (yellow arrows).

Subsequently, the patient was discharged after receiving a second dose of sintilimab (200 mg) and oral anticoagulant therapy (edoxaban tosilate tablets, 30mg/day) on September 25, 2021. The following day, he presented with recurrent fever with peak temperature of 40°C. The patient did not return to the hospital until September 30, at which time his WBC and ANC were normal, but elevated inflammatory markers, including C-reactive protein (42.2 mg/L) and Procalcitonin (12.27 ng/mL), indicated sepsis. However, antibiotic therapy proved to be ineffective.

The patient suddenly developed chest tightness and pain and was subsequently transferred to our hospital for further treatment. On admission, the patient's vital signs were within the normal range except for decreased breath sounds in the left lung during physical examination. In addition, the levels of tumor markers were as follows: carcinoembryonic antigen (CEA) at 4.49 ng/mL, cytokeratin 19 fragment (CYFRA 21–1) at 7.22 ng/mL (normal range: 0–3.3 ng/mL), neuron-specific enolase (NSE) at 15.86 ng/mL, and carbohydrate antigen 125(CA125) at 12.41U/mL, carbohydrate antigen 153(CA153) at 31.40 U/mL(normal range: 0–25 U/mL). Notably, both CA153 and CYFRA21–1 were elevated to levels above their respective normal ranges. Re-evaluation of these tumor markers after treatment can serve as a basis for assessing the efficacy of therapy. The inflammatory marker results upon re-evaluation were as follows: C-reactive protein (7.92 mg/L) and Procalcitonin(0.17 ng/mL). A repeat chest CT scan

conducted on October 5 revealed a mass measuring approximately 6.1×5.2cm in the right lower lung posterior basal segment (Figure 2A), raising concerns of a new neoplasm, with mild perilesional inflammation evident. After 6 days of anticoagulation and heart rate stabilization, coronary artery disease was excluded in this patient using by coronary angiography. Consequently, the patient was subject to the third treatment cycle consisting of carboplatin (0.45g), paclitaxel liposome (270 mg), bevacizumab (300 mg), and sintilimab (200 mg). Soon after midnight he developed a fever with a peak temperature of 40.2°C and exhibited a WBC level of 1.3×10^9/L with an ANC of 0×10^9/L indicating agranulocytosis (Figure 3). These symptoms were originally considered adverse effects of chemotherapy; hence, the treatment was discontinued immediately. However, his WBC showed progressive decline, reaching a nadir of 0.5×10^9/L on October 16, with the ANC remaining at 0×10^9/L throughout; hemoglobin levels were at a low level from October 13 to 21 (Figure 3, Table 1). Additionally, Digital Radiography (DR) findings on October 13 were consistent with the earlier CT results (Figure 2B). This prompted the consideration of the potential bone marrow suppression attributable to immunotherapy. To minimize the risk of infection, the patient was admitted for protective isolation and accommodated in laminar flow beds. Blood cultures were obtained, and the patient was administered intravenous antibiotics (imipenem and cilastatin sodium 1g; vancomycin hydrochloride 500,000 units; caspofungin acetate 50 mg; piperacillin sodium and tazobactam sodium 4.5 g), G-CSF, human albumin(20%, 50 ml), immunoglobulins(5%, reduced from 10 g to 5 g), and blood transfusion(red blood cell suspension 2u). Eating utensils were sterilized and a sodium bicarbonate mouth rinse (sodium bicarbonate and sodium chloride, 250ml, respectively) was given to suppress intraoral disorders. Throughout this period, we closely monitored the patient's blood parameters using peripheral venous blood analysis. The patient refused to undergo a bone marrow examination; hence, the bone marrow morphology test results were lacking. To explore alternative diagnostic avenues, we conducted various tests, including liver function tests and bacterial cultures, and assessment of drug toxicity. Detection of autoimmune disease revealed that the anti-nuclear antibody (ANA) and fungal galactomannan (GM) tests were negative. Additionally, other diagnostic tests such as the G test, Cytomegalovirus (CMV) DNA, and renal profile were within normal limits. A sputum culture conducted on October 18 was positive for Pseudomonas aeruginosa. Notably, the patient had no prior history of agranulocytosis, and his ANC consistently remained within the normal range before and during cancer treatment. Upon review, the patient exhibited bone marrow suppression within 24 hours of chemotherapy, immunotherapy, and anti-angiogenic therapy, which deviated from the typical peak occurrence of chemotherapy-induced bone marrow suppression. A multidisciplinary team (MDT) meeting was organized, and sintilimab-induced agranulocytosis was diagnosed after excluding evidence of autoimmune disease or tumor invasion of the bone marrow.

Considering the patient's history of neutropenia induced by platinum-based chemotherapy, we initiated long-term prophylactic use of recombinant human G-CSF (rhG-CSF) until the ANC returned to normal or near-normal laboratory reference values from its nadir. The patient was started on a treatment regimen consisting of rhG-CSF(5μg/kg, hypodermic injection) for a period of 9 days and methylprednisolone at a daily dosage of 80 mg for 3 days, followed by a subsequent dose reduction to 40 mg over the course of 2 days. Approximately 10 days after the third administration of combined sintilimab therapy, the neutrophil count returned to normal, and no

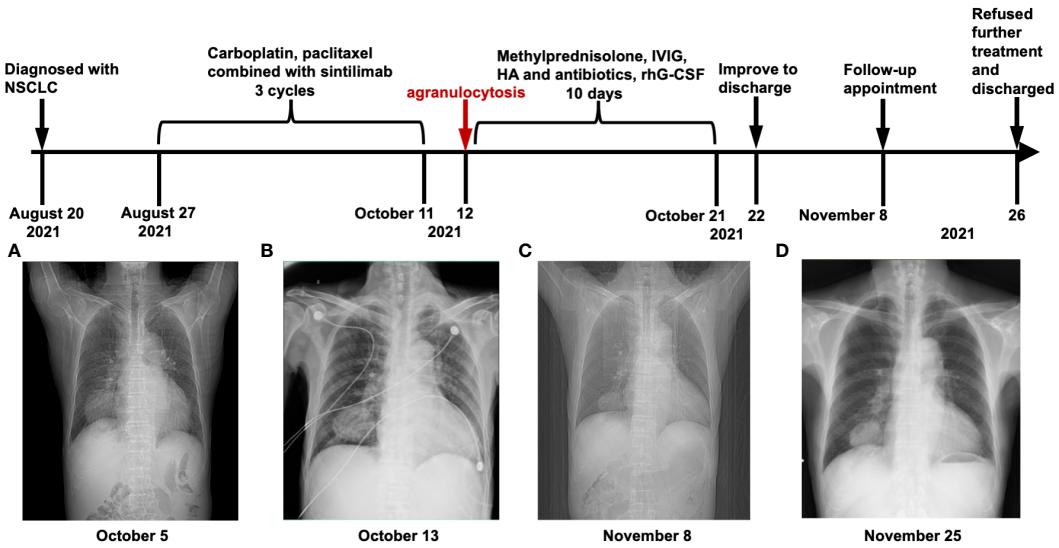

FIGURE 2
Timeline and CT/DR manifestation. **(A)** Chest imaging at initial hospitalization. **(B)** On the first day after the 3rd cycle of immunotherapy, a chest DR image revealed a potential new growth in the lower right lung with mild surrounding obstructive inflammation. **(C)** In the routine tumor follow-up PET-CT image, a right lower lung posterior basal segment cancer mass volume was similar to the 5 October CT image **(A)**, but with reduced obstructive inflammation. **(D)** Chest DR image upon return for treatment: Reduced right lower lung mass and decreased surrounding inflammation compared to the 13 October DR image **(B)**. (NSCLC, non-small cell lung cancer; HA, human albumin; CT, computed tomography; DR, digital radiography; PET-CT, positron emission tomography-computed tomography; Hb, hemoglobin; rhG-CSF, recombinant human granulocyte colony stimulating factor; IVIG, intravenous immunoglobulin).

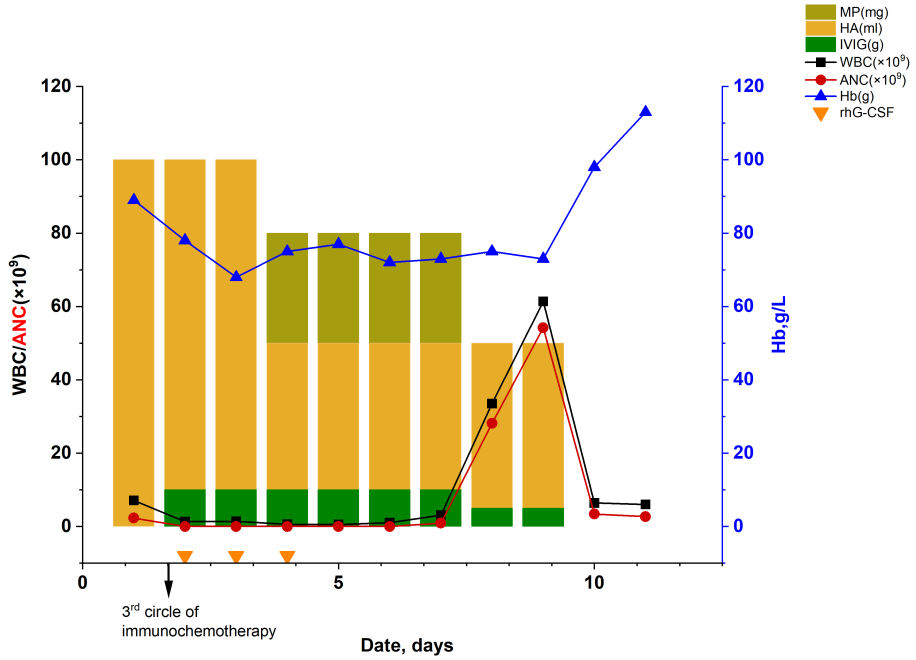

FIGURE 3
Dynamic changes in the routine blood test. (HA, human albumin; WBC, white blood cell count; ANC, absolute neutrophil count;MP, methylprednisolone; IVIG, intravenous immunoglobulin; Hb, hemoglobin; rhG-CSF, recombinant human granulocyte colony stimulating factor).

fever was observed (Figure 3, it is noted that no statistical analysis was employed for the comparison between the lines). The patient was discharged on October 22 and scheduled for a follow-up appointment.

The best response to the third combination of immunotherapies was stable disease(SD). Consequently, the rapid decline in ANC was suspected to be induced by anti-PD-1 antibody. Follow-up positron emission tomography CT (PET-CT) on November 8 revealed that the tumor volume in the right lower lobe peripheral basal segment remained largely unchanged compared to the previous assessment (Figure 2C). Additionally, there was a slight reduction in distal obstructive pneumonia compared to the prior examination.

When the patient returned to our hospital to receive the fourth cycle of chemotherapy on November 25, he no longer received treatment for the previous immune-related responses. Chest CT revealed a decreased mass, measuring approximately 5.6×4.8 cm in the right lower lung, in comparison to the previous assessment, with a slight reduction in perilesional obstructive inflammation (Figure 2D). Subsequent testing of tumor markers revealed carcinoembryonic

TABLE 1 Timeline of blood counts during the third cycle of treatment.

Date	Days of third ICI treatment	WBC(x10^9/L)	ANC(x10^9/L)	Hb(g/L)	RBC(x10^{12}/L)	PLT(x10^9/L)
2021–10-6	-5	5.5	3.1	82	3.81	126
2021–10-9	-2	7.1	2.3	89	4.14	261
2021–10-11	0(3rd)	–	–	–	–	–
2021–10-13	3	1.3	0	78	3.70	302
2021–10-14	4	1.4	0	68	3.17	225
2021–10-15	5	0.6	0	75	3.44	203
2021–10-16	6	0.5	0	77	3.45	188
2021–10-17	7	1	0	72	3.22	165
2021–10-18	8	3.1	0.9	73	3.37	147
2021–10-19	9	33.5	28.1	75	3.43	136
2021–10-21	11	61.4	54.2	73	3.47	113
Normal Ranges	–	4.0–10.0	1.0–8.0	120–160	4.5–5.5	100–400

WBC, white blood cells; ANC, absolute neutrophil count; Hb, hemoglobin; RBC, red blood cells; PLT, platelets; -, no data is available.

antigen (CEA) at 4.63 ng/mL, cytokeratin 19 fragment (CYFRA 21–1) at 4.45 ng/mL (normal range: 0–3.3 ng/mL), neuron-specific enolase (NSE) at 14.89 ng/mL, carbohydrate antigen 125(CA125) at 11.9U/mL, and carbohydrate antigen 153(CA153) at 21.10 U/mL. Compared to pre-treatment results, all tumor markers decreased, with CA153 falling within the normal range; CYFRA21–1 also decreased but remained above normal, indicating, along with the CT results, that the patient showed some response to the treatment; however, the tumor remained active to some extent. The patient experienced fever (38.3°C) again and general malaise following the administration of paclitaxel liposome (270 mg). These symptoms alleviated after discontinuation of the medication. The patient's white blood cell count was 6.0×10^9/L, and ANC was 2.7×10^9/L. Ultimately, the patient chose to discontinue the treatment and was discharged on November 26. During a follow-up telephone consultation, the patient continued to receive regular antineoplastic treatment at the local hospital every three weeks but did not opt for immunotherapy rechallenge.

3 Discussion

NSCLC is the most common clinical subtype of lung cancer, accounting for up to 85% of all lung cancer cases, and over 30% of patients with lung cancer are diagnosed at a locally advanced stage (4). Moreover, conventional clinical treatments often yield suboptimal efficacy in this subset of lung cancer patients, leading to a poor prognosis. Immune checkpoint inhibitors have been approved for the treatment of various malignancies, including lung cancer. Sintilimab can effectively bind to PD-1 and interfere with the interaction between PD-1 and its ligand PD-L1, thereby activating T cell function and exerting antitumor effects. Clinical studies have demonstrated favorable therapeutic efficacy in patients with advanced NSCLC (5). However, the activation of the immune system can contribute to toxic reactions in multiple effector organs, thus affecting organs such as the endocrine system and gastrointestinal tract. Neutropenia, a common adverse reaction to chemotherapy treatment, can easily be confused with ICI-induced agranulocytosis when chemotherapy is used in combination with immunotherapy. This confusion may result in the misuse of treatment and pose a threat to the patient's health and well-being.

The diagnostic criteria and mechanisms underlying ICI-associated neutropenia remain unclear. Currently, the most efficient diagnostic approach for investigating neutropenia typically involves a bone marrow examination. However, in certain cases where the etiology can be explained by patient history and basic laboratory panels, bone marrow examination may not always be clinically necessary for elderly patients. The critical objective was to rule out other potential causes of neutropenia, confirm the diagnosis, and evaluate its severity. In our patient, who had no prior history of rheumatic autoimmune diseases and tested negative for anti-nuclear antibodies upon admission, the development of neutropenia following the first and third cycle of chemotherapy in combination with sintilimab treatment raised concerns. Although the patient exhibited severe decreased WBC and ANC, moderate reduction in red blood cells, and normal platelet count (Table 1), he presented with symptoms of fever and fatigue without bone pain. These findings suggest the possibility of bone marrow infiltration but are not conclusive. Furthermore, the restoration of ANC following G-CSF and antibiotics administration strongly supports the diagnosis of drug-induced agranulocytosis rather than bone marrow infiltration. It is worth noting that the use of medication in our 68-year-old patient carries inherent risks for neutropenia, especially given the increased susceptibility of elderly individuals to chemotherapy-induced neutropenia (6). Although paclitaxel liposomes and carboplatin have been previously associated with neutropenia (7, 8), it is noteworthy that the patient experienced fever even after discontinuing sintilimab during forth cycle of treatment, which may be attributed to prior exposure to platinum-based chemotherapy, advanced or metastatic disease stage, previous chemotherapy exposure, or immune-related effects stemming from immunotherapy.

In this particular case, agranulocytosis was detected within 24 h of the third combined ICI treatment, suggesting that immunotherapy may have increased the risk of myelosuppression. The Chinese Society of Clinical Oncology (CSCO) guidelines for the standardized management of tumor chemoradiotherapy-related neutropenia show that ANC changes in chemotherapy-related neutropenia follow a U-shaped trend approximately 7–14 days after chemotherapy. These levels generally return to normal within 14–21 days (9, 10). In contrast, ICI-related neutropenia can manifest at any time (11) and often presents as grade 3 or 4 neutropenia, which can normalize within two weeks with the use of G-CSF and methylprednisolone (12). The high percentage of patients treated with combined ICI (70%) and earlier and more frequent laboratory testing in these patients indicate that immune-related adverse events generally occur earlier in patients receiving combined ICI (13). Moreover, following administration of the second dose of sintilimab monotherapy, the patient exhibited pyrexia and elevated levels of inflammatory markers on the subsequent day, potentially suggestive of transient neutropenia. Therefore, it is reasonable to hypothesize that the observed neutropenia in this patient following two cycles of chemo-sintilimab combination therapy was likely induced by the synergistic effects of chemotherapeutic agents and ICI. Sintilimab appears to have played a predominant role in precipitating agranulocytosis during the third treatment cycle.

Hem-irAEs can lead to severe neutropenia in patients receiving combined ICIs, rendering them susceptible to bacterial and fungal infections (14). These infections can escalate to septicemia and increase the risk of mortality (15). Notably, four reported cases have been associated with severe neutropenia related to anti-PD-1 antibodies in patients with advanced NSCLC, including three cases linked to nivolumab (16–18) and one to atezolizumab (19). To our knowledge, no case reports of hematotoxicity induced by a combination of sintilimab, carboplatin, and paclitaxel have been published in PubMed, and the pathological features of this combination therapy are not clear. The mechanisms underlying immune-related adverse events induced by PD-1/PD-L1 inhibitors remain poorly understood. Similar to other immune-related adverse events, hematological toxicity is believed to involve the generation of autoreactive T and B cells along with a decrease in the regulatory T cell phenotype (3). Furthermore, the fourth episode of fever could potentially be attributed to acute hypersensitivity reactions to paclitaxel, which commonly manifests immediately after drug

administration. These reactions are associated with the release of proinflammatory cytokines, including IL-6 and TNF-α, which are collectively known as cytokine storms.

The management of irAEs typically involves systemic steroids and symptomatic therapies. Corticosteroids possess immunosuppressive characteristics by exerting pleiotropic effects on the activation, differentiation, and movement of T cells. They inhibit the IL-2 induced activation of effector T cells while promoting the expansion of regulatory T-cells (20). When determining whether to discontinue therapy and administer steroids based on the severity of hem-irAEs (21), consideration should also be given to the potential impact on other irAEs. In our study, the patient with grade 4 neutropenia initially received blood transfusion, antibiotics, and G-CSF therapy. However, methylprednisolone was subsequently administered after failure of initial therapy. This decision was primarily driven by uncertainty in the diagnosis of hem-irAEs, resulting in delayed initiation of steroid therapy. The patient's response to treatment further substantiates the occurrence of immunotherapy-induced neutropenia, given that while chemotherapy induced neutropenia usually improves with the use of antibiotics and G-CSF, immunotherapy-induced neutropenia tends to resolve after steroid administration. G-CSF-based agents can promote the release of mature neutrophils from marginal pools into the peripheral blood and accelerate the differentiation of committed neutrophil precursors in the bone marrow (22). While certain irAEs do not necessarily necessitate the discontinuation of ICI therapy (23), hem-irAEs appear to persist even in the presence of ongoing ICI therapy. In the present case, the patient experienced fever after each ICI treatment, ultimately leading to the patient's decision to discontinue long-term treatment, which in turn accelerated disease progression. Some researchers suggest that downregulation of the immune system with systemic steroids is not recommended for use in immune-related neutropenia (11, 15), whereas others recommend their use with caution (14, 24–26) and in the absence of any evidence of infection (25). It should be noted that steroid use can increase susceptibility to secondary infections.

In conclusion, this case highlights the occurrence of neutropenia, a hematological toxicity, induced by ICIs in combination with chemotherapy. Importantly, the patient responded successfully to short-term steroid therapy. Although severe neutropenia is rare, it is a critical and potentially life-threatening condition requiring prompt clinical intervention.

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 The First Affiliated Hospital of Guangzhou Medical University Ethics 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. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YQ: Conceptualization, Writing – original draft. SL: Data curation, Investigation, Writing – original draft. JC: Investigation, Methodology, Writing – original draft. JP: Resources, Writing – review & editing. JY: Project administration, Supervision, Writing – review & editing.

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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

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Progress of immune checkpoint inhibitors in the treatment of advanced hepatocellular carcinoma

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Among primary liver cancers, hepatocellular carcinoma is the most common pathological type. Its onset is insidious, and most patients have no obvious discomfort in the early stage, so it is found late, and the opportunity for surgical radical treatment is lost, resulting in a poor prognosis. With the introduction of molecular-targeted drugs represented by sorafenib, patients with middle- and late-stage liver cancer have regained the light of day. However, their therapeutic efficacy is relatively low due to the limited target of drug action, toxic side effects, and other reasons. At this time, the emergence of immunotherapy represented by immune checkpoint inhibitors (ICIs) well breaks this embarrassing situation, which mainly achieves the anti-tumor purpose by improving the tumor immune microenvironment. Currently, ICI monotherapy, as well as combination therapy, has been widely used in the clinic, further prolonging the survival of patients with advanced hepatocellular carcinoma. This article reviews the development of monotherapy and combination therapy for ICIs in advanced hepatocellular carcinoma and the latest research progress.

KEYWORDS

hepatocellular carcinoma, immune checkpoint inhibitors, immunotherapy, tumor immune microenvironment, review

1 Introduction

Primary liver cancer is currently the sixth most common malignant tumor in the world, and its fatality rate is the third highest among all malignant tumors (1). According to the latest statistics from the World Health Organization (WHO), about 760,000 people worldwide die of liver cancer every year, and the trend is still on the rise (2). Hepatocellular Carcinoma (HCC) accounts for 90% of primary liver cancers (in this article, liver cancer refers to HCC only), and surgical treatment is still the first treatment of choice for patients with early-stage HCC, with a 5-year survival rate of about 70%-80% (3). However, the onset of HCC

is insidious, most patients are diagnosed with the disease in the middle to late stage, losing the opportunity for radical surgical treatment. In recent years, in the context of precision liver surgery treatment, molecular targeted therapy and immunotherapy have been successively applied to the clinic, bringing light to patients with intermediate and advanced HCC. Since the molecular targeted drugs represented by sorafenib were approved for the treatment of advanced liver cancer in 2007, due to the accumulation of time, they have gradually exposed the problems of limited action targets, toxic side effects, and drug resistance is an urgent need for a new therapeutic modality to break the therapeutic bottleneck of advanced HCC (4–6). Until 2017, the emergence of immunotherapy for treating advanced HCC has opened up a "new world", which is best represented by immune checkpoint inhibitors (ICIs) (7). ICIs inhibit tumor progression mainly by improving the tumor immune microenvironment and enhancing the anti-tumor properties of immune cells. However, due to the unique immunosuppressive tumor microenvironment of hepatocellular carcinoma, the overall response rate of tumor cells to ICIs is not high, so efforts to improve the immune response rate have been made throughout the development of ICIs for hepatocellular carcinoma (8–10). Currently, ICIs mainly include programmed death-1 (PD-1) antibodies, programmed death-1 ligand (PD-L1) antibodies, and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) antibodies. This article outlines the mechanism of action, history of development, and recent research progress of ICIs in treating advanced HCC, points out the potential challenges they face, and looks forward to the future direction of development.

2 ICIs monotherapy

The immune checkpoint molecules are inhibitory receptors that trigger immunosuppressive signaling pathways in immune cells. In activating T cells, immune checkpoint receptors transmit co-inhibitory signals that directly suppress the response of T cells; this is considered one of the main mechanisms of immune escape in HCC. ICIs retard tumor growth by blocking IC and thus improving the immunosuppressive microenvironment of tumors in HCC (Figure 1) (11).

Currently, the primary study population in clinical trials regarding ICIs is patients with advanced hepatocellular carcinoma. Due to hepatocellular carcinoma's unique immunosuppressive microenvironment, its overall immune response rate to ICIs is low. Therefore, the primary purpose of ICI research at this stage is to improve this response rate. The completed single-agent clinical trials of ICIs produced satisfactory results, valuable for guiding the clinical treatment of hepatocellular carcinoma (Table 1). However, the immune response rate still needs to be improved.

2.1 PD-1 inhibitors

2.1.1 Nivolumab

As a fully humanized IgG4 monoclonal antibody, nivolumab inhibits PD-1 on T-cells' surface and activates T-cells' tumor-recognition function to eliminate tumor cells (22, 23). In April 2017, the results of Checkmate040 (NCT01658878), a phase I/II

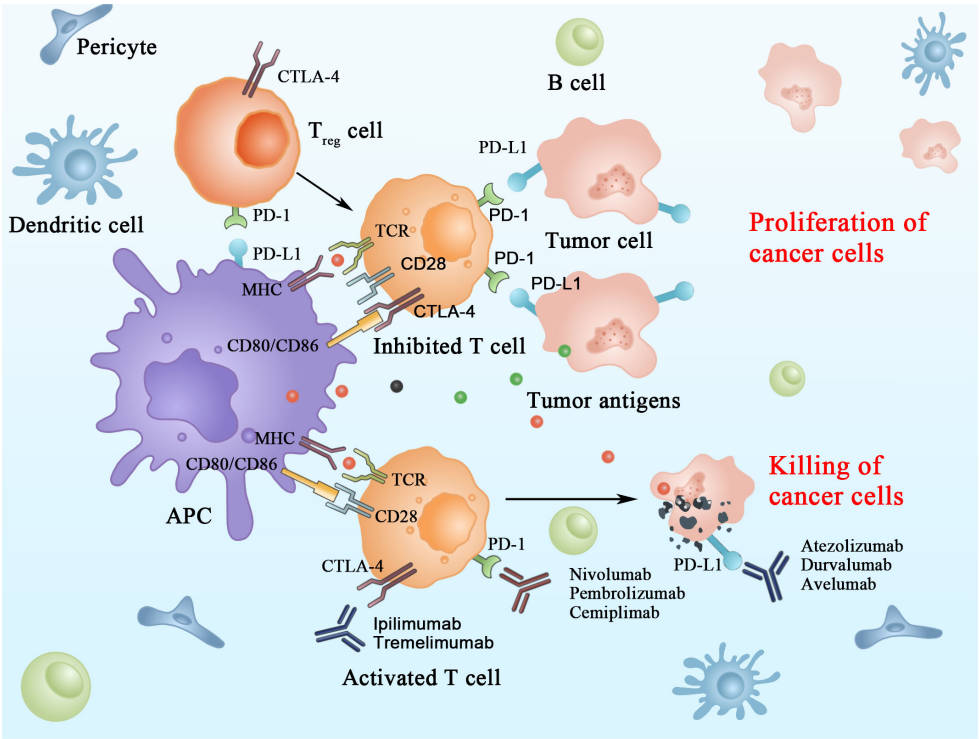

FIGURE 1
Mechanisms of action of immune checkpoint inhibitors in tumor cells.

TABLE 1 Outcomes of clinical trials of ICIs monotherapy in HCC.

Monotherapy	Trial name	Phase	Primary endpoint	n	OS, months	PFS, months	ORR, %	TRAE, %	Reference
Nivolumab	Checkmate040 (NCT10658878)	I/II	Safety,ORR, Tolerance	214	15.0	4.1	20	25	(12)
Nivolumab	Checkmate459 (NCT02576509)	III	OS	371/372	16.4/14.7	3.7/3.8	15/7	22/49	(13)
Pembrolizumb	KEYNOTE-224 (NCT02702414)	II	ORR	104	12.9	4.9	17	24	(14)
Pembrolizumb	KEYNOTE-240	III	OS,PFS	273/135	13.9/10.6	3.0/2.8	18.3/4.4	52.7/46.3	(15)
Pembrolizumb	KEYNOTE-394	III	OS	300/153	14.6/13.0	2.6/2.3	12.7/1.3	66.9/49.7	(16)
Camrelizumab	NCT02989922	II	ORR,OS	217	13.8	2.1	14.7	22	(17)
Tislelizumab	RATIONALE-208	II	ORR	249	13.2	2.7	13	15	(18)
Tislelizumab	NCT03412773	III	OS	342/332	15.9/14.1	36.1/11.0	14.3/5.4	96.2/100	(19)
Sintilimab	ChiCTR2000037655	II	PFS	99/99	–	27.7/15.5	–	12.4/-	(20)
Tremelimumab	NCT01008358	II	OS,PFS	20	8.2	6.48	17.6	45	(21)

study of nivolumab for the treatment of patients with advanced HCC (n = 262), were published. The results showed that in the dose-expansion arm, the confirmed objective response rate (ORR) was 20%, the median duration of response (mDOR) was 9.9 months, and the median progression-free survival (PFS) was 4.1 months. Meanwhile, in the dose-escalation group, the median survival time (mOS) was 15.0 months, the disease control rate (DCR) was 58%, the ORR was 15%, the mDOR was 17.0 months, and the mPFS was 3.4 months (12). Based on the favorable results of the Checkmate040 trial, nivolumab was first approved by the US Food and Drug Administration (FDA) for second-line treatment of advanced HCC in September of the same year (7). In 2019, the European Society for Medical Oncology (ESMO) published the results of the phase III study Checkmate459 (NCT02576509) of patients receiving nivolumab or sorafenib to treat unresectable HCC. The results showed that compared to sorafenib, nivolumab performed better in terms of overall survival time (OS) and ORR (OS: 16.4 vs. 14.7 months; ORR: 15% vs. 7%) with manageable overall toxicity compared to sorafenib (13). The significance of nivolumab as the opening salvo in HCC immunotherapy for patients with advanced disease can be significant. Although nivolumab does not significantly improve survival time in patients with advanced HCC compared to sorafenib, it offers a new treatment option for patients who cannot undergo targeted therapy with a reliable safety profile, and it also provides a good foundation for other subsequent immunotherapies.

2.1.2 Pembrolizumab

Pembrolizumab is a potent humanized IgG4 monoclonal antibody that targets the immune checkpoint PD-1 and blocks its interaction with ligands, thereby preventing tumor cells from evading anti-tumor immunity (24, 25). In June 2018, the results of KEYNOTE-224 (NCT02702414), a phase II study of pembrolizumab for the treatment of patients with advanced HCC (n = 104), were published. The results showed that pembrolizumab had an ORR of 17%, an mPFS of 4.9 months, and an OS of 12.9 months, with 76 (24%) patients experiencing grade 3 or higher treatment-related adverse events (TRAEs) (14). In November 2018, based on the success of the KEYNOTE-224 trial, pembrolizumab became the second drug after nivolumab to receive FDA approval as a second-line therapy for the treatment of unresectable HCC (26). After KEYNOTE-224, phase III studies on pembrolizumab monotherapy for advanced HCC have been conducted. In June 2019, the American Society of Clinical Oncology (ASCO) was the first to publish the results of KEYNOTE-240, a global phase III study of pembrolizumab for the treatment of patients with advanced HCC (n = 278), which was selected for the primary endpoints of OS and PFS. The results showed an ORR of 18.3%, mOS of 13.9 months, and mPFS of 3.0 months, with 147 patients (52.7%) experiencing grade 3 or higher TRAEs (15). Meanwhile, in Asia, KEYNOTE-394, a randomized, double-masked phase III clinical trial of pembrolizumab in patients with advanced HCC (n = 300), is in full swing. The results of the study, which will be presented at ASCO 2022, showed that patients in the pembrolizumab group had a prolonged OS (14.6 vs. 13.0 months) and increased mPFS (2.6 vs. 2.3 months) and a significantly higher ORR (12.7% vs 1.3%), as compared with patients in the placebo group (16). The discovery of pembrolizumab has added a new therapeutic drug for patients with advanced HCC. It has shown promising efficacy and safety, as nivolumab and pembrolizumab have been put into the clinic one after another, and more and more clinical trials of immunotherapeutic drugs for advanced HCC have also been carried out. For some time, immunotherapy for HCC has become a popular medical research.

2.1.3 Camrelizumab

Camrelizumab, or SHR-1210, is an anti-PD-1 IgG4 monoclonal antibody with potent anti-tumor activity (27, 28). With the rise of HCC immunotherapy, PD-1 inhibitors independently developed by China have been introduced and have achieved good efficacy. In February 2020, the results of a phase II study (NCT02989922) on camrelizumab for treating patients with advanced HCC (n=217) were published. The study showed an ORR of 14.7%, a 6-month overall survival of 74.4%, and grade 3 or 4 TRAEs in 47 patients (22%) (17). In this study, camrelizumab showed promising anti-tumor activity and manageable toxicity. Based on the results of this study, camrelizumab was formally approved by the National Medical Products Administration (NMPA) in March of the same year for the treatment of advanced HCC (29). Camrelizumab is the first PD-1 inhibitor independently developed in China and approved for liver cancer indication in China and the third PD-1 inhibitor globally. The approval of camrelizumab has encouraged China's pharmaceutical developers and brought numerous benefits to patients with advanced liver cancer. Compared with other imported PD-1 inhibitors, it has a more affordable price and guaranteed efficacy, which marks the arrival of the era of immunotherapy for Chinese liver cancer patients.

2.1.4 Others

As China's first self-developed PD-1 inhibitor for hepatocellular carcinoma, the launch of camrelizumab has pushed the HCC immunotherapy boom to another wave. Since March 2020, immunotherapeutic drugs independently developed by China, such as tislelizumab, sintilimab, and toripalimab, have been introduced and have shown promising efficacy (30–32). In October 2022, the results of a phase II study (RATIONALE-208) of tislelizumab for treating previously treated patients with advanced HCC (n=249) were reported. The results showed that tislelizumab had an ORR of 13%, a DCR of 53%, and a mOS of 13.2 months, with a total of 38 patients (15%) reporting grade 3 or higher TRAEs, most commonly elevated hepatic transaminases (18). In October 2023, the results of a phase III study (NCT03412773) of tislelizumab in patients with advanced HCC (n=342) were published. Compared with sorafenib, the former showed an overall superiority in mOS, mPFS, and ORR (mOS: 15.9 vs. 14.1 months; mPFS: 36.1 vs. 11.0 months; ORR: 14.3% vs. 5.4%), and the incidence of TRAE was also lower than the latter (96.2% vs. 100%) (19). This result shows that tislelizumab has better anti-tumor activity and safety. In January 2024, the results of a study of sintilimab as adjuvant therapy in resected HCC patients (n=99) with concomitant microvascular invasion (ChiCTR2000037655) were published. The results showed that the mRFS in the sintilimab group was 27.7 months, with 1-year and 2-year survival rates of 99.0% and 87.9%, respectively, and a 12.4% incidence of grade 3 or 4 TRAEs (20). In all of these effective prognostic indicators, the sintilimab group was superior to the active monitoring group, thus demonstrating the effectiveness and safety of sintilimab as a postoperative adjuvant therapy for high-risk HCC patients. There are relatively few clinical studies on these PD-1 inhibitor monotherapies, and it is expected that more clinical studies will be put in place to validate further the efficacy of these PD-1 inhibitor monotherapies in the treatment of advanced HCC.

2.2 PD-L1 inhibitors

PD-L1 is one of the ligands for PD-1, also known as B7-H1 or CD274. The expression of PD-L1 is mainly found in tumor cells, Kupffer's cells, and hepatocytes in HCC (33). As PD-L1 was overexpressed in HCC and combined with PD-1, it inhibited the proliferation and activation of T cells, inactivated T cells. It ultimately led to immune escape, further promoting tumor cell growth (34). Thus, blocking PD-L1 has also emerged as a potential therapeutic strategy for HCC. Currently, two main PD-L1 inhibitors are used for treating advanced HCC, atezolizumab, and durvalumab; both are humanized IgG1 monoclonal antibodies against PD-L1. In June 2020, the results of a phase Ib study (GO30140) on atezolizumab treatment in patients with advanced HCC (n=59) were published. The study showed that the atezolizumab treatment group had an ORR of 17%, mPFS of 3.4 months, and 2 patients (3%) experienced severe TRAEs, which was not as good as the overall outcome of the atezolizumab combined with the bevacizumab treatment group (35). Currently, there are relatively few studies on PD-L1 inhibitor monotherapy for the treatment of advanced HCC, which is still mainly based on combination therapy, and it is expected that more PD-L1 inhibitor monotherapies can be put into clinical studies in the future in order to find a suitable answer.

2.3 CTLA-4 inhibitors

CTLA-4, also known as CD152, is a protein receptor that functions to down-regulate T cells (36). CTLA-4 is expressed not only in activated T cells but also in regulatory T cells. It acts as an "off" switch when bound to CD80 or CD86 on the surface of antigen-presenting cells (37). The two main types of CTLA-4 inhibitors commonly used in the clinic today are tremelimumab, a fully humanized IgG2 monoclonal antibody, and ipilimumab, a fully humanized IgG1 monoclonal antibody, both of which can effectively block CTLA-4 binding. In 2013, the European Association for the Study of the Liver (EASL) annual meeting report published the results of a phase II study (NCT01008358) of tremelimumab for the treatment of patients with hepatitis C-associated HCC (n=20). The study demonstrated that tremelimumab had a DCR of 76.4%, an mOS of 8.2 months and that the treatment was generally well tolerated, with a significant reduction in viral load (21). Due to the limited studies on CTLA-4 inhibitor monotherapy for advanced HCC, its anti-tumor activity and safety cannot be accurately assessed at this time, and more studies are expected to follow to validate it further.

3 ICIs combination therapy

Although the FDA or NMPA has approved several PD-1/PD-L1/CTLA-4 inhibitor monotherapies for use in advanced HCC, their efficacy is still limited and is not the treatment of choice (Figure 2). With further clinical studies on immunotherapy for

hepatocellular carcinoma, immune-combination therapy is a better treatment modality for patients with advanced HCC, which can further improve the therapeutic efficacy (Table 2) (60–65).

3.1 ICIs combined with interventions

Transcatheter arterial chemoembolization (TACE) is an interventional HCC treatment commonly used in treating intermediate and advanced HCC. The chemotherapeutic drugs are delivered directly to the hepatic artery through a catheter. At the same time, the blood supply to the tumor is blocked by using an embolic agent to achieve tumor necrosis and a reduction in its size (66). Using this treatment modality, it is possible to downstage some patients with intermediate to advanced HCC tumors, thus providing the opportunity to achieve radical surgical treatment and prolong survival (67). In addition, TACE can enhance anti-tumor immunity by releasing tumor antigens from killed tumor cells, and immunotherapy can, in turn, strengthen this anti-tumor response, which side-steps the feasibility of TACE in combination with ICIs. Also, TACE has relative limitations, such as low conversion rates, so combination therapy seems more sensible. Several studies have confirmed that ICIs and TACE are efficacious and safe in treating intermediate and advanced HCC (68–70). There is a case report of successful stage reduction of unresectable hepatocellular carcinoma by TACE in combination with tislelizumab, followed by radical surgical resection, with postoperative pathological findings showing complete necrosis of the tumor and no tumor recurrence at 6.0 months postoperatively (71). Based on the promising anti-tumor effects produced by ICIs in combination with interventional therapy, the International Society for Multidisciplinary Interventional Oncology (ISMIO) International Expert Group consensus statement in February 2021 affirmed that TACE combined with the option of systemic therapy regimens can improve the outcome of unresectable HCC (72). In 2023, The Asia-Pacific Primary Liver Cancer Expert Meeting (APPLE) announced the START-FIT (NCT03817736), a phase II study of TACE in combination with stereotactic radiotherapy and avelumab for the treatment of advanced HCC patients (n=33) results. The results showed an ORR of 67%, a DCR of 70%, an mPFS of 21.4 months, an mOS of 30.3 months, and an mDOR of 20.2 months for triple therapy, with 11 (33%) patients experiencing a grade 3 or higher TRAEs, and 4 (12%) patients receiving curative therapy (38). In July of the same year, the results of a study (ChiCTR2000039508) on TACE in combination with TKIs and camrelizumab for treating patients (n=87) with advanced unresectable HCC were published. The results showed an ORR of 71.3%, an mPFS of 10.5 months, and a DCR of 89.7%, as confirmed by mRECIST; ten patients (11.5%) successfully underwent conversion therapy, all achieving R0 resection (39). In April 2024, the results of another phase II study (NCT04599790) of TACE in combination with sintilimab and lenvatinib for advanced HCC (n=30) were published. The results showed an ORR of 60%, mPFS of 8.0 months, DCR of 86.7%, mOS of 18.4 months, and grade 3 or higher TRAEs in 12 patients (40%) (40). These studies have demonstrated the synergistic anti-tumor effect of TACE combined with ICIs, which may allow unresectable advanced HCC patients to gain access to conversion therapy and prolong survival. In addition, there is also the phase II study of TACE in combination with nivolumab for intermediate-stage HCC (IMMUTACE) (73) and the phase III LEAP-012 (NCT04246177) study of TACE in combination with lenvatinib and pembrolizumab for intermediate stage HCC (74), which have

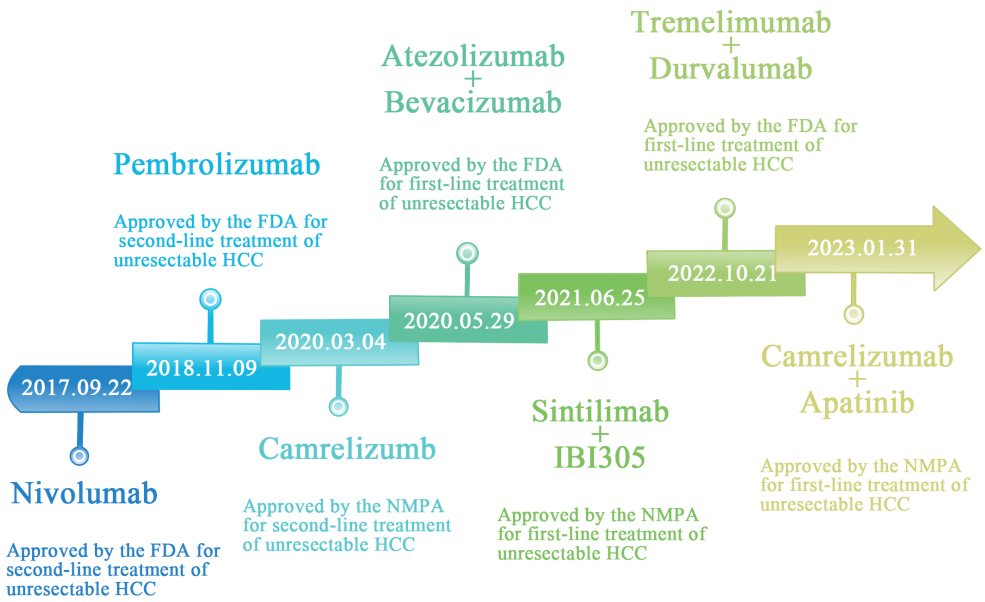

FIGURE 2
FDA and NMPA approval of immune checkpoint inhibition schedule for unresectable hepatocellular carcinoma.

also achieved good results but has not yet clarified the advantageous population receiving TACE combined with ICIs for advanced HCC and the advantages and disadvantages of each combination therapy have not been clarified. More relevant studies will follow to validate the above questions and provide clear answers.

3.2 ICIs combined with radiofrequency ablation

Radiofrequency ablation (RFA) therapy is a commonly used local treatment for early-stage HCC, especially for HCC patients with tumor diameters <3 cm is reproducible, minimally invasive, and has low complications (75). RFA treatment activates systemic anti-tumor immune responses and inhibits the immune escape of tumor cells; however, due to the weakness of these responses, they do not allow complete control of the tumor, contributing to the high recurrence rate of RFA (76, 77). Recurrence rates as high as 50% to 70% within 20 to 30 months after successful RFA have been reported, suggesting that single ablative therapy does not appear to be a perfect option for the treatment of early HCC (78). Based on the mechanism by which RFA causes HCC recurrence, it is easy to think of the feasibility of combination immunotherapy. Several studies have demonstrated the synergistic anti-tumor effect of RFA combined with immunotherapy (79–81). In 2017, results from a study (NCT01853618) of the CTLA-4 inhibitor tremelimumab in combination with RFA for advanced HCC (n=32) were published. The study showed that patients treated with the combination had a 6-month progression-free survival time rate of 57.1%, a 12-month progression-free survival time rate of 33.1%, a median OS of 12.3 months, and a significant reduction in viral load in 12 of 14 patients with quantifiable hepatitis C (41). In February 2022, the results of NIVOLVE (UMIN000026648), a phase II study of adjuvant nivolumab after surgical resection/radiofrequency ablation for the

TABLE 2 Outcomes of clinical trials of ICIs combination therapy in HCC.

Combination therapy	Trial name	Phase	Primary endpoint	n	OS, months	PFS, months	ORR,%	TRAE,%	Reference
TACE									
TACE plus SBRT and avelumab	START-FIT (NCT03817736)	II	Proportion of patients who may be cured	33	30.3	21.4	67	33	(38)
TACE plus TKI and camrelizumab	CHiCTR2000039508		PFS,ORR	87	–	10.5	71.3	67.8	(39)
TACE plus Lenvatinib and sintilimab	NCT04599790	II	PFS	30	18.4	8.0	60	40	(40)
RFA									
RFA plus tremelimumab	NCT01853618		OS,PFS	32	12.3	7.4	26.3	–	(41)
RFA plus nivolumab	NIVOLVE (UMIN000026648)	II	RFS	53	–	26.3	–	18.9	(42)
RFA plus pembrolizumb	IMMULAB (NCT03753659)	II	ORR	30	–	–	13.3	–	(43)
RFA plus toripalimab	ChiCTR1900027807		RFS	20/20	–	15.4/8.0	–	–	(44)
RT [^{90}Y]									
RT plus nivolumab	CA209-678 (NCT03033446)	II	ORR	36	20.2	27.6	36	14	(45)
RT plus durvalumab	SOLID	I/IIa	TTP	24	–	6.9	83.3	8.7	(46)
RT plus pembrolizumab	HCRNGI15-225 (NCT03099564)		PFS	27	20.3	9.95	30.8	48.1	(47)
HAIC									
HAIC plus lenvatinib and toripalimab	NCT04044313	II	PFS	36	17.9	10.4	63.9	11.1	(48)
HAIC plus camrelizumab and apatinib	NCT04191889	II	ORR	35	–	10.38	77.1	37.1	(49)

(Continued)

TABLE 2 Continued

Combination therapy	Trial name	Phase	Primary endpoint	n	OS, months	PFS, months	ORR,%	TRAE,%	Reference
Anti-VEGF									
Bevacizumab plus atezolizumab	G030140 (NCT02715531)	Ib	ORR,PFS	104	17.1	12.4	36	–	(35)
Bevacizumab plus atezolizumab	IMbrave150 (NCT03434379)	III	ORR,PFS	336	67.2	6.8	–	56.5	(50)
IBI305 plus sintilimab	ORIENT-32 (NCT03794440)	II/III	Safety,OS, PFS	380/191	-/10.4	4.6/2.8	21/4	14/18	(51)
TKI									
Lenvatinib plus pembrolizumab	NCT03006926	Ib	Safety,Tolerance, ORR,DOR	104	22	8.6	36	67	(52)
Lenvatinib plus pembrolizumab	LEAP-002 (NCT03713593)	III	OS,PFS	395/399	21.2/19.0	8.2/8.0	–	17/17	(53)
Apatinib plus camrelizumab	NCT03092895	II	Safety,Tolerance	28	13.2	3.7	10.7	92.9	(54)
Apatinib plus camrelizumab	CARES-310 (NCT03764293)	III	OS,PFS	272/271	22.1/15.2	5.6/3.7	25/6	88/68	(55)
Lenvatinib plus tislelizumab	NCT04401800	II	ORR	64	–	8.2	38.7	28.1	(56)
ICIs									
Nivolumab plus ipilimumab	NCT03222076	II	Safety,Tolerance	14/13	–	19.53/9.4	–	43/23	(57)
Durvalumab plus tremelimumab	NCT02519348	I/II	Safety	332	18.73	2.17	24	37.8	(58)
Durvalumab plus tremelimumab	HIMALAYA (NCT03298451)	III	OS	393	16.4	3.8	20.1	25.8	(59)

treatment of patients with HCC (n=53), were published. The study showed that patients in the combination therapy group had a 1-year RFS of 26.3 months, an 18.9% incidence of grade 3-4 TRAEs, and an overall favorable treatment outcome (42). In February 2023, the results of IMMULAB (NCT03753659), a phase II study of pembrolizumab in combination with radiofrequency ablation for the treatment of patients (n=30) with early-stage HCC, were published. According to RECIST v1.1, the confirmed ORR was 13.3%, which did not meet the provisional mOS (43). In September 2023, the results of a prospective controlled study (ChiCTR1900027807) of toripalimab combined with radiofrequency ablation for treating recurrent HCC were published. The results showed that mRFS was higher in the combination therapy group compared to single RFA treatment (15.4 vs. 8.0 months, HR:0.44, P<0.05) (44). Comprehensive studies on RFA combined with ICIs in recent years have shown that the combination of RFA and ICIs can effectively make up for some of the limitations of RFA, reduce the recurrence rate of tumors, prolong the survival period, and have a controllable safety profile. However, it is not without the lack of persuasiveness due to the small sample size that more multi-center and large-sample studies are expected to further validate the benefits of ICIs in combination with RFA therapy.

3.3 ICIs combined with radiotherapy

Radiotherapy is divided into two types: internal radiotherapy and external radiotherapy, which are suitable for patients with advanced HCC, especially those with combined portal vein cancer thrombosis. In recent years, radiotherapy has achieved good results in treating advanced HCC. Among them, selective internal radiotherapy (SIRT) using yttrium [90Y] resin microspheres has been a famous study in HCC in recent years. SIRT injects the radionuclide yttrium [90Y] microspheres containing beta-emitting radionuclides into the tumor tissue via the hepatic artery. Yttrium [90Y] microspheres kill the tumor cells by releasing short-range radiation and cause minimal damage to the normal liver tissue, characterized by a fast onset of action, minimal damage, and precise positioning (82). Yttrium [90Y] was first marketed in Australia in 1998 and has subsequently been used primarily as a palliative treatment for unresectable HCC, and in the last few years, has emerged as a potential down-staging strategy for unresectable hepatocellular carcinoma due to its findings of efficacy in tumor shrinkage and liver hypertrophy (83). In 2022, China's first yttrium [90Y] resin microsphere intervention, led by Academician Jiahong Dong, was implemented in Boao LeCheng, Hainan, and successfully downstaged a patient with Chinese liver cancer stage (CNLC) IIIa to

stage Ia with radical surgical treatment. The tumor cells in the resected specimen were almost entirely necrotic (84). These results demonstrate the feasibility and safety of yttrium [90Y] microspheres for treating advanced HCC. Studies have confirmed that the systemic immune system is activated during radiotherapy, and the combination of ICIs further enhances the therapeutic efficacy and synergistic anti-tumor effect with a reliable safety profile (85, 86). In October 2021, the results of a phase II study CA209-678 (NCT03033446) of radioembolism using yttrium [90Y] resin microspheres in combination with nivolumab for the treatment of patients (n=36) with advanced HCC were published, the primary endpoint of this study was ORR and the secondary endpoint was PFS. The study showed an ORR of 30.6%, mPFS of 20.2 months, and grade 3 or higher TRAEs in 5 patients (14%) (45). In September 2023, the results of SOLID, an I/IIa study of durvalumab in combination with yttrium [90Y] resin microspheres for the treatment of patients (n=24) with locally advanced unresectable HCC, were published. The study showed mPFS of 6.9 months, ORR of 83.3%, DCR of 91.7%, failure to achieve mOS, and grade 3 TRAEs in 2 (8.7%) patients (46). In February 2024, the results of a preliminary study HCRNGI15-225 (NCT03099564) on pembrolizumab in combination with yttrium [90Y] resin microspheres for the treatment of patients (n=27) with advanced HCC were published, showing an mPFS of 9.95 months, an mOS of 20.30 months, an ORR of 30.8% and a DCR of 84.6%, and grade 3 or higher TRAEs occurred in 13 of 27 patients (48.1%) (47). Results have also been published from studies of sintilimab and tislelizumab in combination with radiation therapy, which have shown good efficacy (87, 88). In recent years, the combination of radiotherapy and ICIs has been increasingly used in the treatment of advanced HCC, and its ability to enable some patients to complete tumor downstaging for radical treatment and further prolong the survival of patients has become a hot research topic.

3.4 ICIs combined with chemotherapy

Hepatic arterial infusion chemotherapy (HAIC) is one of the primary means of treatment for intermediate and advanced HCC. The primary chemotherapy regimen approved in China is FOLFOX4, which selectively administers chemotherapeutic drugs (including oxaliplatin, fluorouracil, and folinic acid) to the blood-supplying arteries of intrahepatic tumors mainly through an arterial catheter to increase the local concentration, thus exerting a potent anti-tumor effect, and possessing therapeutic characteristics of precise targeting and low toxicity (89–91). Some studies have confirmed that oxaliplatin can induce immunogenic cell death and modulate the tumor cell microenvironment, making oxaliplatin-containing FOLFOX4 chemotherapy regimens combined with ICIs a potential treatment option for unresectable advanced HCC (92). In 2022, the results of a study from China evaluating the efficacy and safety of atezolizumab and bevacizumab in combination with HAIC for the treatment of advanced HCC were published, which enrolled a total of 52 eligible patients with advanced HCC for triple therapy. The results showed an ORR of 67.3%, mPFS of 10.6 months, OS was not achieved, all TRAEs were controlled, and further analysis concluded that extrahepatic metastases were an independent risk factor associated with PFS (93). In the same year, the results of another China's phase II study (NCT04044313) on the combination of lapatinib and toripalimab with HAIC in patients with advanced HCC (n=36) were also published. The results showed that mPFS was 10.4 months, mOS was 17.9 months, ORR was 63.9%, and mDOC was 14.4 months, with 4 (11.1%) patients experiencing grade 3 or higher TRAEs and no treatment-related deaths (48). In 2023, the results of a phase II study (NCT04191889) on the combination of camrelizumab and apatinib with HAIC in patients with advanced HCC (n=35) were published. The study showed an ORR of 77.1%, DCR of 97.1%, mPFS of 10.38 months, and failure to achieve mOS. A total of 13 patients (37.1%) developed grade 3 or higher TRAEs, and six patients (17.1%) achieved disease downstaging and radical surgery after triple therapy (49). The relevant studies in recent years show that the current combination of ICIs and molecular targeting with HAIC for the treatment of advanced HCC has an excellent synergistic effect, which can further improve the anti-tumor activity and have controllable safety. However, based on the limited number of studies on the combination of the three treatments, more studies are still needed to determine the value of their clinical application.

3.5 ICIs combined with targeted therapy

3.5.1 ICIs combined with angiogenesis inhibitors

Prior to the introduction of immunotherapy, molecularly targeted therapies had been the sole therapeutic modality for the systemic treatment of advanced HCC, remaining a monopoly for a decade. Targeted therapies block the growth and proliferation of liver cancer cells by targeting specific signal transduction pathways in liver cancer and adopting a point-to-point approach whereby the drug binds to specific receptors or molecules on the surface of liver cancer cells (94, 95). *Bevacizumab* is an angiogenesis inhibitor, which not only inhibits angiogenesis and thus reduces the blood supply to the tumor but also regulates the tumor's immune response, a mechanism of action that offers the possibility of subsequent combination with ICIs for the treatment of advanced HCC (96). In 2018, ASCO was the first to publish the results of the phase Ib study GO30140 (NCT02715531) of atezolizumab in combination with bevacizumab (T + A) for the treatment of patients (n=104) with unresectable HCC, the results showed that the combination of the two had a manageable safety profile with a PFS of 12.4 months, a mOS of 17.1 months, an ORR of 36% and a DCR of 71% (35). Due to the synergistic anti-tumor effect of the "T+A" combination regimen shown in the GO30140 study, follow-up studies were soon to follow. In November 2019, ESMO published the results of the phase III study IMbrave150 (NCT03434379) of the "T+A" combination therapy for the treatment of patients (n=336) with unresectable HCC, atezolizumab in combination with bevacizumab showed better DFS and OS rates compared to sorafenib (DFS: 6.8 vs 4.3 months;1-year overall survival rate: 67.2% vs 54.6%) (50). The IMbrave150 further confirms that the combination of the two has

good anti-tumor activity in treating advanced HCC. Based on the success of the IMbrave150 study, the FDA and NMPA approved the "T+A" regimen in May and October 2020, respectively, for the treatment of unresectable HCC without prior systemic therapy (97). University societies and guidelines recommend this combination regimen (T + A) as a first-line treatment for advanced HCC (98–103). In October 2020, Cinda Biologics announced the results of ORIENT-32 (NCT03794440), a Phase II-III study of sintilimab in combination with IBI305 (a bevacizumab analog) for the treatment of unresectable HCC. Compared with patients in the sorafenib-treated group, sintilimab in combination with IBI305 significantly improved mPFS and ORR (mPFS: 4.6 vs 2.8 months; ORR: 21% vs 4%), and although sintilimab in combination with IBI305 did not achieve the prespecified mOS, it was still superior to the sorafenib group by 10.4 months (51). Based on the success of the ORIENT-32 trial, in June 2021, the NMPA approved sintilimab in combination with IBI305 as the first-line treatment for advanced HCC. In addition, the results of the phase II study (NCT04843943) on sintilimab in combination with bevacizumab as a conversion therapy for resectable intermediate-stage HCC, led by academician Fan Jia, were presented for the first time at ESMO 2022. The results of the study showed that the ORR and DCR were 23.3% and 90%, respectively, and a total of 13 patients (43.3%) met the criteria for hepatic resection and underwent surgical treatment, after which the patients recovered well and had no recurrence for the time being (104). Currently, ICIs combined with angiogenesis inhibitors have been widely used in intermediate and advanced HCC and have achieved promising therapeutic results. The combination of the two has synergistic solid anti-tumor activity. It can achieve tumor downstaging for intermediate-stage HCC patients with the opportunity to achieve radical surgical treatment and further prolong the patient's survival.

3.5.2 ICIs combined with tyrosine kinase inhibitors

With the continuous exploration of ICIs in combination with molecular targeted therapy for the treatment of HCC, TKIs in combination with ICIs are feasible and effective in treating advanced HCC. TKIs inhibit the growth and proliferation of tumor cells and promote apoptosis mainly by inhibiting cellular signal transduction (105). Its combination with ICIs has a synergistic anti-tumor effect, further improving the survival of patients with advanced HCC while ensuring safety. In 2019, the ESMO Annual Meeting presented for the first time the results of the phase Ib study (NCT03006926) of pembrolizumab plus lenvatinib for the treatment of patients (n=104) with unresectable HCC. With a confirmed ORR of 36% according to RECIST v1.1, an mDOR of 12.6 months, an mPFS of 8.6 months, and a mOS of 22.0 months, 67 percent of patients experienced grade 3 or higher TRAEs (52). In 2022, the results of the phase III study LEAP-002 (NCT03713593) of pembrolizumab in combination with lenvatinib for the treatment of patients with advanced HCC (n=395) were published, showing a mOS of 21.2 months and an mPFS of 8.2 months, which were both better than in the placebo group but fell short of the pre-determined thresholds (53). At the same time, our researchers have been actively involved and have achieved results that have attracted the world's attention. In 2021, the results of a phase II study (NCT03092895) of kamrelizumab in combination with apatinib for treating advanced primary hepatocellular carcinoma were published. The study showed an ORR of 10.7 percent, an mPFS of 3.7 months, and an mOS of 13.2 months, with 26 patients experiencing grade 3 or higher TRAEs (54). At the ESMO Annual Meeting 2022, the results of the phase III study CARES-310 (NCT03764293) on kamrelizumab in combination with apatinib for unresectable HCC were presented by Prof Shukui Qin, which showed that compared to sorafenib, the combination therapy had an ORR of 25%, a DCR of 78%, an mOS of 22.1 months and an mPFS of 5.6 months, which were all significantly better than the former (55). Based on the excellent results CARES-310, the CSCO Liver Cancer Guidelines recommended it as a first-line treatment for advanced HCC in the same year (100). In January 2023, the NMPA formally approved the first-line treatment of advanced HCC, achieving a significant breakthrough in treating advanced HCC with ICIs combined with TKIs. In addition, the phase II study of tislelizumab in combination with lenvatinib for treating unresectable HCC (NCT04401800) also achieved good results, showing good anti-tumor activity and tolerability (56). At present, the clinical studies of ICIs combined with small-molecule TKIs for the treatment of advanced HCC have had a series of successive reports, especially the karelizumab combined with apatinib regimen proposed by Prof. Shukui Qin in China, the only large clinical study of ICIs combined with small molecule TKIs for advanced HCC that has obtained positive dual endpoints of OS and PFS to date, adds another reliable treatment option for patients with advanced HCC and has landmark status in the treatment of hepatocellular carcinoma.

3.6 Dual-immunity combination therapy

Compared to the limited availability of ICIs as monotherapy, dual-immunity combination therapy achieves "1 + 1>2" efficacy. In October 2020, the results of a Checkmate040 randomized clinical trial were published, which showed better anti-tumor activity and safety in the nivolumab combined with the ipilimumab treatment group compared to monotherapy (106). In January 2022, results from a phase II study (NCT03222076) of nivolumab in combination with ipilimumab for resectable HCC were published, with the combination having superior mPFS (19.5 vs 9.4 months) and a manageable overall safety profile compared to nivolumab monotherapy (57). The above study blocked both PD-1 and CTLA-4 immune checkpoints, which further inhibited the immune escape of tumor cells and delayed tumor growth. In July 2021, the results of a phase I/II study (NCT02519348) of durvalumab plus tremelimumab in the treatment of patients with unresectable HCC (n=332) were published. The study showed an ORR of 24%, an mOS of 18.73 months, an mPFS of 2.17 months, and an incidence of TRAEs of grade 3 or higher of 38.7% with the combination of both treatments (58). In 2022, the results of HIMALAYA (NCT03298451), a phase III study of durvalumab

plus tremelimumab for treating unresectable HCC, were published. The study results showed an ORR of 20.1%, mPFS of 3.8 months, and mOS of 16.4 months. Subsequent follow-up observation found that the 3-year overall survival rate and 4-year overall survival rate of the combination therapy group were 30.7% and 25.2%, respectively, which were significantly higher than those of the sorafenib group, which were 19.8% and 15.1% (59, 107). Based on the success of the HIMALAYA study, the FDA formally approved tremelimumab in combination with durvalumab for the first-line treatment of unresectable HCC in October 2022 (108). This is the first time a PD-L1 inhibitor and a CTLA-4 inhibitor have been combined, making this regimen the second FDA-approved first-line regimen for treating advanced HCC after "T+A",which will benefit more patients.

4 Conclusions and perspectives

Due to the high metastasis and recurrence of liver cancer, the global mortality rate of liver cancer patients has remained high every year. The emergence of immunotherapy represented by ICIs has brought light to patients with advanced liver cancer and, at the same time, broken the monopoly of molecular targeted therapy for advanced liver cancer, opening up a new pattern of liver cancer treatment. ICIs inhibit tumor growth and proliferation by blocking immune checkpoints, thereby inhibiting the immune escape of tumor cells from immune cells, significantly increasing the overall survival and disease-free survival of patients and improving the quality of their survival. Compared with the limited efficacy of PD-1/PD-L1/CTLA-4 inhibitor monotherapy, immuno-combination therapy has better efficacy for patients with advanced HCC, and some patients can even achieve tumor downstaging and radical surgical treatment through combination therapy. Although immunotherapy has benefited many liver cancer patients, it still faces many challenges, such as drug resistance during treatment, the advantageous population suitable for each combination therapy, which is still unclear, and the occurrence of immune-related adverse events after treatment. Significantly, since the vast majority of liver cancer patients in China develop from hepatitis B, finding ICI monoclonal antibodies that are more suitable for our patients has become significant. In addition, it has been confirmed that ICI treatment can reactivate the hepatitis virus in a small number of patients, resulting in fulminant hepatitis or even liver failure, and how to eliminate this phenomenon is also a subsequent problem to be solved. All these issues need to be validated by ongoing multi-center, large-sample, prospective controlled studies and in-depth clinical and basic research to individualize treatment. In addition to this, immunotherapy should not be limited to immune checkpoint inhibitors, but relay cell therapy and tumor vaccines have also been the subject of immunological research on advanced liver cancer in recent years, and the results of these studies are also worth looking forward to. Due to the complexity of the pathogenesis and the high degree of malignancy of hepatocellular carcinoma, it is essential to clearly understand that although immunotherapy offers a ray of hope for patients with advanced HCC, ultimately, the efficacy of drug treatment is limited, earlier detection and diagnosis is the top priority, and this requires an in-depth study of the pathogenesis of liver cancer, and then make targeted prevention. We look forward to an early breakthrough in this research direction to reduce the incidence and mortality of liver cancer.

Author contributions

TL: Conceptualization, Data curation, Investigation, Software, Writing – original draft, Writing – review & editing, Methodology. GM: Investigation, Writing – review & editing. SM: Investigation, Writing – review & editing. JY: Investigation, Writing – review & editing. LY: Conceptualization, Writing – review & editing. RH: Conceptualization, Writing – review & editing. XZ: Conceptualization, Writing – review & editing. YC: Supervision, Writing – review & editing.

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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

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Efficacy and safety of a novel TKI (anlotinib) for the treatment of advanced digestive system neoplasms: a systematic review and meta-analysis

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Objective: To systematically evaluate the efficacy and safety of anlotinib targeted therapy for the treatment of patients with advanced digestive system neoplasms (DSNs).

Methods: Clinical trials were extracted from PubMed, the Cochrane Library, Web of Science, Embase, China National Knowledge Infrastructure (CNKI) and the Wanfang database up to October 2023. Outcome measures, including therapeutic efficacy, quality of life (QOL) and adverse events, were extracted and evaluated.

Results: Twenty trials, including 1,613 advanced DSNs patients, were included. The results indicated that, compared with conventional treatment alone, the combination of anlotinib targeted therapy with conventional treatment significantly improved the patients' 6-months overall survival (OS, OR=1.76, CI=1.53 to 2.02, $P<0.00001$), overall response (ORR, OR=1.76, CI=1.53 to 2.02, $P<0.00001$) and disease control rate (DCR, OR=1.51, 95% CI=1.25 to 1.84, $P<0.0001$). Moreover, the group that received the combined therapy had higher rates of hypertension ($P<0.00001$), proteinuria ($P<0.00001$), fatigue ($P<0.00001$), diarrhea ($P<0.00001$), hypertriglyceridemia ($P=0.02$), alanine aminotransfease (ALT)increased ($P=0.004$), aspartate transaminase (AST) increased ($P=0.006$), anorexia ($P<0.00001$), weight loss ($P=0.002$), abdominal pain ($P=0.0006$), hypothyroidism ($P=0.02$), prolonged QT interval ($P=0.04$). Analyses of other adverse events, such as gastrointestinal reaction, leukopenia, and neutropenia, did not reveal significant differences ($P>0.05$).

Conclusion: The combination of anlotinib targeted therapy and conventional treatment is more effective for DSNs treatment than conventional treatment alone. However, this combined treatment could lead to greater rates of hypertension, albuminuria and hand-foot syndrome. Therefore, the benefits and risks should be considered before treatment.

KEYWORDS

anlotinib, target therapy, conventional treatment, digestive system neoplasms, meta-analysis

1 Introduction

Digestive system neoplasms (DSNs) are an important part of the incidence and mortality rate of cancer in the world, and cause 3,524,932 deaths in 2020, which accounts for 18% of all cancer deaths worldwide (1–3). This category comprises colorectal cancer, gastric cancer, liver cancer, esophageal cancer, and pancreatic cancer, which are the third, sixth, seventh, tenth, and fourteenth most common cancers, respectively (4). Gastrointestinal malignant tumor is a common tumor of the digestive system in the clinic, which threatens the human's life and health seriously (5). The three main modalities (chemotherapy, targeted therapy and immunotherapy) had been widely used in treating patients with DSNs (6). Despite the extraordinary improvements carried out in diagnostic and therapeutic management of DSNs in the past few decades, the 5-year survival rate of patients is still very low (1, 7). Since DSNs are mostly detected only at advanced stages, early extensive invasion and distant metastasis, as well as a profound resistance towards multi-drugs contribute to poor prognosis for the patients (8–10). Therefore, the effective and new therapeutic strategies targeting DSNs should be developed.

In recent years, molecular-targeted agents have attracted substantial attention to improve the anti-cancer specificity and efficacy and significantly reduce non-selective resistance and toxicity (11). Targeted therapy is a type of cancer treatment that uses drugs or other substances by targeting cancer-specific genes, proteins, or the tissue environment that control cancer cells' growth, division and spreading (12, 13). Compared to traditional chemotherapy drugs, targeted anti-tumor drugs can specifically act on cancer cells with high efficacy and little damage is done to normal cells (14). As a result of the rapid innovations and advancements in the field of tumor biology, more and more attention has been focused on the new modality of tumor molecular-targeted therapy for advanced cancer (11). Multiple clinical studies have confirmed that molecular targeted therapy combined with conventional treatment methods has better effects on cancer patient (15–18).

Over the past few decades, increasing evidence has indicated the important role of neovascularization in proliferation, migration, and invasion of various solid tumors (19). Vascular endothelial growth factor (VEGF), fibroblast growth factor, platelet-derived growth factor and their corresponding receptors play an important role in the process of vascular growth. Therefore, vascular-targeted therapy against these growth factors and their receptors is one of the important strategies for patients with advanced DSNs. Anlotinib is a novel and oral small-molecule multitarget tyrosine kinase inhibitor (TKI), which is able to inhibit both tumor angiogenesis and proliferation by targeting vascular endothelial growth factor receptor (VEGFR) 1/2/3, stem cell-factor receptor, platelet-derived growth factor receptors (PDGFR)-α, and fibroblast growth factor receptor (FGFR) 1/2/3 (20, 21). Anlotinib has now been approved for the treatment of lung cancer, soft tissue sarcoma, and other solid tumors (21, 22). It was independently developed by Chia Tai Tianqing Pharmaceutical Group, and has been approved by the China National Medical Products Administration for patients in China since May 2018. In several clinical trials, anlotinib therapy combined with conventional chemotherapy exhibited more prominent therapeutic effects for patients with advanced DSNs than conventional treatment alone (23–25). However, systematic review of clinical trials assessing the therapeutic efficacy of anlotinib in combination with chemotherapy in advanced DSNs patients remains scarce.

In this study, we conducted a systematic review and meta-analysis to investigate the efficacy and safety of combined use of anlotinib with conventional chemotherapy in patients with advanced DSNs to provide a scientific reference for the design of future clinical trials.

2 Methods

This meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (26). No further ethical approval is required since the program does not require the recruitment of patients and the collection of personal information.

2.1 Search strategy

Related Literatures were searched across nine electronic databases, including Cochrane Library, Web of Science, Embase, Medline, PubMed, Chinese Scientific Journal Database (VIP), Wanfang database, Chinese Biological Medicine Database (CBM) and China National Knowledge Infrastructure (CNKI). Publications in English and Chinese dated from the inception of the database to October 2023 were shortlisted using the following search terms: "anlotinib" combined with "gastric cancer" or "colorectal cancer" or "gastrointestinal cancer" or "liver cancer" or "esophageal cancer" or "pancreatic cancer" or "digestive system neoplasms" without restriction on the language.

2.2 Eligibility criteria

2.2.1 Inclusion criteria

Inclusion criteria for this review were (1): Randomized controlled trials (RCTs) concerning DSNs patients were included; (2) Patients are diagnosed as DSNs by pathology. The nationality, race, gender, and age of the patients included in the study are not limited; (3) Articles involving more than 40 DSNs patients; (4) Literatures comparing the clinical outcomes of regular treatments plus anlotinib targeted therapy (experimental group) with regular treatments alone (control group); (5) Overall response rate (ORR), disease control rate (DCR) and treatment–related adverse effects must be included in each study.

2.2.1 Exclusion criteria

Exclusion criteria were: (1) Studies not focus on anlotinib were excluded; (2) Inappropriate criteria in experimental or control

group were excluded; (3) Articles without sufficient available data were excluded; (4) Non-RCTs, literature reviews, meta-analysis, meeting abstracts, case reports, repeated studies and experimental model researches were excluded.

2.3 Quality assessment

To ensure the quality of the meta-analysis, the quality of the included RCTs was evaluated according to the Cochrane Handbook tool (27).

2.4 Types of outcome measures

The primary outcomes in present analysis included short-term and long-term clinical efficacy, and adverse effects (AEs) according to the World Health Organization criteria and Response Evaluation Criteria in Solid Tumors 1.1 (RECIST Criteria 1.1) (28). The primary outcomes were: (1) Short-term clinical efficacy: the short-term tumor response included overall response rate (ORR, the sum of complete response and partial response) and disease control rate (DCR, the sum of complete response, partial response and stable disease); (2) Long-term clinical efficacy: 1-5 year overall survival (OS) defined as the time from the date of randomization to death from any cause; (3) Treatment–related adverse effects; (4) Quality of life (QOL): QOL was evaluated using Karnofsky score.

2.5 Data extraction and management

The following data were extracted from eligible studies: (1) Study characteristics such as name of the first author, patient ages, year of publication, number of cases, and study parameter types; (2) Details of the interventions such as intervention technique as well as dosage, administration route, and duration of anlotinib treatment; (3) Outcomes measures and other parameters that included the OS, ORR, DCR, Karnofsky performance score (KPS), and AEs. We attempted to contact the authors to request missing or incomplete data. If the relevant data could not be acquired, the studies were excluded from the analysis.

2.6 Statistical analysis

Stata 16.0 (Stata Corp., College Station, TX, USA) and Review Manager 5.3 (Nordic Cochran Centre, Copenhagen, Denmark) statistical software were used for statistical analyses. Dichotomous data were represented by the risk ratio (RR) with the respective 95% confidence interval (CI), whereas continuous variables were expressed as mean difference (MD) with 95% CI. $P<0.05$ indicates difference with statistical significance. Heterogeneity among studies was estimated using the Cochran's Q statistic and I^2 tests, and $I^2 >$ 50% or $P<0.1$ indicated a high statistical heterogeneity (29). A fixed-effects model was used to pool the estimates when heterogeneity was absent ($I^2 < 50\%$). Otherwise, a random effects model was selected.

Any publication bias was investigated using funnel plots and the Begg and Egger tests for parameters that were reported in more than 10 studies (30–32). A trim-and-fill method was used to coordinate the estimates from unpublished studies if publication bias existed, and the adjusted results were compared with the original pooled RR (33). Subgroup analysis was conducted to investigate the influence of cancer types, and therapeutic regimens.

3 Results

3.1 Search results

A total of 1,017 articles were identified with initial retrieve. 843 papers were excluded due to duplication. After title and abstract review, 109 articles were further excluded because they were not clinical trials (n=35) or were unrelated studies (n=43) or were literature review and meta-analysis (n=14) or were meeting abstract and case report (n=17), leaving 65 studies as potentially relevant. After detailed assessment of full texts, articles were not RCTs (n=15), studies with a sample size of less than 30 (n=6); publications with inappropriate criteria of experimental or control group (n=17), and trials with insufficient data (n=7) were excluded. Finally, 20 trials (23, 25, 34–51) involving 1,613 DSNs patients were included in this analysis (Figure 1).

3.2 Patient characteristics

In total, 934 DSNs patients were treated by regular treatments in combination with anlotinib targeted therapy, while 679 patients were treated by regular treatments alone. Detailed information of the involved studies and DSNs patients is shown in Tables 1, 2. All included trials except one (49) clearly introduce the dosage and duration of anlotinib treatment.

3.3 Quality assessment

The assessment of bias risk is shown in Figure 2. Among the studies involved in the present analysis, nineteen were determined to have a low risk of bias and the remaining one did not offer a clear description of the randomization process. The selection and attrition risks of involved trials were low. None of the trials included in the present analysis provided a clear description of the performance and detection risks. Among the trials, one were considered to present unclear risk, owing to selective reporting, whereas four studies were considered as high risk, on account of the lack of data pertaining to the primary outcome measures.

3.4 OS assessments

Eight clinical trials (23, 25, 35, 37, 40, 41, 48, 49) involving 1,004 cases compared the OS between the two groups (Figure 3). The

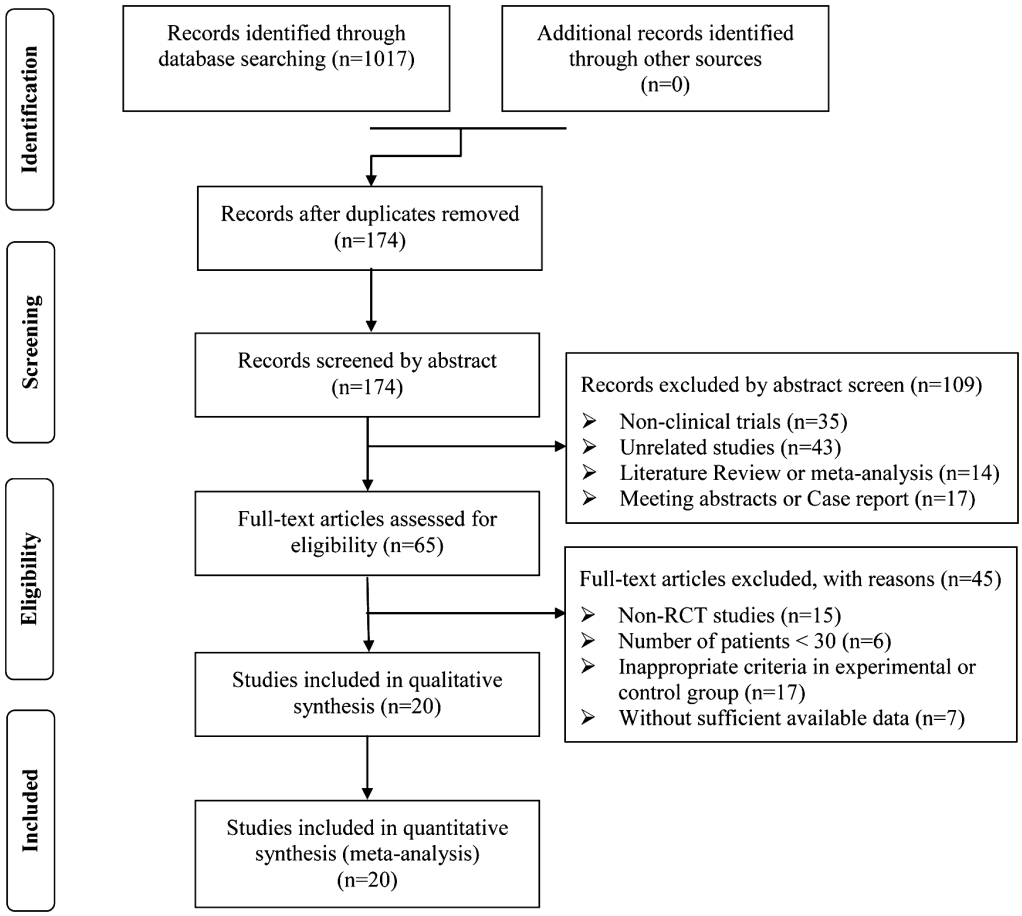

FIGURE 1
Flow diagram of the selection process.

analysis result of OS was shown in Figure 3A. Compared with regular treatments, the combination of regular treatments and anlotinib can increase 6-, 12-, 18-, 24-, and 36-months OS, but only 6-months OS reaches a significant level (6-months OS: RR=1.13, 95% CI=1.01-1.26, *P*=0.04; 12-months OS: RR=1.28, 95% CI=0.97-1.67, *P*=0.08; 18-months OS: RR=0.99, 95% CI=0.46-2.16, P=0.99; 24-months OS: RR=0.96, 95% CI=0.48-1.93, *P*=0.92; 36-months OS: RR=1.79, 95% CI=0.91-3.54, *P*=0.09). 12-, 18-, and 24-months OS (12-months OS: *P*=0.005, I^2 = 68%; 18-months OS: *P*= 0.05, I^2 = 61%; 24-months OS: *P*=0.04, I^2 = 61%) displayed statistical heterogeneity, as per the heterogeneity test. Hence, a random-effects model was used in the meta-analysis. Otherwise, the fixed-effect model was used in case of 6- and 36-months OS.

3.5 ORR and DCR assessments

Eighteen clinical trials (23, 34–43, 45–51) involving 1,420 cases compared the ORR and DCR between the two groups (Figures 4, 5). Our pooled results showed that patients underwent combined therapy had significantly improved ORR and DCR (ORR: RR=1.76, 95% CI=1.53-2.02, *P*<0.00001; DCR: RR=1.51, 95% CI =1.25-1.84, *P*<0.0001) compared with regular treatments alone. DCR (*P*= 0.30, I^2 = 13%) displayed slightly significant heterogeneity, as per the heterogeneity test. Hence, a fixed-effect model was used in the meta-analysis. Otherwise, the random-effects model was used in case of DCR.

3.6 KPS score

Five trials (36, 39, 46, 50, 51) involving 264 DSNs patients evaluated the QOL according to the KPS Score. As shown in Figure 6, the KPS score of DSNs patients in the combined group were higher than that of the control group, but the difference was not statistically significant (MD = 8.86, 95% CI = -2.32-20.05, *P*=0.12). *P*<0.00001 and I^2 = 98% indicated that there was significant heterogeneity among the studies; thus a random effect model was employed.

3.7 Adverse events assessment

Seventeen trials (23, 25, 34–44, 47, 48, 50, 51) involving 1,486 DSNs patients evaluated the safety of anlotinib mediated therapy.

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TABLE 1 Clinical information from the eligible trials in the meta-analysis.

Included studies	Tumor type	Tumor stage	Patients Con/Exp	Age (year)		Parameter types
				Control group	Experimental group	
Chi Y 2021 (23)	CC	TNM (IV)	137/282	55.2±10.8 (mean)	56.2 ±10.5 (mean)	OS, ORR, DCR, AE
Dai KJ 2022 (34)	CC	NK	44/44	51.31 ± 8.64 (mean)	50.79 ± 9.19 (mean)	ORR, DCR, AE
Huang J 2020	EC	TNM (IV)	55/109	45–76	43–74	OS, AE
Lan L 2020 (35)	GC	TNM (II-IV)	20/60	43-72	41-73	OS, ORR, DCR, AE
Liu L 2023 (36)	HC	BCLC (B/C)	40/40	54.12±8.95 (mean)	55.28±8.42 (mean)	ORR, DCR, KPS, AE
Liu YJ 2021 (37)	EC	TNM (II/III)	25/23	48.58 ± 2.35	48.26 ± 2.62	OS, ORR, DCR, AE
Lu JY 2021 (38)	HC	Advanced Stage	30/30	64.37±3.19 (mean)	64.63±3.82 (mean)	ORR, DCR, AE
Pang H 2022 (39)	EC	Advanced Stage	28/29	NK	NK	ORR, DCR, KPS, AE
Pei SF 2023 (40)	EC	TNM (III/IV)	53/53	53.6±6.8 (mean)	54.1±7.2 (mean)	OS, ORR, DCR, AE
Wang C 2020 (41)	EC	TNM (IV)	30/30	≥60 25	≥60 22	ORR, DCR, AE
Wang ZY 2019 (42)	EC	TNM (II/III)	18/18	49.1 ±7.3	48.3±8.4	OS, ORR, DCR, AE
Xiong HP 2022 (43)	EC	Advanced Stage	20/21	57.69±6.52	58.10±6.78	ORR, DCR, AE
Xu YW 2021 (44)	EC	TNM (II/III)	34/34	52.36±5.74	52.69±5.58	AE
Xue WL 2019 (45)	GC	TNM (III/IV)	18/18	55.2±2.5	54.7±2.3	ORR, DCR
Xue WL 2020a (46)	EC	TNM (II-IV)	17/18	52.6±2.9	51.7±3.1	ORR, DCR, KPS
Xue WL 2020b (47)	CC	NK	17/17	51.3±3.2 (mean)	50.9±3.1 (mean)	ORR, DCR, AE
Yang WW 2022 (48)	CC	Advanced Stage	19/34	<65 (89.47)	<65 (88.24)	OS, ORR, DCR, AE
Zhang XW 2020 (49)	EC	Advanced Stage	28/28	59.36±7.10	59.64±7.01	OS, ORR, DCR
Zhao HJ 2021 (50)	EC	TNM (IV)	26/26	53.72±9.81	55.68±11.76	ORR, DCR, KPS, AE
Zhou CS 2022 (51)	GC	TNM (III/IV)	20/20	72.1±2.8	74.3±3.3	ORR, DCR, KPS, AE

Control group, Conventional treatment group; Experimental group, Anlotinib combined conventional treatment group.
CC, colorectal cancer; EC, esophageal cancer; HC, Hepatocellular cancer; GC, gastric cancer; TNM, tumor node metastasis classification; BCLC, Barcelona clinic liver cancer staging classification; NK, unknown; KPS, karnofsky performance score; OS, Overall survival; ORR, overall response rate; DCR, disease control rate; AE, adverse events.

As shown in Table 3, the patients who underwent combination therapy exhibited higher incidences of hypertension (RR=2.53, 95% CI=1.87 to 3.41, P<0.00001), proteinuria (RR=2.15, 95% CI=1.63 to 2.82, P<0.00001), fatigue (RR=1.69, 95% CI=1.40 to 2.04, P<0.00001), diarrhea (RR=2.68, 95% CI=1.90 to 3.77, P<0.00001), hypertriglyceridemia (RR=1.96, 95% CI=1.10 to 3.47, P=0.02), ALT increased (RR=1.93, 95% CI=1.23 to 3.03, P=0.004), AST increased (RR=1.74, 95% CI=1.17 to 2.57, P=0.006), anorexia (RR=2.23, 95% CI=1.62 to 3.08, P<0.00001), weight loss (RR=3.32, 95% CI=1.53 to 7.18, P=0.002), abdominal pain (RR=2.50, 95% CI=1.48 to 4.24, P=0.0006), hypothyroidism (RR=4.60, 95% CI=1.30 to 16.27, P=0.02), and prolonged QT interval (RR=1.67, 95% CI=1.03 to 2.71, P=0.04) compared to the patients who underwent conventional therapy. The analysis of gastrointestinal reaction (RR=1.18, 95% CI=0.97 to 1.42, P=0.09), leukopenia (RR=1.41, 95% CI=0.94 to 2.09, P=0.09), neutropenia (RR=1.39, 95% CI=0.52 to 3.71, P=0.52), hemoglobinopenia (RR=0.77, 95% CI=0.48 to 1.22, P=0.26), thrombocytopenia (RR=1.15, 95% CI=0.48 to 2.74, P=0.75), vomiting and Nausea (RR=1.18, 95% CI=0.81 to 1.72, P=0.39), hypercholesterolemia (RR=1.26, 95% CI=0.90 to 1.77, P=0.17), hand-foot syndrome (RR=2.98, 95% CI=0.60 to 14.78, P=0.18), oropharyngeal pain (RR=1.30, 95% CI=0.83 to 2.03, P=0.25), hepatic function damage (RR=1.21, 95% CI=0.86 to 1.69, P=0.28), myelosuppression (RR=1.39, 95% CI=0.83 to 2.35, P=0.21), and Rash (RR=1.97, 95% CI=0.70 to 5.58, P=0.20) did not reveal any significant difference between the two groups. The incidence of neutropenia (P=0.09, I^2 = 50%), hypertriglyceridemia (P=0.10, I^2 = 51%), hand-foot syndrome (P=0.09, I^2 = 54%), myelosuppression (P=0.03, I^2 = 63%), hypothyroidism (P=0.003, I^2 = 79%) and rash (P=0.05, I^2 = 58%) showed mid to high level heterogeneity, as per the heterogeneity test. Consequently, a random-effects model was used to pool the results in the present meta-analysis. Otherwise, the fixed-effect model was used.

3.8 Publication bias

Publication bias was assessed by Begg's and Egger's regression tests, and was detected in indicators such as ORR, DCR and partial side effect indicators (number of included studies > 7). A trim-and-fill analysis was performed, in order to determine whether the publication bias affected the pooled risk. The adjusted RR indicated

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TABLE 2 Information of anlotinib combined with conventional treatment.

Included studies	Therapeutic regimen		Enrollment Period
	Experimental group	Control group	
Chi Y 2021 (23)	Anlotinib (12mg per d, per os; d 1-14; 21 days per cycle [a, b, c])	Placebo	2014.12-2016.8
Dai KJ 2022 (34)	Anlotinib (12mg per d, per os; d 1-14; 21 days per cycle; 2 cycles)+capecitabine.	Capecitabine (1250 mg/m^2)	2018.9-2020.3
Huang J 2020	Anlotinib (12mg per d, per os; d 1-14; 21 days per cycle [a, d])	Placebo	2016.1-2018.5
Lan L 2020 (35)	Anlotinib (12mg per d, per os; d 1-14; 21 days per cycle [b])	Placebo	2015.2-2016.5
Liu L 2023 (36)	Anlotinib (12mg per d, per os; d 1-14; 21 days per cycle) + TACE.	TACE (Epirubicin, 10mg; Oxaliplatin, 50mg)	2020.1-2022.1
Liu YJ 2021 (37)	Anlotinib (12mg per d, per os; d 1-14; 21 days per cycle; 2 cycles) + Chemotherapy + Radiotherapy	Chemotherapy (Paclitaxel, 50 mg//m^2; Carboplatin, AUC = 2) + Radiotherapy (1.8-2.0 Gy/d 5 days per week; 54-60 Gy in total)	2018.9-2020.9
Lu JY 2021 (38)	Anlotinib (12mg per d, per os; d 1-14; 21 days per cycle [a])+TACE.	TACE	2014.1-2016.1
Pang H 2022 (39)	Anlotinib (8mg per d, per os; d 1-14; 21 days per cycle [a]) + Radiotherapy.	Radiotherapy (2.0 Gy/d 5 days per week; 60 Gy in total)	2019.7-2021.7
Pei SF 2023 (40)	Anlotinib (12 mg per d, per os; d 1-14; 21 days per cycle) + Chemotherapy + Radiotherapy	Chemotherapy (Cisplatin, 80mg/m^2; 5- Fluorouracil, 1000mg/m^2) + Radiotherapy (1.8-2.0 Gy/d 5 days per week; 54-60 Gy in total)	2017.1-2019.1
Wang C 2020 (41)	Anlotinib (12 mg per d, per os; d 1-14; 21 days per cycle; 2 cycles [a])+S-1	S-1, 80 mg /m^2 ·d	2018.6-2019. 9
Wang ZY 2019 (42)	Anlotinib (12 mg per d, per os; d 1-14; 21 days per cycle) + Radiotherapy.	Radiotherapy (1.8-2.0 Gy/d 5 days per week; 63.4-68 Gy in total)	2017.6-2017.9
Xiong HP 2022 (43)	Anlotinib (12 mg per d, per os; d 1-14; 21 days per cycle [a]) + Camrelizumab	Camrelizumab, 200 mg, once every 3 weeks.	2019.2-2021. 9
Xu YW 2021 (44)	Anlotinib (12 mg per d, per os; d 1-14; 21 days per cycle) + Chemotherapy + Radiotherapy	Chemotherapy (Cisplatin 75mg/m^2, Fluorouracil 750-1000mg/m^2) + Radiotherapy (1.8-2.0 Gy/d 5 days per week; 54-60 Gy in total)	2018.1-2019.4
Xue WL 2019 (45)	Anlotinib (12 mg per d, per os; d 1-14; 21 days per cycle; 3 cycles) + Chemotherapy	Chemotherapy (Fluorouracil, 500 mg/m^2)	2018.1-2019.5
Xue WL 2020a (46)	Anlotinib (12 mg per d, per os; d 1-14; 21 days per cycle; 2 cycles) + Chemotherapy+ Radiotherapy	Chemotherapy (Capecitabine,1000 mg/m^2 ·d) + Radiotherapy (2.0 Gy/d 5 days per week; 64 Gy in total)	2018.1-2019. 1
Xue WL 2020b (47)	Anlotinib (12 mg per d, per os; d 1-14; 21 days per cycle; 2 cycles) + Chemotherapy	Chemotherapy (Capecitabine,2500 mg/m^2 ·d)	2018.1-2019.1
Yang WW 2022 (48)	Anlotinib (12mg per d, per os; d 1-14; 21 days per cycle [a, b, c]) + Chemotherapy	Chemotherapy (Fluorouracil, Oxaliplatin/Irinotecan) + Placebo	2014.9-2016.8
Zhang XW 2020 (49)	Anlotinib (ND) + Radiotherapy	Radiotherapy (2.0 Gy/d 5 days per week; 55-65 Gy in total)	2017.1-2018.1
Zhao HJ 2021 (50)	Anlotinib (12 mg per d, per os; d 1-14; 21 days per cycle; 2 cycles) + Chemotherapy	Chemotherapy (Irinotecan 125mg /m^2)	2018.10-2018.10
Zhou CS 2022 (51)	Anlotinib (12 mg per d, per os; d 1-14; 21 days per cycle; 3 cycles) + S-1	S-1 (40-60 mg/time, 2 times/day)	2018.10-2020.10

Control group, Conventional treatment group; Experimental group, Anlotinib combined conventional treatment group; a: The treatment continued until PD or intolerable toxicity; b: If the patient could not tolerate 12mg/day, then the dose could be reduced to 10 mg/day or 8 mg/day; c: If the dose of 8 mg/day was not tolerated, then treatment was terminated in accordance with the RECIST; d: Treatment interruptions and dose modifications due to treatment-related toxicities were allowed.
TACE, Transcatheter hepatic arterial chemoembolization; PD, Progressive disease; NK, unknown; S-1, Gimeracil and Oteracil Porassium Capsules.

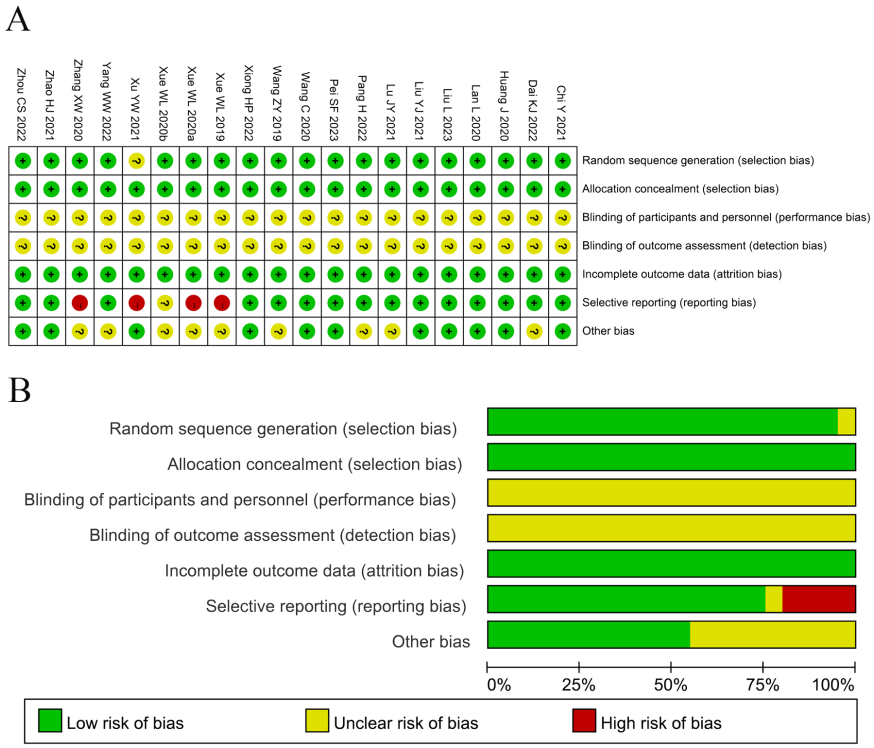

FIGURE 2
(A) Risk of bias summary: Review of the authors' judgments about each risk of bias item for the included studies. **(B)** Risk of bias graph: Review of the authors' judgments about each risk of bias item presented as percentages across all included studies. Each color represents a different level of bias: red for high-risk, green for low-risk, and yellow for unclear risk of bias.

same trend with the result of the primary analysis, reflecting the reliability of our primary conclusions (Table 4).

3.9 Sensitivity analysis

Sensitivity analysis was performed to explore an individual study's influence on the pooled results by deleting one single study each time from pooled analysis. As shown in Figure 7, the results revealed that none of the individual studies significantly affected the primary outcome measures, which implied statistically robust results.

We also conducted subgroup analysis to explore the source of heterogeneity in ORR and DCR with respect to cancer types, and therapeutic regimens. As shown in Table 5, our analysis indicates that the selection of tumor types and the formulation of treatment plans may have a certain impact on the efficacy of anlotinib targeted therapy.

4 Discussion

With the studying development of tumor molecular biology and epigenetic in recent years, increasing numbers of first-line treatment agents, including gefitinib, erlotinib and anlotinib, been suggested for improving therapeutic effects for patients with malignancies (52–54). VEGF is a key mediator of tumor angiogenesis, in which it is up-regulated by oncogene expression, a variety of growth factors and also hypoxia (55). It is essential for endothelial cell functions associated with angiogenesis and plays an important role in angiogenesis, tumor progression and vascular permeability (56, 57). VEGF and their receptors are regarded as the most well-known regulators of neovascularization. VEGF binding to VEGFR provides cell proliferation and vascular tissue formation by the subsequent tyrosine kinase pathway (58). VEGF/VEGFR-related signal pathways leads to endothelial cell differentiation, migration, proliferation, and survival involved in angiogenesis (59). The VEGF/VEGFR system is of great importance in regulating and controlling tumor angiogenesis, and anti-VEGF/VEGFR therapy for cancer are now widely used in the clinical field (60). Researchers have confirmed that the expressions of VEGF and VEGFR signaling pathway exhibited significant correlations with poor prognosis for cancer patients (61–63). Therefore, VEGF/VEGFR axis displays an attractive and potential target for anti-angiogenesis and anti-cancer drug design.

Drugs known as vascular endothelial growth factor receptor tyrosine kinase inhibitor (VEGFR-TKI) can inhibit VEGFR, which have recently been approved and used in treating various cancers, such as renal cell carcinoma (RCC) and liver cancer (64, 65). VEGFR-TKI inhibit angiogenesis induced by tumor cells, leading to the inhibition of cell proliferation and shrinkage of tumors. Thus, VEGFR-TKI are an important option for the treatment of cancer. The VEGFR family includes VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), VEGFR-3 (Flt-4), and VEGFR co-receptors neuropilin 1 and

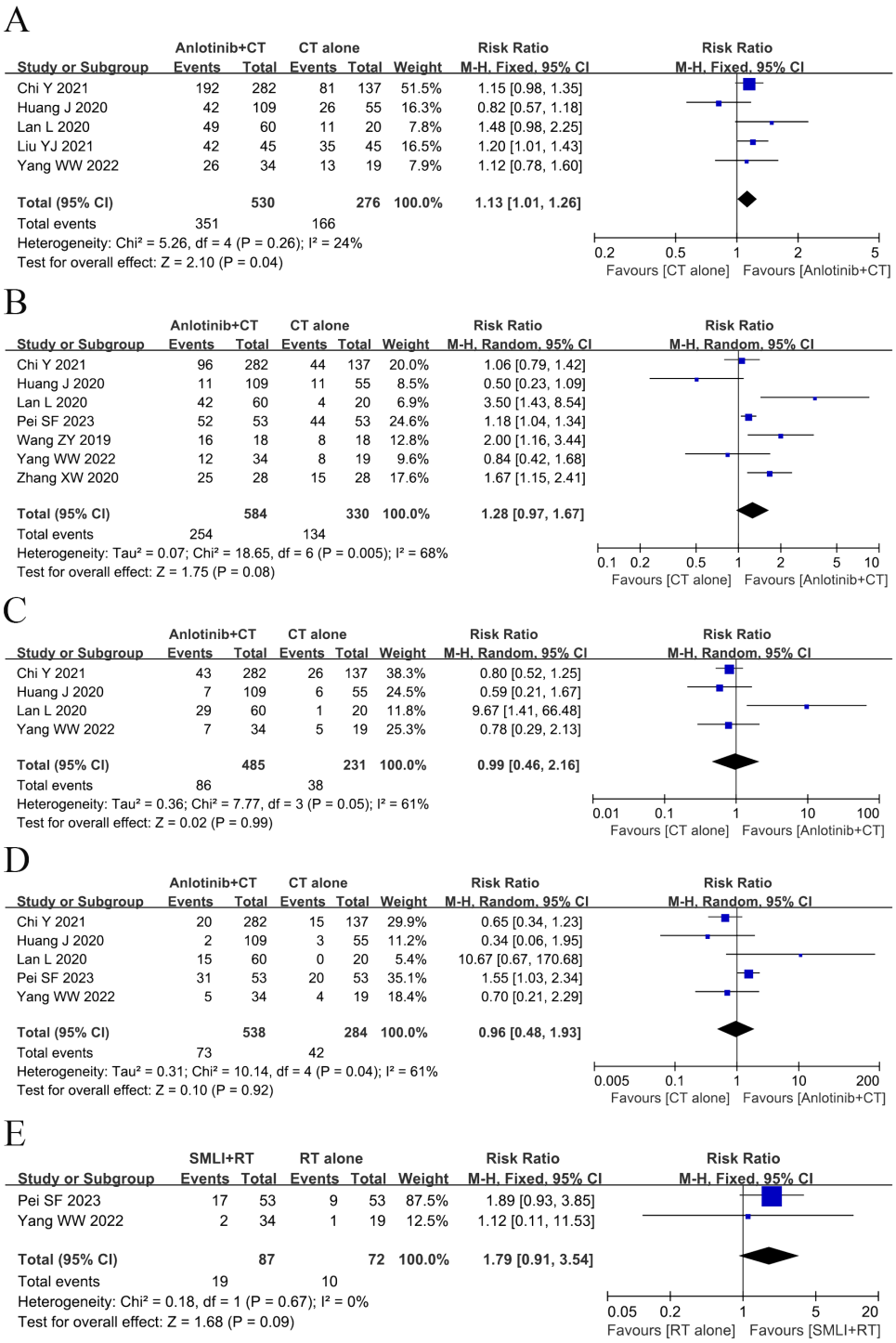

FIGURE 3
Forest plot of the comparison of the overall survival (OS) between the experimental and control group. **(A)** 6-months OS, **(B)** 12-months OS, **(C)** 18-months OS, **(D)** 24-months OS, and **(E)** 36-months OS. Control group, conventional treatment group; experimental group, anlotinib combined conventional treatment group; CI, confidence interval. The fixed-effects meta-analysis model (Mantel–Haenszel method) was used.

2 (66). Among these receptors, VEGFR-2, as an important tyrosine transmembrane protein, is aberrantly expressed in many malignant tumors, and it play an important role in the occurrence, development, and growth of tumors and drug resistance (67). Anlotinib is a novel inhibitor of VEGFR-2 tyrosine kinase with inhibitory effects on angiogenesis and tumor growth, which targets the intracellular ATP binding site of the receptor (68). Several studies have demonstrated that anlotinib has shown good efficacy and tolerability in patients with advanced DSNs (69, 70). Although a number of statistical analyses of clinical trials have been published,

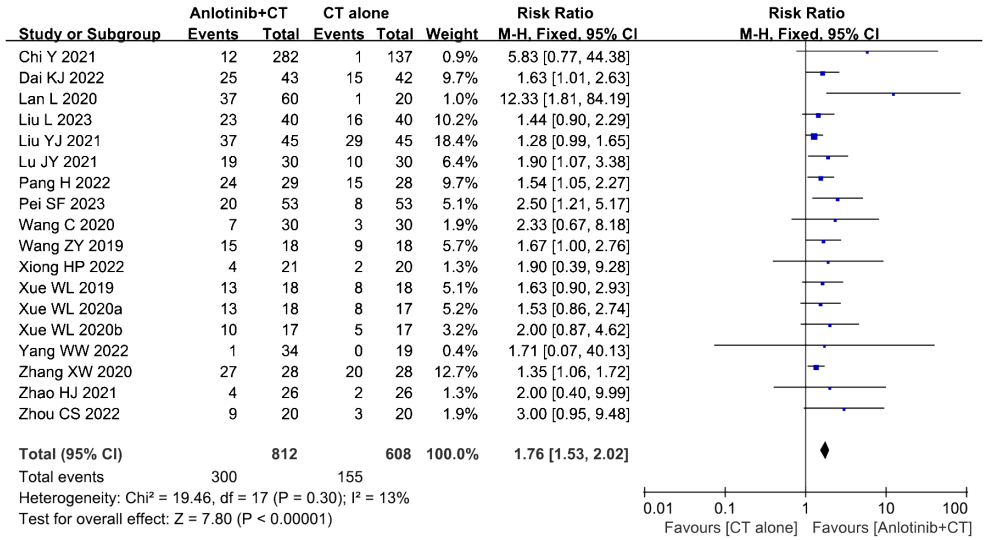

FIGURE 4
Forest plot of the comparison of the overall response rate (ORR) between the experimental and control group. Control group, conventional treatment group; experimental group, anlotinib combined conventional treatment group; CI, confidence interval. The fixed-effects meta-analysis model (Mantel–Haenszel method) was used.

the therapeutic and toxic effects have not been systematically demonstrated and evaluated due to the impact of sample size and variability among these clinical trials. Additionally, a variety of different protocols and equation models in these clinical trials may have led to different therapeutic effects. In the present study, an extensive and analytical online search was performed followed by rigorous contrasting and combining analyses to provide a systematical and comprehensive conclusion.

In this study, the efficacy and safety of anlotinib as maintenance therapy for advanced DSNs patients was analyzed and reported from 20 randomized controlled trials. Our meta-analysis revealed that the combined treatment of anlotinib with conventional chemotherapy is associated with a more favorable efficacy compared with conventional treatment alone. The patients who were treated with combined treatment exhibited markedly increased 6-months OS, ORR and DCR (P<0.05). In this analysis, the QOL of patients was also evaluated, and it was found that although the use of anlotinib can improve aspects of quality of life in patients to some extent, but this improvement did not reach a significant difference. These results indicated that the exact efficacy

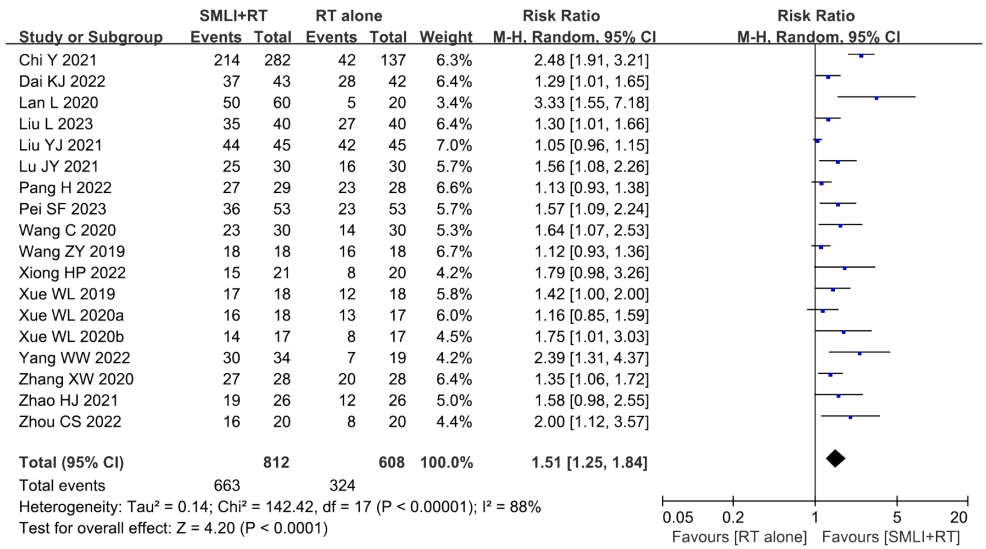

FIGURE 5
Forest plot of the comparison of the disease control rate (DCR) between the experimental and control group. Control group, conventional treatment group; experimental group, anlotinib combined conventional treatment group; CI, confidence interval. The fixed-effects meta-analysis model (Mantel–Haenszel method) was used.

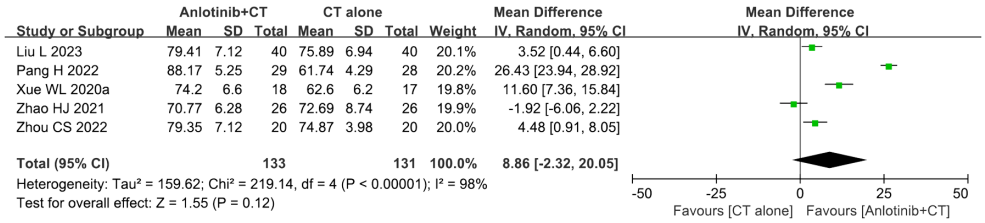

FIGURE 6
Forest plot of the comparison of the Karnofsky performance score (KPS) between the experimental and control groups. Control group, conventional treatment group; experimental group, anlotinib combined conventional treatment group; CI, confidence interval. The fixed-effects meta-analysis model (Mantel–Haenszel method) was used.

TABLE 3 Comparison of adverse events between the experimental and control group.

Adverse events	Experimental group	Control group	Analysis method	Heterogeneity		Risk Ratio (RR)	95% CI	*P*-value
	No. patients (n)	No. patients (n)		I^2 (%)	*P*-value			
Hypertension	413	302	Fixed	45	0.06	2.53	1.87-3.41	<0.00001
Proteinuria	635	419	Fixed	0	0.52	2.15	1.63-2.82	<0.00001
Gastrointestinal reaction	236	233	Fixed	3	0.40	1.18	0.97-1.42	0.09
Leukopenia	266	197	Fixed	35	0.17	1.41	0.94-2.09	0.09
Neutropenia	246	177	Random	50	0.09	1.39	0.52-3.71	0.52
Hemoglobinopenia	385	240	Fixed	0	0.70	0.77	0.48-1.22	0.26
Thrombocytopenia	94	79	Fixed	0	0.98	1.15	0.48-2.74	0.75
Fatigue	545	329	Fixed	0	0.85	1.69	1.40-2.04	<0.00001
Diarrhea	578	324	Fixed	6	0.38	2.68	1.90-3.77	<0.00001
Vomiting and Nausea	562	308	Fixed	0	0.86	1.18	0.81-1.72	0.39
Hypertriglyceridemia	451	237	Random	51	0.10	1.96	1.10-3.47	0.02
Hypercholesterolemia	451	237	Fixed	0	0.69	1.26	0.90-1.77	0.17
ALT increased	425	211	Fixed	0	0.49	1.93	1.23-3.03	0.004
AST increased	425	211	Fixed	13	0.32	1.74	1.17-2.57	0.006
Anorexia	465	251	Fixed	27	0.25	2.23	1.62-3.08	<0.00001
Weight loss	391	192	Fixed	0	0.89	3.32	1.53-7.18	0.002
Hand-foot syndrome	129	128	Random	54	0.09	2.98	0.60-14.78	0.18
Oropharyngeal pain	136	135	Fixed	0	0.55	1.30	0.83-2.03	0.25
Abdominal pain	425	211	Fixed	7	0.34	2.50	1.48-4.24	0.0006
Hepatic function damage	212	171	Fixed	0	0.48	1.21	0.86-1.69	0.28
Myelosuppression	172	171	Random	63	0.03	1.39	0.83-2.35	0.21
Hypothyroidism	451	237	Random	79	0.003	4.60	1.30-16.27	0.02
Rash	521	306	Random	58	0.05	1.97	0.70-5.58	0.20
Prolonged QT interval	425	211	Fixed	0	0.90	1.67	1.03-2.71	0.04

Control group, Conventional treatment group; Experimental group, Anlotinib combined conventional treatment group.

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TABLE 4 Summary of publication bias.

Publication Bias	ORR	DCR	12-OS	Adverse events				
				Hypertension	Diarrhea	Fatigue	Proteinuria	Vomiting and Nausea
Begg	0.012	< 0.001	1.000	0.592	0.266	0.072	0.602	0.764
Egger	< 0.001	< 0.001	0.698	0.298	0.289	0.359	0.458	0.721
Trim and fill analysis								
before	*P* < 0.001	< 0.001						
after	*P* < 0.001	< 0.001						

OS, Overall survival; ORR, overall response rate; DCR, disease control rate.

of anlotinib targeted therapy for DSNs patients still needs further research. Safety is the top priority for implementation of clinical treatment, and it is also the key factor for the development of anlotinib targeted therapy. Regarding adverse events and severe toxicities, our analytical results revealed that there were no significant differences in most of the adverse event indicators between the two groups. Consistent with previous reports (22, 25, 71–74), the most common AEs associated with anlotinib are hypertension, proteinuria, loss of appetite, fatigue, diarrhea, dyslipidemia, increased liver transaminase, and hypothyroidism. Most of the AEs are grade 1-2, and only a few patients with grade 3-4 adverse reactions need to reduce the dose of anlotinib, indicating that the side effects of anlotinib were tolerable. All included trials did not report treatment-related deaths. This may indicate that the

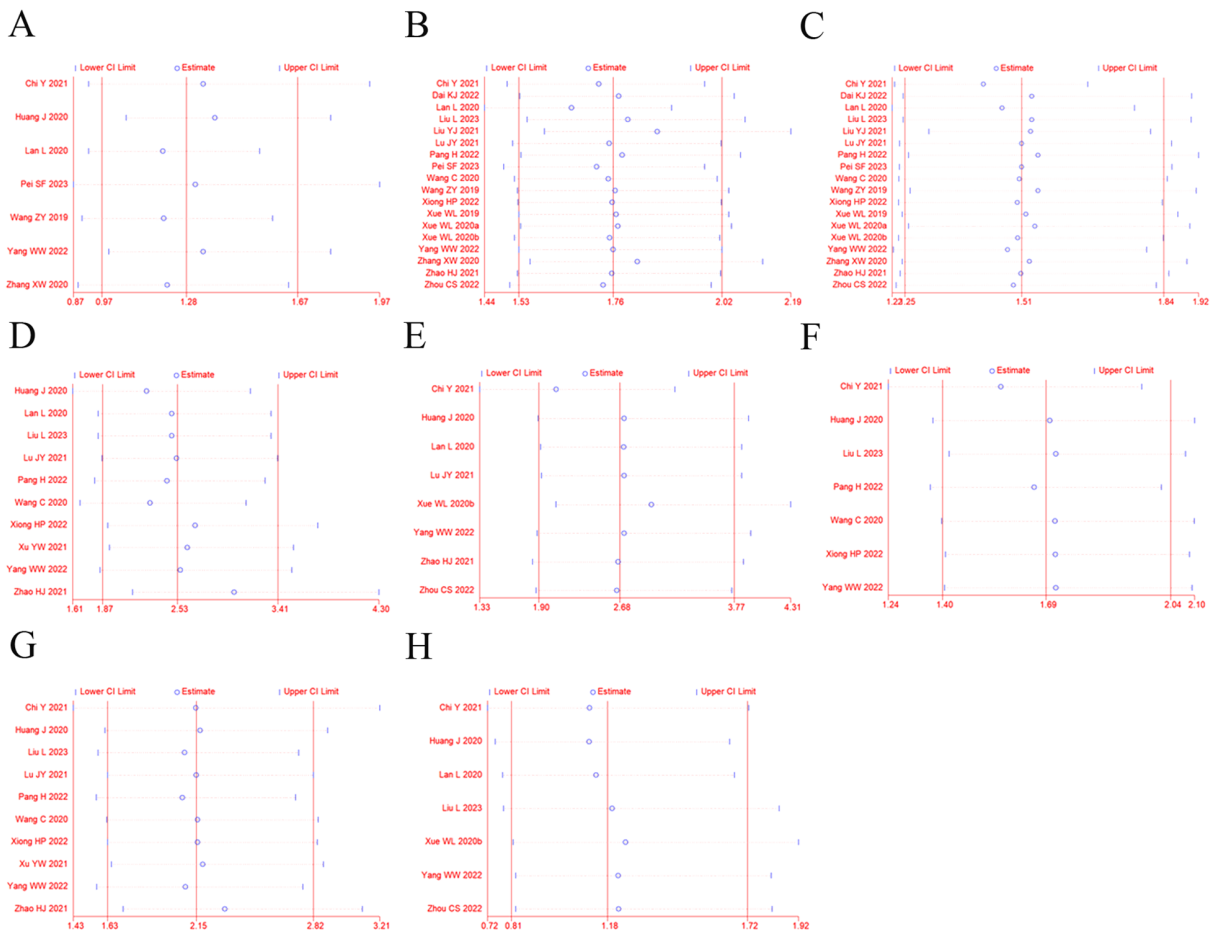

FIGURE 7
Sensitivity analysis for ORR **(A)**, DCR **(B)**, 12-OS **(C)**, hypertension **(D)**, diarrhea **(E)**, fatigue **(F)**, proteinuria **(G)**, and vomiting and nausea **(H)**.

TABLE 5 Subgroup analyses of ORR and DCR between the experimental and control group.

Parameter	Factors at study level	Exp group	Con group	Analysis method	Heterogeneity		Odds Ratio (OR)	95% CI	*P*-value
		No. patients (n)	No. patients (n)		I^2 (%)	*P*-value			
ORR	**Type of cancer**								
	Gastric cancer	98	58	Random ndam	72	0.03	3.15	0.91 to 10.86	0.07
	Colorectal cancer	376	215	Fixed	0	0.63	1.97	1.29 to 3.00	0.002
	Hepatocellular Carcinoma	70	70	Fixed	0	0.46	1.62	1.13 to 2.32	0.009
	Esophageal cancer	268	265	Fixed	0	0.66	1.55	1.32 to 1.83	<0.00001
	Therapeutic regimen								
	Anlotinib+Placebo	342	157	Fixed	0	0.59	9.26	2.29 to 37.35	0.002
	Anlotinib+Radiotherapy	75	74	Fixed	0	0.67	1.48	1.21 to 1.82	0.0001
	Anlotinib+Chemotherapy	258	242	Fixed	0	0.98	1.76	1.39 to 2.24	<0.00001
	Anlotinib +Chemoradiotherapy	116	115	Fixed	47	0.15	1.54	1.20 to 1.97	0.0006
DCR	**Type of cancer**								
	Gastric cancer	98	58	Random	69	0.04	1.98	1.12 to 3.50	0.02
	Colorectal cancer	376	215	Random	82	0.0008	1.88	1.22 to 2.91	0.004
	Hepatocellular Carcinoma	70	70	Fixed	0	0.40	1.40 40	1.13 to 1.72	0.002
	Esophageal cancer	268	265	Fixed	74	0.0001	1.28	1.08 to 1.51	0.004
	Therapeutic regimen								
	Anlotinib+Placebo	342	157	Fixed	0	0.47	2.58	2.01 to 3.30	<0.00001
	Anlotinib+Radiotherapy	75	74	Fixed	0	0.42	1.20	1.06 to 1.36	0.004
	Anlotinib+Chemotherapy	258	242	Fixed	0	0.50	1.54	1.36 to 1.75	<0.00001
	Anlotinib +Chemoradiotherapy	116	115	Random	85	0.002	1.22	0.84 to 1.76	0.29

Control group, Conventional treatment group; Experimental group, Anlotinib combined conventional treatment group.
ORR, overall response rate; DCR, disease control rate.

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AE associated with anlotinib is tolerable. To summarize, AEs related to the drug still need to be treated with caution, especially some of the intolerable grade 3 or above AEs. However, on the whole, AEs associated with anlotinib were controllable and the advantages of the use of anlotinib for advanced DSNs outweigh the disadvantages.

Some main factors, such as different treatment regimens and tumor types, may influence the therapeutic effects of anlotinib targeted therapy. The results in our subgroup analysis suggest that anlotinib has a weaker therapeutic effect on patients with advanced gastric cancer compared to other DSNs. However, currently published studies that have probed the influences of these factors on the curative effect of anlotinib targeted therapy are still insufficient. Thus, these issues should be further researched and explored. Furthermore, the determination of the optimal therapeutic strategy will be valuable for DSNs treatment.

There are some limitations in our analysis. First, the number of DSNs patients included in this study is not sufficiently large, and the follow-up time was short. Second, the different trials evaluated the therapeutic efficacy using different outcomes, so it was difficult to summarize the results on the same scale, which led to shrunken statistical sample sizes. Third, our data were partly extracted from published papers rather than original patient records, which mean that we were not able to avoid analytical bias based on the information presented in the articles. Due to the above limitations, future studies and generated data will be valuable to verify the safety and efficacy of anlotinib targeted therapy.

In summary, our study confirmed that the combined treatment of anlotinib with conventional chemotherapy may offer an effective treatment for advanced DSNs patients. Anlotinib targeted therapy markedly enhances the short-term treatment efficacy (ORR and DCR) of conventional treatment for advanced DSNs, but its long-term clinical efficacy remains to be studied further. Moreover, this combined treatment could lead to greater rates of adverse events, such as hypertension, proteinuria and fatigue. Therefore, the potential risks and benefits of treatment options should be considered before treatment.

Data availability statement

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

Author contributions

CZ: Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing. WW: Investigation, Resources, Writing – original draft, Writing – review & editing. YM: Data curation, Project administration, Writing – original draft. MM: Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing.

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

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Efficacy and safety of chemoradiotherapy plus immune checkpoint inhibitors for the treatment of locally advanced cervical cancer: a systematic review and meta-analysis

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Background: Radiotherapy plus concurrent chemotherapy is a standard method for treating locally advanced cervical cancer (LACC). Immune checkpoint inhibitors (ICIs) are widely applied in the treatment of recurrent cervical cancer, metastatic cervical cancer or LACC. The efficacy and safety of radiotherapy plus immunotherapy for LACC require further investigation. The objective of this review and meta-analysis was to analyze the efficacy and safety of concurrent chemoradiotherapy (CCRT) combined with ICIs for treating LACC on the basis of the results of randomized controlled trials (RCTs).

Methods: We comprehensively searched electronic databases to identify RCTs that focused on CCRT plus ICIs for LACC treatment. The outcomes included the objective response rate (ORR) and progression-free survival (PFS), overall survival (OS) and adverse events (AEs). A standard method for systematic review and meta-analysis was used. Review Manager 5.4 was used for data combination and analyses.

Results: Three RCTs involving 1882 participants with LACC were identified and included in the systematic review and meta-analysis. CCRT plus ICIs improved the rates of PFS (hazard ratio [HR]: 0.76, 95% confidence interval [CI]: CI: 0.64, 0.91, P = 0.002) and OS (HR: 0.7695% CI (95% CI 0.58–0.99, P = 0.04) in patients with LACC. Compared with the control group, the CCRT plus immunotherapy group had an increased ORR (OR: 1.37, 95% CI: 1.02,1.85, P=0.04). The two methods had similar rates (HR=1.99, 95% CI: 0.99, 1.43; P=0.07) of treatment-

related grade 3 or higher AEs. The CCRT plus immunotherapy group had a higher rate than did the control group (HR: 2.68, 95% CI: 1.38, 5.21; *P*=0.004) in terms of any grade immunotherapy-related AEs.

Conclusions: CCRT plus ICIs is efficacious and safe for the management of LACC. The addition of ICIs to CCRT improved the rates of PFS and OS in patients with LACC. The adverse effects of immunotherapy-related AEs should be strictly examined and managed in a timely manner.

KEYWORDS

chemotherapy, randomized controlled trials, cervical cancer, radiotherapy, immune checkpoint inhibitors

Introduction

Cervical cancer is the fourth most common malignant tumor in the world and poses a serious threat to human health. Cervical cancer is the fourth leading cause of cancer death among women. According to statistics, there are approximately 600000 new cases of cervical cancer worldwide each year, with 90% of cases occurring in low- and middle-income countries (1–3). Early cervical cancer can be cured through surgery, but approximately half of patients are locally advanced at initial diagnosis (4–6). Concurrent chemoradiotherapy (CCRT) based on cisplatin combined with brachytherapy is the standard treatment for locally advanced cervical cancer (LACC). However, after completion of CCRT, the prognosis of these patients remains poor, with a 5-year OS rate of approximately 65–70% and nearly 40% of patients experience recurrence or metastasis (7–9). Reducing distant metastasis and improving the long-term survival rate of patients with LACC remain urgent clinical issues that need to be addressed. Immune checkpoint inhibitors (ICIs) (such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death-1 (PD-1), and PD-L1 inhibitors) have emerged as important strategies for various cancers (10, 11). Mounting evidence indicates that immunotherapy has good effectiveness and safety in treating malignant tumors such as melanoma (12), lung cancer (13), and liver cancer (14). In recurrent, metastatic cervical cancer (R/M CC), the Keynote-826 trial demonstrated that immunotherapy is safe and effective in the treatment of R/M CC, improving OS and PFS (15, 16). Some studies have applied ICIs in LACC treatment and confirmed that immunotherapy plays a certain antitumor role, with compelling results (17–19).

However, there is still a lack of sufficient clinical evidence on the efficacy and safety of CCRT combined with ICIs in LACC patients. In this systematic study and meta-analysis, we systematically elucidated the efficacy of CCRT combined with immunotherapy in LACC patients on the basis of published randomized controlled trials (RCTs).

Objectives and research question

Therefore, this review aimed to summarize the clinical trials that have focused on CCRT combined with ICIs for the management of LACC.

Methods and materials

Study registration

This meta-analysis protocol was registered on PROSPERO (ID: 560803). This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

Search strategy

We conducted a systematic search of the Web of Science, PubMed, EMBASE, ClinicalTrial, ScienceDirect and Cochrane Library databases. The search terms included cervical cancer, immunotherapy, checkpoint inhibitor, radiotherapy and chemotherapy. The latest search was conducted on 22 June 2024. First, a repeated evaluation of the literature obtained from the search was conducted. After removing duplicates, a reviewer screened the titles of the studies to identify potentially suitable studies. Two reviewers subsequently independently screened the records on the basis of the abstracts/full texts. If there was any disagreement regarding the included literature, it was resolved through discussion.

Participants, interventions, and comparator

Patients aged >18 years who had an LACC diagnosis confirmed by pathology were included. Patients who experienced recurrence were excluded.

Intervention

Treatment group

Patients who received CCRT with concurrent immunotherapy.

Control group

Patients who received CCRT without concurrent immunotherapy.

Outcomes

Primary outcomes

Objective response rate (ORR).

Progression-free survival (PFS).

Overall survival (OS).

Secondary outcomes

Adverse events (AEs) included all-grade treatment-related AEs, treatment-related grade 3 or higher AEs, all-grade immunotherapy-related AEs (irAEs), grade 3 or higher irAEs and individual toxicity ≥grade 3.

Furthermore, the inclusion criteria were as follows: (1) studies including women diagnosed with cervical cancer by pathology; (2) studies including at least 20 patients; (3) studies published in English since 2015; (4) studies reporting safety or survival data; and (6) RCTs. The exclusion criteria were as follows: comments, editorials, guidelines, opinions, letters, and meeting summaries.

Quality assessment

The Cochrane tool was applied to assess the quality of the RCTs (20). The bias assessment included selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias assessments; these items were evaluated by two independent reviewers, and any disagreements were resolved by discussion among the review group.

Data extraction

All the data were extracted via standardized methods. The extracted information included the first author of the study, publication year, sample size, treatment method and medication, Eastern Cooperative Oncology Group (ECOG) performance status score, Federation International of Gynecology and Obstetrics (FIGO) stage, histology, nodal involvement, follow-up times and results of interest (ORR, PFS, OS, and AEs). The secondary outcomes of interest included locoregional progression events, distant progression events and toxicity. Data on the outcomes of interest were extracted by two independent reviewers. All reviewers resolved any disagreements through discussion.

Statistical analysis

Statistical analysis was conducted via RevMan 5.4 (Nordic Cochrane Centre). The risk ratio (HR) and its 95% confidence interval (CI) were used to describe survival outcomes. The odds ratio (OR) and its 95% CI were used to evaluate AEs and ORRs. I^2 was used to evaluate heterogeneity, and 25%, 50%, and 75% values were considered low, medium, and high, respectively (21). If I^2 was <25%, a fixed-effects model was used for data analysis; otherwise, a random-effects model was used. A P value<0.05 was considered statistically significant. Subgroup analysis and sensitivity analysis were subsequently conducted. Egger and Begg tests were used to evaluate publication bias (22).

Results

Study selection and characteristics

Overall, three RCTs, involving 1882 participants with LACC, were included in this review and meta-analysis (23–25). A total of 942 patients were included in the CCRT plus ICIs group, and 940 patients were included in the control group. The follow-up time ranged from 4.6 months to 18.5 months, and 1336 patients had an ECOG performance status score of 0. A total of 544 patients had an ECOG performance status score of 1, and two patients had an ECOG performance status score of 2. A total of 1569 patients had cervical squamous cell carcinoma, 768 patients had FIGO stage IB2-IIB disease, and 1480 patients had positive lymph nodes. The basic information of the included studies is shown in Table 1. The selection process is outlined in Figure 1, and the risk of bias evaluation is presented in Figure 2.

Objective response rate

Two RCTs (24, 25) described the ORR. As described by Lorusso et al. (24), the ORR was 79% and 76% in the intervention group and the control group, respectively. Monk et al. (25) indicated that the ORR was 83% and 81% in the CRT plus immunotherapy group and the control group, respectively. Pooled data from the two studies (24, 25) indicated that the CCRT with concurrent immunotherapy group had an increased ORR compared with that of the control group (OR: 1.37, 95% CI: 1.02, 1.85), and the *P* value was 0.04 (Figure 3A). A random-effects model was used for analysis because of high heterogeneity (Chi^2 = 1.74, I^2 = 43%, P=0.19).

Progression-free survival

Only two RCTs (24, 25) reported PFS. Lorusso et al. (24) indicated that the PFS rates were 22% and 29% in the intervention group and the control group, respectively, with an HR of 0·70 (95% CI: 0·55–0·89). Monk et al. (25) reported that the 12-month PFS rate was 76·0% in the intervention group and 73·3% in the control group, with an HR of 0·84 (95% CI 0·65–1·08). In summary, the results of two RCTs (24, 25) suggested that the CCRT with concurrent immunotherapy group had an improved PFS rate compared with that of the control group (HR: 0.76 (95% CI: 0.64, 0.91), *P* value=0.002), as shown in Figure 3B, and the analysis revealed no significant heterogeneity (Chi^2 = 1.15, I^2 = 13%, P=0.28).

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TABLE 1 the basic information of included randomized controlled trials(RCTs).

Study	Design	Treatment	Case	Median age	ECOG Score 0/1/2	FIGO stage I-II/ III-IV	Histology Non-squamous[#]/ squamous	Nodal status N0/N+	Followed-up time(m)
Lorusso et al.	Phase 3, double-blind	CCRT plus pembrolizumab	529	49 (40–57)	380/149/0	235/294	96/433	84/445	17.9
		CCRT	531	50 (41–59)	397/134/0	227/304	80/451	93/438	17.9
Duska et al.	phase2, open label	CCRT plus pembrolizumab	28	49 (28-74)	21/7/0	20/8	4/24	12/16	4.6
		CCRT following pembrolizumab	24	49 (28-74)	18/5/1	21/3	5/19	13/11	9.2
Monk et al.	phase3, double-blind	CCRT Plus Durvalumab	385	50 (41-57)	265/119/1	135/250	63/322	106/279	18.5
		CCRT	385	48 (40–57)	255/130/0	130/255	65/320	94/291	18.4

CCRT, concurrent chemoradiotherapy; ECOG, Eastern Cooperative Oncology Group; FIGO, Federation International of Gynecology and Obstetrics;
[#] Includes adenocarcinoma and adenosquamous carcinoma.

Overall survival

Two RCTs reported results on OS (24, 25). Lorusso et al. (24) indicated that a total of 44 (8%) patients in the intervention group and 59 (11%) patients in the control group experienced OS events, with an HR of 0·73 (95% CI: 0·49–1·07). Monk et al. (25) reported that the death rate was 15% in the intervention group and 19% in the control group, with an HR of 0·78 and a 95% CI of 0.55-1.10. The combined data (24, 25) indicated that the CCRT with concurrent immunotherapy group had a favorable OS rate compared with that of the control group, with an HR of 0.76 (95% CI 0.58-0.99) and a *P* value of 0.04. A fixed-effects model was used for analysis because there was no heterogeneity (Chi^2 = 0.05, I^2 = 0, P=0.82), as presented in Figure 3C.

Local progression events and distant progression events

Only Monk et al. described local progression events. There were 42 and 40 local progression events in the CCRT with concurrent immunotherapy group and the control group, respectively. The analysis revealed that the OR for local progression events was 1.06 (95% CI: 0.67, 1.67), and the P value was 0.82. Only Monk et al.

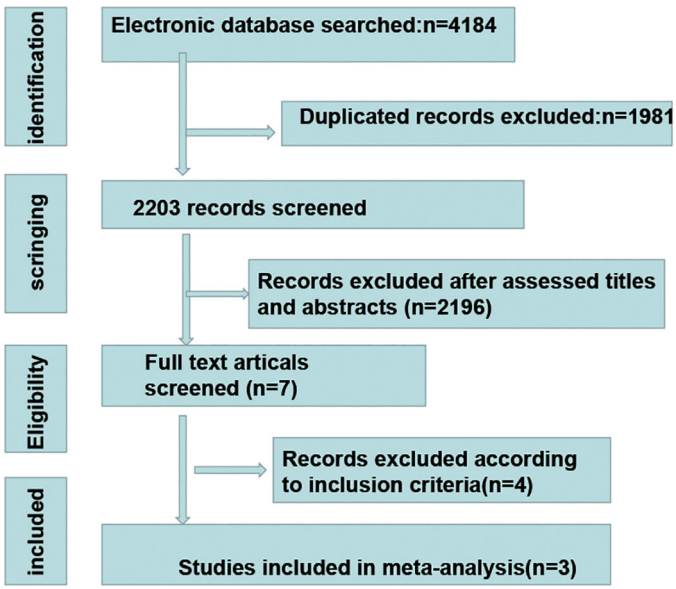

FIGURE 1
The process of study selection.

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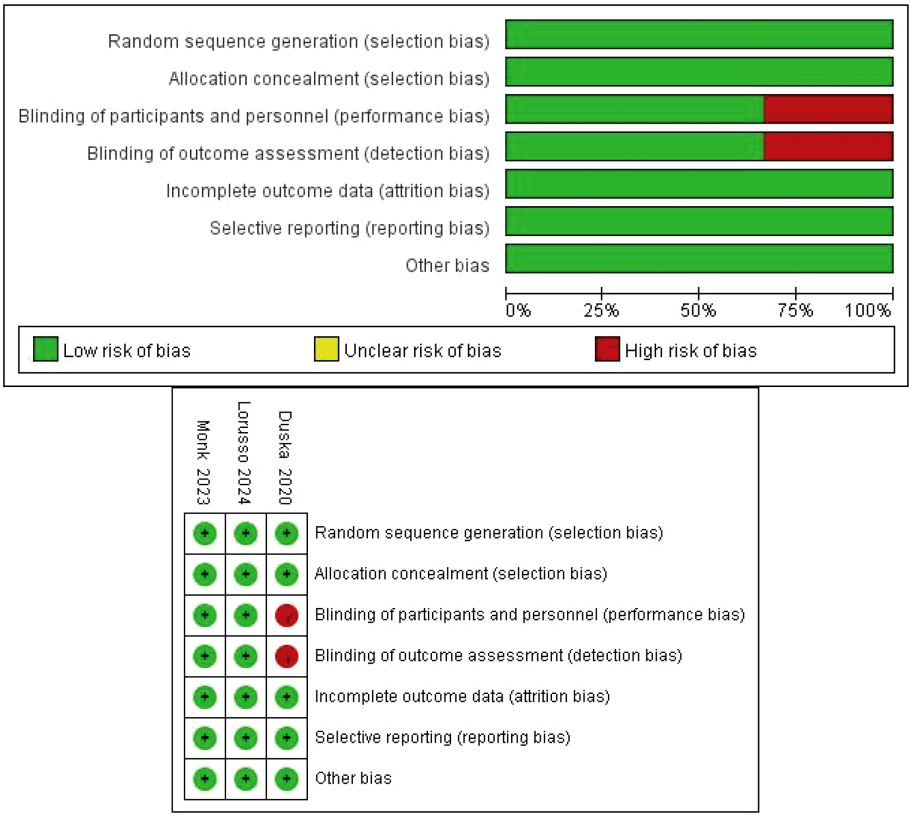

FIGURE 2
Risk of bias assessment.

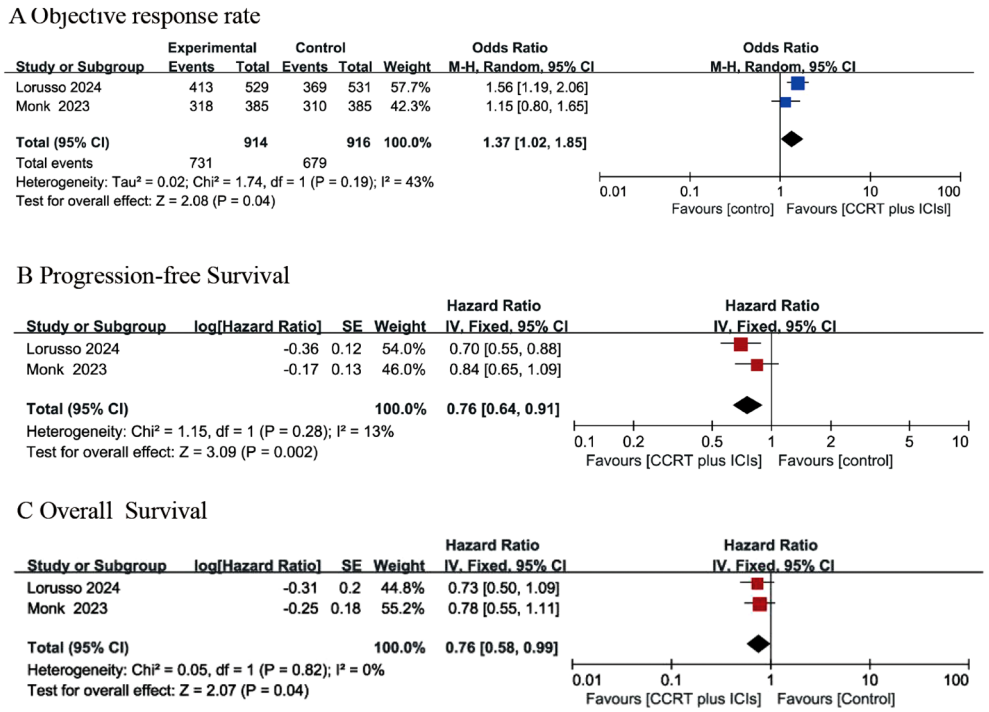

FIGURE 3
Forest plots for objective response rate **(A)**, progression-free survival **(B)** and overall survival **(C)** between concurrent chemoradiotherapy (CCRT) plus ICIs and control group.

provided data on distant progression events. There were 52 and 69 distant progression events in the experimental group and the control group, respectively. The pooled data indicated that the OR for distant progression events was 0.71 (95% CI: 0.48, 1.05), and the P value was 0.09.

Adverse events

Three RCTs (23–25) described any grade of treatment-related AE. The data revealed that the two strategies had comparable grades of treatment-related AE (HR: 1.22, 95% CI: 0.55, 2.67; *P*=0.62) (Supplementary Figure S1). For treatment-related Grade 3 or higher AEs, the pooled data indicated that the two methods had similar rates (HR=1.99, 95% CI: 0.99, 1.43; *P*=0.07), but CCRT plus ICIs tended to have a higher rate (Supplementary Figure S2). With respect to any-grade irAEs, the CCRT combined with immunotherapy group had a higher rate compared to that of control group (HR: 2.68, 95% CI: 1.38, 5.21, *P*=0.004), and a random-effects model was used for analysis (I^2 = 80, *P*=0.007) (Supplementary Figure S3). In terms of grade 3 or higher immunotherapy-related treatment AEs, Duska et al. (23) reported one case of grade 3 hyperthyroidism in the control group and no AEs in the CRT with concurrent immunotherapy group. As described by Lorusso et al. (24), the incidence of grade 3 or higher immunotherapy-related AEs was 4% and 1% in the intervention group and the control group, respectively.

Two studies provided details of toxicities (24, 25). With respect to grade ≥3 nausea, anemia, diarrhea, a decreased white blood cell count, a decreased neutrophil count, neutropenia, leukopenia, a decreased platelet count, hyperthyroidism and colitis, comparisons between the CCRT with concurrent immunotherapy group and the control group are provided in Table 2. The two groups had similar rates of toxicity.

Sensitivity analysis and subgroup analysis

We conducted sensitivity and subgroup analyses on PFS on the basis of age (≥65 versus <65 years), type of radiotherapy plan design (intensity modulated radiation therapy (IMRT)/volumetric modulated arc therapy (VMAT) versus non-IMRT/VMAT), and FIGO stage (IB2-IIB versus III-IV). The results are provided in Table 3. In the subgroup of patients aged less than 65 years and radiotherapy plan design by the IMRT/VMAT, radiotherapy combined with immunotherapy improved PFS compared with that of the control group. In the subgroup of patients with other factors (such as an age >65 years, a non-IMRT/VMAT plan, and CCRT plus immunotherapy), the PFS rate was similar to that of the control group.

Publication bias

A funnel plot of treatment-related grade 3 or higher AEs was used to evaluate publication bias in the included studies, and all the results within the 95% CIs revealed no significant publication bias (Figure 4). Because only three RCTs were included in this meta-analysis, we did not apply Egger or Begg tests for precise testing of publication bias.

Discussion

Summary of the main findings

Compared with the control group, patients who received CCRT with concurrent immunotherapy had longer OS (0.7695% CI (95% CI 0.58–0.99, *P*=0.04) and PFS (HR: 0.76, 95% CI: 0.64, 0.91, *P* = 0.002). The CCRT with concurrent immunotherapy group had an

TABLE 2 Meta-analysis of Grade ≥3 toxicity between concurrent chemoradiotherapy plus immunotherapy and control group.

Items Grade ≥3 toxicity	No.Of Trials	Effect model	OR and Its 95% CI	Z value	P value	Heterogeneity		
						Chi^2	I^2 (%)	P
Nausea	2	Random-effect	1.16 (0.39,3.43)	0.26	0.79	1.43	30	0.23
Anaemia	2	Fixed-effect	1.28 (0.99,1.65)	1.89	0.06	0.19	0	0.66
Diarrhoea	2	Random-effect	2.46 (0.19,31.46)	0.69	0.49	3.26	69	0.07
Decreased white blood cell count	2	Fixed-effect	0.86 (0.67,1.10)	1.19	0.24	0.34	0	0.56
Decreased neutrophil count	2	Fixed-effect	0.92 (0.69,1.22)	0.56	0.57	0.53	0	0.47
Neutropenia	2	Random-effect	1.31 (0.85,2.03)	1.21	0.23	1.45	31	0.23
Leukopenia	2	Fixed-effect	1.13 (0.82,1.55)	0.74	0.46	0.43	0	0.51
Decreased platelet count	2	Random-effect	1.33 (0.55,3.23)	0.63	0.53	2.37	58	0.12
Hyperthyroidism	2	Fixed-effect	5.03 (0.59,43.10)	1.47	0.14	0.00	0	1.00
Colitis	2	Random-effect	2.10 (0.25,17.27)	0.69	0.49	1.90	47	0.17

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TABLE 3 Subgroup and sensitive analysis on progression-free survival.

Items	No. Of studies	Effects model	HR and Its 95% CI	Z value	P value	Heterogeneity		
						Chi^2	I^2 (%)	P
Age<65	2	Fixed-effect	0.77 (0.64,0.92)	2.83	0.005	0.58	0	0.45
age≥65	2	Fixed-effect	0.72 (0.41,1.25)	1.17	0.24	0.92	0	0.34
IMRT/VMAT	2	Random-effect	0.76 (0.59,0.97)	2.22	0.03	1.59	37	0.21
Non-IMRT/VMAT	2	Fixed-effect	0.83 (0.53,1.31)	0.78	0.43	0.13	0	0.72
FIGO stage IB2-IIB	2	Fixed-effec	0.90 (0.67,1.19)	0.75	0.45	0.03	0	0.87
FIGO stage III-IV	2	Random-effect	0.78 (0.45,1.36)	0.87	0.38	9.80	90	0.002

FIGO, Federation International of Gynecology and Obstetrics; IMRT, Intensity Modulated Radiation Therapy; VMAT, Volumetric Modulated Arc Therapy.

increased ORR compared with that of the control group (OR: 1.37, 95% CI: 1.02, 1.85, *P*=0.04). The two groups had similar rates of treatment-related Grade 3 or higher AEs (HR=1.99, 95% CI: 0.99, 1.43; *P*=0.07). CCRT plus ICIs was associated with a higher rate of any-grade irAEs (HR: 2.68, 95% CI: 1.38, 5.21; *P*=0.004).

Radiation therapy is often used to treat patients with cervical cancer. CCRT is the standard treatment for locally advanced nonsurgical cervical cancer (26). ICIs (CTLA-4, PD-1, or PD-L1) (27) are widely used to treat solid tumors (28), with the aim of utilizing host immunity to combat cancer, making them promising strategies for treating solid tumors. ICI treatment is an effective treatment method for cervical cancer (29). As confirmed in the KEYNOTE-826 phase III trial (16), the combination of pembrolizumab (an anti-PD-1 inhibitor) and first-line chemotherapy significantly improved the PFS of patients with R/M CC from 8.2 months to 10.4 months, and the 2-year OS rate also increased from 40.4% to 50.4%. Similarly, the midterm analysis of GOG-3016 revealed that (30), compared with chemotherapy, the PD-1 inhibitor cimipril monoclonal antibody improved OS in patients with R/M CC receiving second-line treatment. An increasing number of clinical trials have shown that ICIs have certain safety and efficacy in the treatment of cervical cancer.

In terms of the ORR, the ORR ranged from 76% to 83% in LACC patients in the included studies (24, 25). In patients with R/M CC who received pembrolizumab, the median ORR was 22.39%, ranging from 12.2% to 42% (31–33). In patients with cervical cancer receiving nivolumab, the ORR ranged from 15.8% to 93.8% (34). An excellent ORR (93.8%) was reported in the NICOL trial, in which patients with LACC were administered nivolumab in combination with CCRT (35). One trial (35) reported that the 2-year PFS rate was 75%. Our analysis revealed that CCRT with concurrent immunotherapy significantly increased the ORR (OR: 1.37, 95% CI: 1.02, 1.85; *P*=0.04). The combination of radiation therapy and immunotherapy for the treatment of cervical cancer is receiving widespread attention. Another study of stereotactic radiotherapy (SBRT) combined with atezolizumab (an anti-PD-L1 drug) in the treatment of R/M CC confirmed a median PFS of 4.5 months and a 6-month PFS rate of 46% [38]. The combination of CCRT with ICIs can significantly upregulate immune activation markers, leading to a significant increase in central and effector memory T cells and

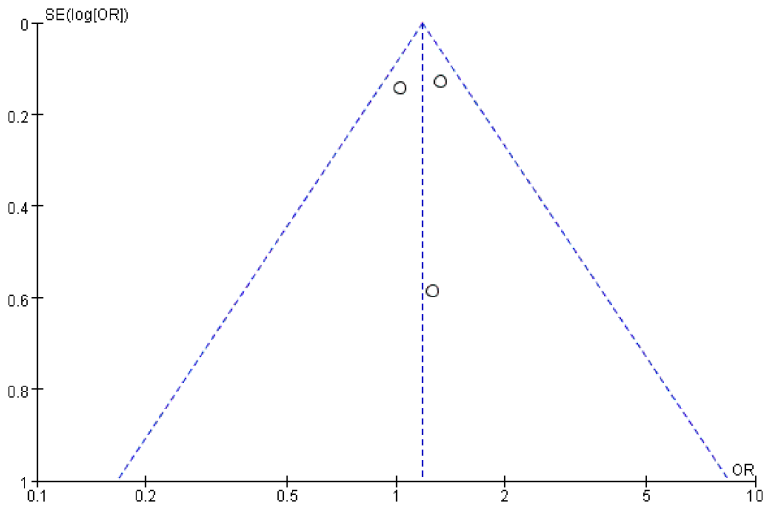

FIGURE 4
Publication bias was assessed by funnel plot of treatment-related grade 3 or higher adverse events.

evidence of immune-modulating activity (36). The PRIMMO phase II trial investigated the efficacy of pembrolizumab combined with SBRT and immunomodulatory drug combinations in patients with R/M CC. The main ORR is 11.1%. The progression-free survival period is 4.1 weeks, and these drugs exhibit persistent and effective antitumor activity (37). A recent review revealed that ICIs improved PFS in patients with cervical cancer (HR, 0.68; 95% CI, 0.59–0.79) compared with the control treatment (38). These results are consistent with our finding that CCRT plus immunotherapy was associated with longer PFS than was the control treatment. A phase 2 RCT (39) reported that the PFS was 2.8 months and 1.9 months in patients with R/M CC who received ragolumab plus atezolizumab or atezolizumab, respectively. In another phase 2 trial of 27 patients with R/M CC treated with sintilimab in combination with chemotherapy, the ORR was 44.4%, and the median PFS was 5.2 months (40). Our subgroup analysis revealed that patients aged less than 65 years who received CCRT plus immunotherapy had longer PFS than did the controls. Moreover, in patients with the IMRT/VMAT radiotherapy plan, those who received CCRT plus immunotherapy also had a longer PFS. The development of new technologies in radiation therapy allows the delivery of higher doses, lowering toxicity and resulting in survival benefits (41). Some studies reported that VMAT combined with guided adaptive brachytherapy resulted in satisfactory PFS and OS in LACC patients (42). Moreover, compared with three-dimensional conformal radiotherapy (3D-CRT), the IMRT technique has a lower degree of radiotherapy toxicity in LACC patients (43). However, in a randomized trial, the effects of the two techniques (IMRT versus 3D-CRT) on relapse-free survival and disease-free survival did not differ, and whether IMRT treatment improved PFS compared with 3D-CRT needs further investigation (44).

In terms of OS, Lorusso et al. reported that the estimated 2-year OS rate was 87% in the intervention group and 81% in the control group and that the median OS was not reached in either group. In patients with cervical cancer who received nivolumab, the median OS ranged from 14.5 months to 21.9 months (34). In patients with R/M CC treated with pembrolizumab, the median OS ranged from 9.4 months to 11.2 months (31). Our study indicated that CCRT plus ICIs improved OS compared with the control treatment, which was consistent with the findings of previous studies (38).

Immunotherapy provides clinical benefits for cancer patients, and owing to its mechanism of action, it inevitably produces a series of side effects. These side effects may affect various organs or systems throughout the body, including the gastrointestinal tract, heart, skin, liver, endocrine system, and lungs (26). The occurrence and onset of immune-mediated adverse reactions depend on various factors, including cancer type, dosage, and ICI category, as well as patient-specific factors. For treatment-related grade 3 or higher AEs, the pooled data indicated that the two methods had similar rates (HR=1.99, 95% CI:0.99, 1.43, P=0.07), but CCRT plus ICIs had a higher rate, indicating that CCRT plus immunotherapy might increase toxicity. However, with respect to individual toxicity, such as ≥ grade 3 nausea, anemia, diarrhea, and a decreased white blood cell count, the two groups presented similar rates. A recent study indicated that ICIs combined with chemotherapy increased the incidence of all-grade AEs (HR 1.11 [1.09; 1.12]) but did not increase the treatment-related mortality rate (45). Toxicity can be safely managed with suitable methods (46). In most cases, ICI treatment can be closely monitored in the presence of mild irAEs. If level 3 toxicity occurs, the use of ICIs should be suspended. In the presence of level 4 toxicity, permanent cessation of ICI therapy is usually recommended; however, if endocrine function is abnormal due to immunity, it can be controlled through hormone replacement. The phase 2 studies included in this study indicate that pembrolizumab combined with CCRT is safe and effective in the treatment of LACC. Among the 52 patients included, 88% experienced treatment-related grade 2 or higher AEs, with approximately 22% experiencing at least one grade 4 AE and 23 experiencing at least one grade 3 AE. With the combination of atezolizumab and SBRT for patients with R/M CC, all patients completed the scheduled treatment with controllable tolerability. Among the most common grade 2 or above AEs, the most common were leukopenia (31%), fatigue (23%) and hypothyroidism (15%) (47). The PRIMMO phase II trial confirmed that pembrolizumab combined with SBRT treatment is safe and effective (30), which is consistent with our meta-analysis results.

Limitations

This study has several limitations. First, only three RCTs were included in this meta-analysis, and one study did not report survival outcomes. This limited the statistical power. Second, the ICIs included in this meta-analysis were different drugs (pembrolizumab and durvalumab), which might explain the differences. Third, PD-L1 expression is an important biomarker for the prediction of treatment effects. Due to limited data, we did not conduct subgroup analysis on the basis of the PD-L1 level. In addition, this analysis included only fully published papers published in English, and studies with negative results might be ignored. Furthermore, in some analyses, high heterogeneity may exist, and some subgroup analyses do not yield positive results; these results should be interpreted with caution.

Conclusions

Compared with the control treatment, CCRT plus ICIs significantly improved survival outcomes and increased the ORR. Similar rates of treatment-related grade 3 or higher AEs and toxicities were observed between the two groups. Moreover, large, well-designed RCTs are needed to further confirm the efficacy and safety of CCRT plus ICIs in LACC patients.

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.

Author contributions

ZZ: Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. JR: Data curation, Formal analysis, Resources, Software, Writing – original draft. MF: Conceptualization, Data curation, Writing – original draft. JL: Data curation, Formal analysis, Methodology, Writing – original draft. GL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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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/fimmu.2024.1459693/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Forest plots for any grade of treatment adverse events between concurrent chemoradiotherapy (CCRT) plus ICIs and control group.

SUPPLEMENTARY FIGURE 2
Forest plots for treatment-related Grade 3 or higher adverse events between concurrent chemoradiotherapy (CCRT) plus ICIs and control group.

SUPPLEMENTARY FIGURE 3
Forest plots for all grade immunotherapy-related adverse events between concurrent chemoradiotherapy (CCRT) plus ICIs and control group. PRISMA Checklist.

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Impact of combinatorial immunotherapies in breast cancer: a systematic review and meta-analysis

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Background: Breast cancer has the highest mortality rate among all cancers affecting females worldwide. Several new effective therapeutic strategies are being developed to minimize the number of breast cancer-related deaths and improve the quality of life of breast cancer patients. However, resistance to conventional therapies in breast cancer patients remains a challenge which could be due to several reasons, including changes in the tumor microenvironment. Attention is being diverted towards minimizing the resistance, toxicity, and improving the affordability of therapeutics for better breast cancer management. This includes personalized medicine, target-specific drug delivery systems, combinational therapies and artificial intelligence based screening and disease prediction. Nowadays, researchers and clinicians are also exploring the use of combinatorial immunotherapies in breast cancer patients, which have shown encouraging results in terms of improved survival outcomes. This study attempts to analyze the role of combinational immunotherapies in breast cancer patients, and offer insights into their effectiveness in breast cancer management.

Methodology: We conducted a systematic review and meta-analysis for which we selected the randomized clinical trials (RCTs) focused on completed Phase I/II/III/IV clinical trials investigating combination immunotherapies for breast cancer. The analysis aimed to assess the efficacy of combination therapies in comparison to mono-therapies, focusing on overall survival (OS), and progression-free survival (PFS).

Results: We observed that, combination immunotherapies significantly (P<0.05) improved OS as compared to single-drug therapies in the Phase I with overall Risk ratio (RR) of 16.17 (CI 2.23,117.50), Phase II with an overall RR of 19.19 (CI 11.76,31.30) and for phase III overall RR 22.27 (CI 13.60,36.37). In the case of PFS, it was significant with RR: 12.35 (CI 2.14, 71.26) in Phase I RR 6.10 (CI 4.31, 8.64) in

phase II, RR 8.95 (CI 6.09, 13.16) in phase III and RR 14.82 (CI 6.49, 33.82) in Phase IV of clinical trials.

Conclusion: The observed improvements in overall survival and progression-free survival suggest that combination immunotherapies could serve as a better approach to breast cancer management.

KEYWORDS

Combinational therapy, immunotherapy, breast cancer, systematic review, meta-analysis

1 Introduction

As per Globocan 2022, among all cancers, breast cancer is one of the leading causes of death in females (1–3), due to various confounding factors, such as age, lifestyle, use of oral contraceptives, lack of physical activities, obesity, high Body Mass Index including epigenetic changes resulting into complexities, heterogenicity, and drug resistance have necessitated the use of a wide range of immunotherapeutic drugs, targeted radiation, and chemotherapies (4–7). The advent of the genomics era has significantly revolutionized the generation of cancer therapeutics. A better understanding of cancer genetics and epigenetics is crucial for the development of effective cancer prevention strategies, precision diagnostics, and therapeutic regimens (8). Targeted drug therapies, gene therapy, and cancer vaccines are available as part of cancer treatment. However, over the time, cancer cells develop resistance to these treatments or undergo genetic changes, making them less effective and increasing the risk of mortality. Finding new strategies to overcome these challenges is the need of the hour to improve cancer treatment outcomes (9, 10). To address these challenges, attempts are being made to develop new treatment approaches, such as precision medicine, personalized therapies, and combination therapy, to enhance treatment outcomes (11, 12).

Conventional therapies for treating breast cancer patients exhibit varying response rates depending upon the stages and receptor profiles of breast cancer, as well as genetic changes in cancer cells (13, 14). These reasons highlight the complexity of cancer treatment outcomes and underscore the need for personalized and tailored approaches to improve the chances of successful responses in each patient (8). Ongoing research has led to innovative combination drug therapies, such as combination immunotherapy, where more than one molecule targets different immune response pathways or different pathways to improve the effectiveness of treatment, overcome drug resistance, and reduce the likelihood of relapse. The integration of innovative therapies with existing treatments offers a potential pathway to significantly improve survival rates and reduce the overall burden of breast cancer (15, 16). The results of combination therapies have the potential to improve treatment outcomes and offer a more comprehensive approach to manage complex diseases such as breast cancer (17–20), and may reduce the mortality rate of breast cancer (Figure 1).

Moreover, the breast tumor microenvironment (TME) in breast cancer is a critical determinant of tumor progression, metastasis, and therapy resistance. Its complex interplay of cellular and non-cellular components creates a supportive niche for tumor growth and poses significant challenges to effective treatment. Targeting the TME, in addition to the cancer cells themselves, represents a promising strategy for overcoming resistance and improving therapeutic outcomes in breast cancer (21). Literature also suggests that combination immunotherapy offers a multifaceted approach to overcome therapy resistance in the tumor microenvironment. By targeting various components of the TME —such as immune suppression, stromal interactions, hypoxia, and antigen presentation, combination therapies can enhance the effectiveness of immunotherapy and lead to more durable responses in breast cancer. This strategy not only improves the efficacy of treatment but also addresses the underlying mechanisms of resistance, potentially leading to better clinical outcomes (22).

The emergence of personalized medicine and combination therapies has become a pivotal strategy in modern cancer treatment. Personalized medicine tailors treatment to the individual characteristics of each patient, including genetic, biomarker, and phenotypic information, allowing for more precise and effective interventions. This approach is particularly important in breast cancer, where heterogeneity among patients requires targeted therapies that addresses specific tumor profiles. The integration of personalized medicine with combination therapies enhances treatment efficacy, reduces the likelihood of resistance, and improves patient outcomes by offering a more comprehensive and tailored approach to cancer management (23, 24).

Hence, to know the effectiveness and impact of combination immunotherapy, the current systematic review and meta-analysis was focused extensively on the completed clinical trials of phases I/II/III and IV in breast cancer, where immunotherapies are used in combination. The study revealed significant outcomes in terms of overall survival (OS), and progression-free survival (PFS) in combination immunotherapies. The results of this study hold the potential to improve cancer treatment and provide insights to develop new

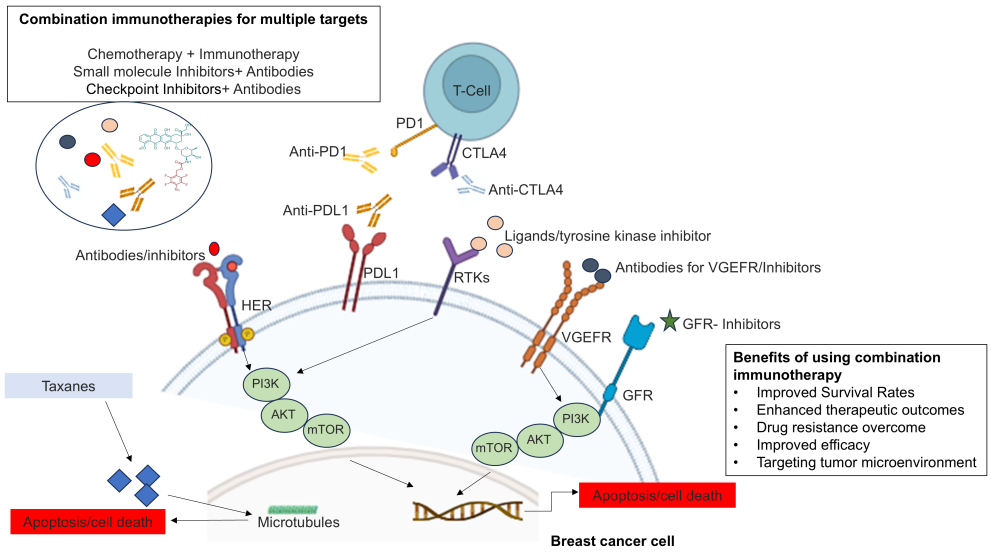

FIGURE 1
Overview of combination immunotherapy in breast cancer treatment.

therapies, which can ultimately improve cancer patient outcomes, especially in breast cancer. This study may also open new avenues of research in combinational immunotherapies in breast cancer with different types of stages and receptor profiles, as well as other cancers that are hard to treat due to several genetic changes and drug resistance.

2 Materials and methodology

2.1 Literature search strategy

A systematic review and meta-analysis study was performed as per the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines for ensuring transparency, rigor, and consistency (Figure 2) (25, 26). The literature search was done through the database *"Clinicaltrials.gov.in" and PubMed* as per the PRISMA guidelines. The keywords used to identify the completed studies on *"Clinicaltrials.gov.in"* and PubMed were *"Combination therapy", Combinational Immunotherapy" in* "breast cancer".

The patients, intervention, comparison, outcome, and study design (PICOS) were followed to design the study.

a. Patients: The studies included known breast cancer patients (females only).
b. Interventions: Those studies were included that have an intervention with a drug combination with an immunotherapy drug.
c. Comparators: The included studies were focused on immunotherapy compared with combination therapy (chemotherapy/radiation/inhibitors/hormonal therapy/endocrine therapy/immunotherapy + immunotherapy).
d. Outcome Measures: Overall survival (OS) and progression-free survival (PFS).
e. Study design: Only randomized controlled trials (RCTs) were included.

2.2 Data retrieval

Screening of the studies was performed by the two authors (SS & JR) on the basis of inclusion and exclusion criteria, and their results were evaluated. A final decision was made and compared with the third author's (VK) opinion. Only those studies that have statistical analysis for overall survival (OS) and progression-free survival (PFS) in patients treated with single immunotherapy versus a combination of immunotherapy with other molecules (two or more) were selected.

2.2.1 Inclusion criteria

Studies were included to compare the results of *"patients treated with one therapy versus a combination of immunotherapies with another molecule" of "randomized control clinical trials Phase I/II/III/IV" and "completed" in breast cancer.*

Additionally, only those studies that had (a) statistical median values with 95% CI intervals results of OS and PFS and (b) studies that had a combination of immunotherapies or combination of any therapy with immunotherapy were included.

2.2.2 Exclusion criteria

Studies were excluded on the basis of pre-determined exclusion criteria listed below:

a. Any duplicate study.
b. Studies other than breast cancer.
c. Results posted only for single therapy in breast cancer.
d. Terminated clinical trials studies.

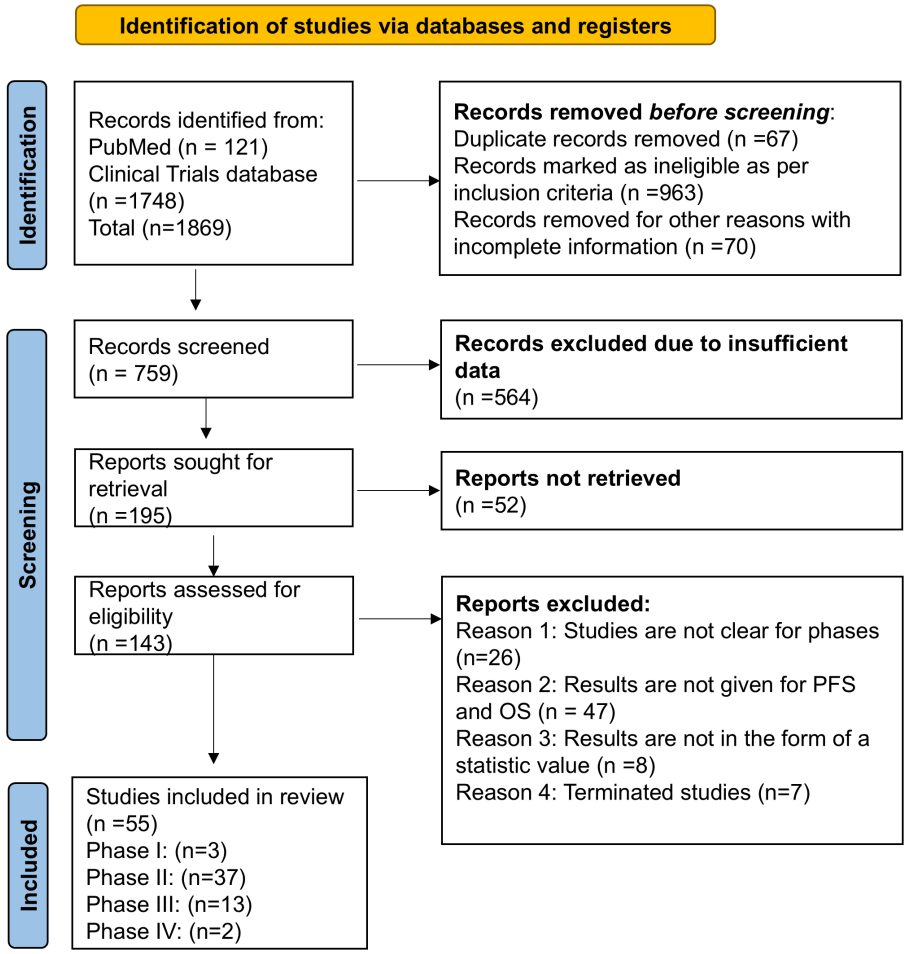

FIGURE 2
PRISMA flowchart for searching the clinical database and selection process for overall survival and progression-free survival in completed clinical trial phase I/II/III/IV in breast cancer.

e. Studies that did not have statistical median values and 95% CI intervals.
f. Studies that did not have outcomes in the form of OS and PFS.

2.2.3 Quality assessment

Quality assessment of all the included studies has been done via CONSORT questionnaire for the randomized clinical trial. All included studies hold a quality score ranging from 22 to 25, which indicates that these were of high quality for the purpose of meta-analysis (27) (Supplementary Table 1).

We also assessed the risk of bias for randomized controlled trials (RCTs) using the Cochrane Collaboration's Risk of Bias (RoB) tool in Review Manager software (version 5.3) (https://community.cochrane.org/help/tools-and-software/revman-5). The evaluation covered seven key domains: random sequence generation (to identify selection bias), allocation concealment (to detect selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other biases (such as funding sources). The results of this assessment are shown in Supplementary Figure 3.

2.3 Statistical analysis

The OS and PFS of patients treated with the combination of immunotherapies (with another molecule or multiple immunotherapy) versus single immunotherapy alone were investigated with the help of statistical median value with 95% confidence intervals. The statistical data of our outcome was observed and determined through overall RR and heterogeneity (I^2 statistics) in the form of percentage value. All the statistical analysis has been carried out using RevMan 5.3 software in which $p<0.05$ was considered significant.

3 Results

3.1 Search criteria and study selection

The initial search focused on retrieving the studies from 2013 to 2024, where 1869 studies were identified, and on the basis of inclusion

TABLE 1 Details of all breast cancer randomized clinical trials for combinational immunotherapies included for the analysis (Source: Clinicaltrials.gov.in and PubMed).

S. No.	Study ID	Year	Sample size	Type of breast cancer	Drug combination	Median; 95% CI interval	Median; 95% CI interval	Outcome
Clinical trial phase I in breast cancer						PFS	OS	
1	NCT01975831	2022	104	Non-triple negative Breast Cancer	Durvalumab + Tremelimumab	56(27 to 223)	267 (35 to 589)	Number of Subjects with treatment-emergent Adverse Events (TEAEs) [Time Frame: Up to 36 months] Progression-free Survival [Time Frame: Up to 5 years] Overall Survival [Time Frame: Up to 5 years]
2	NCT00426556	2014	88	Metastatic Breast Cancer	Everolimus + Trastuzumab + Paclitaxel	5.52 (4.99 to 7.69)	18.07 (12.85 to 24.11)	PFS will be censored at the date of last adequate tumor assessment., every 8 - 9 weeks until disease progression or a new lesion is identified| OS was to be reported at extension and after 3-year follow-up. The Kaplan-Meier median was used to analyze the OS., every 3 months until death
3	NCT03256344 (PMID: 36863095)	2024	36	Metastatic Triple Negative Breast Cancer	Talimogene Laherparepvec + Atezolizumab	5.4 (1.0 to NA)	19.2 (1.5 to NA)	Progression-free Survival (PFS) [Time Frame: Every 12 weeks (± 28 days) up to approximately 3.5 years.] Overall Survival (OS) [Time Frame: Every 12 weeks (± 28 days) up to approximately 3.5 years.]
Clinical trial phase II in breast cancer								
1	NCT02513472	2022	258	Neoplasm	Eribulin Mesylate + Pembrolizumab	4.1 (2.3 to 4.4)	15.5 (12.5 to 18.7)	Objective Response Rate (ORR) [Time Frame: From date of first dose of study drug administration to date of first documentation of disease progression or death, whichever occurred first (up to 3 years 11 months)]
2	NCT03167619	2022	45	Triple Negative Breast Cancer	Olaparib + Durvalumab	0.11 (0.07 to 0.19)	18.27 (8.18 to NA)	Overall Survival (Olaparib in Combination With Durvalumab) [Time Frame: From date of randomization until death or last patient contact, approximately 2 years] To determine the efficacy of maintenance olaparib in combination with durvalumab following platinum based chemotherapy as assessed by overall survival (OS).
3	NCT00733408	2018	59	Estrogen Receptor-negative Breast Cancer| HER2-negative Breast Cancer| Progesterone Receptor-negative Breast Cancer| Recurrent Breast Cancer|Stage IV Breast Cancer| Triple-negative Breast Cancer	Paclitaxel albumin-stabilized nanoparticle formulation + Bevacizumab + Erlotinib hydrochloride	9.1(7.2 to 11.1)	18.1 (15.6 to 21.7)	Overall Survival [Time Frame: Time from date of registration to date of death due to any cause, assessed up to 8 years] Kaplan-Meier survival curves will be used. Percentage of Participants With Response [Time Frame: Up to 8 years]
4	NCT02657343	2022	25	HER2-positive Breast Cancer	Ribociclib + T-DM1; Ribociclib + Trastuzumab: Fulvestrant	10.4(2.7 to 19.3)	7.9 (3.4 to NA)	Maximum Tolerated Dose (Mtd) And/Or Recommended Phase2 Dose (RP2D) [Time Frame: 2 years] Clinical Benefit Rate (CBR) By RECIST [Time Frame: 2]

(Continued)

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TABLE 1 Continued

S. No.	Study ID	Year	Sample size	Type of breast cancer	Drug combination	Median; 95% CI interval	Median; 95% CI interval	Outcome
Clinical trial phase II in breast cancer								
5	NCT02536339	2021	40	HER2-Positive Metastatic Breast Cancer	Pertuzumab + Trastuzumab	16.26(0.03 to 55.20)	27.17 (0.82 to 57.49)	Serum pertuzumab concentrations [Time Frame: Pre-dose and post-dose during Weeks 1, 4, 10, and 16] Serum trastuzumab concentrations [Time Frame: Pre-dose and post-dose during Weeks 1, 4, 10, and 16]
6	NCT02924883	2021	202	Metastatic Breast Cancer	Trastuzumab Emtansine + Placebo, Trastuzumab Emtansine + Atezolizumab	6.8 (4.0 to 11.1); 8.2 (5.8 to 10.7)	NA	Progression Free Survival (PFS) as Determined by Investigator's Tumor Assessment Using Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 [Time Frame: From Baseline until disease progression or death (up to approximately 28 months)]
7	NCT02536794	2022	30	Estrogen Receptor Negative|Estrogen Receptor Positive| HER2/Neu Negative| Recurrent Breast Carcinoma|Stage IV Breast Cancer	MEDI4736 + Tremelimumab	4.86(3.09 to 7.89)	11.3 (7.16 to 36.6)	Toxicity of MEDI4736 in combination with Tremelimumab [Time Frame: Up to 6 months after last treatment] Toxicity will be evaluated by the number, frequency, and severity of adverse events as defined by the NCI Common Terminology Criteria for Adverse Events or CTCAE version 4.03
8	NCT02648477	2024	30	Estrogen Receptor Negative|Estrogen Receptor Positive| HER2/Neu Negative| Progesterone Receptor Negative| Progesterone Receptor Positive| Stage IV Breast Cancer|Triple-Negative Breast Carcinoma	Cohort 1 (Pembrolizumab, Doxorubicin Hydrochloride) Triple Negative Breast Cancer Cohort 2 (Pembrolizumab, Anti-estrogen Therapy) HR + HER2- Breast Cancer	5.2 (4.7 to NA): 1.8 (1.6 to 2.6)	15.6 (13.3 to NA): 17.2 (9.4 to NA)	Clinical Benefit Rate [Time Frame: Up to 6 months] Overall Survival (OS) [Time Frame: Up to 3 years] Progression-free Survival (PFS) [Time Frame: Up to 3 years]
9	NCT01670877	2022	56	Neoplasms	Neratinib + Fulvestrant + Trastuzumab	20 (8 to NA) 24 (15.7 to 31)		This phase II study will test cancer to see if it has a HER2 mutation and, if so, see how HER2 mutated cancer responds to treatment with neratinib.
10	NCT03321981	2024	105	Breast Cancer Metastatic	Zenocutuzumab + Trastuzumab + Vinorelbine + Endocrine therapy	5.59 (4.11 to 7.39) 1.45 (1.45 to 2.73)	26.41 (17.51 to NA)	A total of up to 40 patients evaluable for efficacy are included in the Cohort 2.

(Continued)

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TABLE 1 Continued

S. No.	Study ID	Year	Sample size	Type of breast cancer	Drug combination	Median; 95% CI interval	Median; 95% CI interval	Outcome
Clinical trial phase II in breast cancer								
11	NCT01605396	2019	80	Neoplasms	Ridaforolimus + Dalotuzumab + Exemestane	23.29 (8.71 to 38.43)		The primary hypothesis of the study is that the triplet of ridaforolimus, dalotuzumab and exemestane will improve progression free survival (PFS) compared to ridaforolimus and exemestane.
12	NCT00670982	2013	29	HER2-Positive, Metastatic Breast Cancer	Ridaforolimus + Dalotuzumab + Exemestane	7.8 (3.5 to 22.0)		The purpose of this research study is to determine the effects of the combination of bevacizumab, vinorelbine, and trastuzumab on participants and their cancer.
13	NCT01201265	2016	40	Triple Negative Metastatic Breast Cancer	Bevacizumab+ Carboplatin + Gemcitabine	255 (157 to 465)	475.0 (358.0 to 759.0)	This multicenter study will assess the efficacy and safety of bevacizumab in combination with gemcitabine and cisplatin as first line treatment in participants with triple negative metastatic breast cancer. Participants will receive bevacizumab at a dose of 15 mg/kg intravenously (iv) every 3 weeks, plus gemcitabine (1000 mg/m2 iv) and carboplatin (iv to an area under curve [AUC]=2) on Days 1 and 8 of each 3-week cycle. Anticipated time on study treatment is until disease progression.
14	NCT00004888	2014	84	Recurrent Breast Cancer|Stage IV Breast Cancer |	Pegylated liposomal doxorubicin hydrochloride + Docetaxel + Trastuzumab	10.6 (5.6 to 15.7)	31.8 (23.7 to 44.9)	Phase II trial to study the effectiveness of combination chemotherapy with or without trastuzumab in treating women who have metastatic breast cancer. Drugs used in chemotherapy use different ways to stop tumor cells from dividing so they stop growing or die. Monoclonal antibodies such as trastuzumab can locate tumor cells and either kill them or deliver tumor-killing substances to them without harming normal cells.
15	NCT00654836	2017	32	Recurrent or Metastatic Breast Cancer	Bevacizumab + Carboplatin + ABI-007	16 (9.80 to 22.20)	21(13.48 to 28.52)	
16	NCT00699491	2018	48	|Recurrent Breast Carcinoma|Stage IV Breast Cancer AJCC v6 and v7	Cixutumumab + Laboratory Biomarker Analysis + Pharmacological Study + Temsirolimus	2.0 (1.5 to 3.0)		This phase I/II trial is studying the side effects and best dose of cixutumumab when given together with temsirolimus and to see how well they work in treating patients with breast cancer that has recurred (come back) at or near the same place as the original (primary) tumor or has spread to other places in the body. Monoclonal antibodies, such as cixutumumab, can block tumor growth in different ways by targeting certain cells. Temsirolimus may stop the growth of tumor cells by blocking some of the enzymes needed for cell growth. Giving cixutumumab together with temsirolimus may be a better treatment for breast cancer.
17	NCT01427933	2014	141	Metastatic Breast Cancer	Ramucirumab (IMC-1121B) +: Eribulin	4.4 (3.1 to 6.7)	13.5 (10.4 to 17.9)	PURPOSE: This phase II trial is studying how well giving carboplatin and paclitaxel together with bevacizumab works in treating patients with locally recurrent or metastatic breast cancer.
18	NCT01234402	2019	153	Metastatic Breast Cancer	Ramucirumab DP + IMC-18F1 + Capecitabine	22.1 (12.1 to 36.1) 7.3 (6.3 to 13.0)	67.4 (41.3 to 82.6) 62.1 (41.0 to 84.0)	An open-label, multicenter, randomized, Phase 2 trial in which participant with unresectable, locally advanced or metastatic breast cancer who have been previously treated with anthracycline and taxane therapy receive ramucirumab DP or Icrucumab (IMC-18F1) administered on an every-21-day cycle (in combination with oral capecitabine therapy; capecitabine is administered twice a day on Days 1-14 of each

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TABLE 1 Continued

S. No.	Study ID	Year	Sample size	Type of breast cancer	Drug combination	Median; 95% CI interval	Median; 95% CI interval	Outcome
Clinical trial phase II in breast cancer								
								cycle). Approximately 150 participants will be randomized in a 1:1:1 ratio to either ramucirumab DP or Icrucumab (IMC-18F1) in combination with capecitabine (Arm A and Arm B, respectively) or capecitabine monotherapy (Arm C). Randomization will be stratified by triple-negative receptor status (estrogen receptor-negative, progesterone receptor-negative, and human epidermal growth factor receptor-2 [HER2/neu]-negative) (yes/no) and receipt of prior antiangiogenic therapy.
19	NCT00662129	2017	50	Metastatic Breast Cancer	Bevacizumab + Gemcitabine hydrochloride + Paclitaxel albuminstabilized nanoparticle formulation	0.792 (0.647 to 0.882)	24.4 (18.2 to 29.3)	Drugs used in chemotherapy, such as gemcitabine and paclitaxel albumin-stabilized nanoparticle formulation, work in different ways to stop the growth of tumor cells, either by killing the cells or by stopping them from dividing. Monoclonal antibodies, such as bevacizumab, can block tumor growth in different ways. Some block the ability of tumor cells to grow and spread. Others find tumor cells and help kill them or carry tumor-killing substances to them. Giving combination chemotherapy together with bevacizumab may kill more tumor cells.
20	NCT00846027	2014	90	HER-2 negative breast cancer.	Bevacizumab + Paclitaxel + Gemcitabine	11.51 (9.01 to 17.59)	27.39 (21.86 to NA)	This single-arm study assessed the efficacy and safety of first-line treatment with Avastin (bevacizumab) in combination with taxane-based chemotherapy (paclitaxel and gemcitabine) in patients with HER-2 negative breast cancer. Patients received Avastin 10 mg/kg iv, paclitaxel 150 mg/m^2 iv, and gemcitabine 200 mg/m^2 iv on Day 1 and Day 15 of each 4-week treatment cycle until disease progression, death, or withdrawal of consent.
21	NCT01306942	2019	37	HER2 positive Metastatic Breast Cancer	Dasatinib + Trastuzumab + Paclitaxel	23.9 (10.3 to NA)		PURPOSE: This phase II trial is studying how well giving paclitaxel albumin-stabilized nanoparticle formulation and gemcitabine together with bevacizumab works in treating patients with metastatic breast cancer.
22	NCT00444587	2016	114	HER2 positive Metastatic Breast Cancer	Secondline chemotherapy + Trastuzumab[Herceptin]		717 (589 to 1057)	This 2 arm study will compare the efficacy and safety of continuation or discontinuation of Herceptin treatment in combination with 2nd line chemotherapy, in patients with HER2 positive metastatic breast cancer whose condition has progressed on 1st line chemotherapy plus Herceptin. Patients will be randomized either to continue or discontinue Herceptin treatment (6mg/kg iv infusion every 3 weeks) while receiving second-line chemotherapy of the investigator's choice. The anticipated time on study treatment is until disease progression, and the target sample size is 100-500 individuals.
23	NCT00811135	2015	88	HER2-Positive Breast Cancer	Bevacizumab[Avastin] + Capecitabine[Xeloda] + Trastuzumab [Herceptin]	14.2 (10.5 to 14.9)	31.8 (26.3 to 38.2)	This single arm study will assess the efficacy and safety of Avastin in combination with Herceptin and Xeloda as first-line treatment of patients with HER2-positive locally recurrent or metastatic breast cancer. Patients will receive 3-weekly treatment cycles of Herceptin (8mg/kg iv on day 1 of first cycle, followed by 6mg/kg iv maintenance dose on day 1 of subsequent cycles), Xeloda (1000mg/m2 bid po on days 1-14 of each treatment cycle) and Avastin (15mg/kg on day 2 of first treatment cycle,and on day 1 of each subsequent cycle).The anticipated time on study treatment is until disease progression, and the target sample size is <100 individuals.

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TABLE 1 Continued

S. No.	Study ID	Year	Sample size	Type of breast cancer	Drug combination	Median; 95% CI interval	Median; 95% CI interval	Outcome
Clinical trial phase II in breast cancer								
24	NCT02260531	2021	36	Metastatic	Cabozantinib + Trastuzumab	4.1 (2.8 to 6.2)	13.8 (8.2 to NA)	This research study is evaluating the effectiveness of the drug called cabozantinib (alone or in combination with trastuzumab) as a possible treatment for advanced breast cancer in which the cancer has spread to the brain.
25	NCT00193063	2014	41	HER2 positive Metastatic Breast Cancer	Trastuzumab + Gemcitabine	4 (1.9 to 5.3)	21 (11.5 to 30.5)	Due to its remarkable activity as salvage treatment in women with metastatic breast cancer as well as the additive activity observed for gemcitabine administered in combination with trastuzumab, the clinical activity of the combination of gemcitabine administered with trastuzumab represents an exciting and ideal combination to further evaluate in Her 2 over-expressing metastatic breast cancer patients.
26	NCT02322814	2019	169	Metastatic Triple Negative Breast Cancer	Cobimetinib + Paclitaxel + Placebo + Atezolizumab + Nab-Paclitaxel		15.57 (14.26 to NA)	This three-cohort, multi-stage, randomized, Phase II, multicenter trial will evaluate the safety and tolerability and estimate the efficacy of cobimetinib plus paclitaxel versus placebo plus paclitaxel in Cohort I, of cobimetinib plus atezolizumab plus paclitaxel in Cohort II, and of cobimetinib plus atezolizumab plus nab-paclitaxel in Cohort III in participants with metastatic or locally advanced, triple-negative adenocarcinoma of the breast who have not received prior systemic therapy for metastatic breast cancer (MBC). Participants may continue on study treatment until the development of progressive disease (PD) or the loss of clinical benefit, unacceptable toxicity, and/or consent withdrawal. The Cohort I target sample size is 12 participants for the safety run-in stage and approximately 90 participants in the expansion stage. Each of Cohorts II and III will consist of a safety run-in stage of approximately 15 participants followed by an expansion stage of approximately 15 participants
27	NCT01491737	2020	258	HER2-Positive and Hormone Receptor-Positive Advanced (Metastatic or Locally Advanced) Breast Cancer	Pertuzumab + Trastuzumab + Aromatase Inhibitor + Induction Chemotherapy	20.63 (14.39 to 28.35)	60.16 (47.21 to 79.01)	This randomized, open-label, two-arm, multi-center, Phase II study will evaluate the efficacy and safety of pertuzumab in combination with trastuzumab plus an aromatase inhibitor (AI) in first-line participants with HER2-positive and hormone receptor-positive advanced breast cancer. Participants will be randomized to one of two treatment arms; Arm A (pertuzumab in combination with trastuzumab plus an AI) or Arm B (trastuzumab plus an AI). Participants may also receive induction chemotherapy (a taxane, either docetaxel or paclitaxel) at the investigator's discretion in combination with the assigned treatment arm. The anticipated time on study treatment is until disease progression, unacceptable toxicity, withdrawal of consent, or death whichever occurs first.
28	NCT03025880	2023	26	HER2-negative ABC	Pembrolizumab + Gemcitabine	3.1 (2 to 4.3)	8.7 (6.5 to 11.7)	This is a multicenter phase II trial, with an initial exploratory run-in-phase, to evaluate the efficacy and safety of pembrolizumab in combination with gemcitabine in patients with HER2-negative ABC that have previously received anthracyclines and taxanes (unless clinically contraindicated). In hormone receptor positive patients, previous treatment with 2 or more lines of hormone therapy will also be required. Patients must have at least one measurable lesion that can be accurately assessed at baseline and is suitable for repeated assessment by CT, MRI or plan X-ray. Approximately 53 patients (up to a maximum of 65 patients depending on the results of the run-in-phase) will be included in this trial

(Continued)

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TABLE 1 Continued

S. No.	Study ID	Year	Sample size	Type of breast cancer	Drug combination	Median; 95% CI interval	Median; 95% CI interval	Outcome
Clinical trial phase II in breast cancer								
29	NCT01565083	2016	213	HER2 positive Breast Cancer	Pertuzumab + Trastuzumab + Vinorelbine	14.3 (11.2 to 17.5) 11.5 (10.3 to 15.8)		This two-cohort, open-label, multicenter, phase 2 study will assess the safety and efficacy of pertuzumab given in combination with trastuzumab (Herceptin) and vinorelbine in first line participants with metastatic or locally advanced HER2-positive breast cancer. Participants will receive pertuzumab and trastuzumab administered sequentially as separate intravenous (IV) infusions (followed by vinorelbine) and conventional sequential administration of pertuzumab and trastuzumab in separate infusion bags, followed by vinorelbine.
30	NCT03121352	2023	30	Metastatic Triple Negative Breast Cancer	Carboplatin + Nab-paclitaxel + Pembrolizumab	5.8 (4.7 to 8.5)		The purpose of this study is to see how effective the combination of the two chemotherapy drugs (carboplatin and nab-paclitaxel) are when added to a third drug, pembrolizumab. Pembrolizumab is an investigational (experimental) drug that works by reinvigorating the immune system, allowing it to target and destroy cancer cells. Pembrolizumab is experimental because it is not approved by the Food and Drug Administration (FDA) for this type of breast cancer treatment.
31	NCT00331552	2017	30	HER2-positive Breast Cancerr| Recurrent Breast Cancer|Stage IV Breast Cancer	Pegylatedliposomal doxorubicin hydrochloride + Cyclophosphamide + Trastuzumab	0.16 (0.033 to 0.77)	0.49 (0.32 to 0.76)	Drugs used in chemotherapy, such as doxorubicin hydrochloride liposome and cyclophosphamide, work in different ways to stop the growth of tumor cells, either by killing the cells or by stopping them from dividing. Monoclonal antibodies, such as trastuzumab, can block tumor growth in different ways. Some block the ability of tumor cells to grow and spread. Others find tumor cells and help kill them or carry tumor-killing substances to them. Giving more than one drug (combination chemotherapy) together with trastuzumab may be a better way to block tumor growth.
32	NCT01305941	2018	32	HER-2 Positive Breast Cancer	Everolimus + Vinorelbine + Trastuzumab		1.01 (.57 to 1.78)	Purpose: This study is a single-arm, open-label phase II clinical trial testing the hypothesis that daily everolimus plus weekly vinorelbine and trastuzumab will be effective, safe, and tolerable among patients with human epidermal growth factor receptor 2 (HER2)-positive breast cancer brain metastases. Once enrolled, patients will receive everolimus PO daily in combination with weekly intravenous (IV) vinorelbine and trastuzumab. Cycles will be repeated every 3 weeks (21 days). At the time of progression, patients will come off study. Participants: Up to 35 adults over 21 with HER-2 positive breast cancer that has metastasized to the brain.
33	NCT02971761	2024	18	Androgen Receptor Positive| Estrogen Receptor Negative|HER2/ Neu Negative| Metastatic Triple-Negative Breast Carcinoma| Progesterone Receptor	Enobosarm + Laboratory Biomarker Analysis + Pembrolizumab	2.6 (1.9 to 3.1)	25.5(10.4 to 30.9)	This phase II trial studies the side effects and how well pembrolizumab and enobosarm work in treating patients with androgen receptor positive triple negative breast cancer that has spread to other places in the body (metastatic). Immunotherapy with monoclonal antibodies, such as pembrolizumab, may help the body's immune system attack the cancer, and may interfere with the ability of tumor cells to grow and spread. Androgen can cause the growth of breast cancer cells. Hormone therapy using enobosarm may fight breast cancer by blocking the use of androgen by the tumor cells. Giving pembrolizumab and enobosarm may work better than pembrolizumab alone in treating patients with androgen receptor positive triple negative breast cancer.

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TABLE 1 Continued

S. No.	Study ID	Year	Sample size	Type of breast cancer	Drug combination	Median; 95% CI interval	Median; 95% CI interval	Outcome
Clinical trial phase II in breast cancer								
				Negative|Stage IV Breast Cancer AJCC v6 and v7				
34	NCT03147287	2024	220	Metastatic Breast Cancer	Fulvestrant + Palbociclib + Avelumab	8.1(3.2 to 10.7)		This research study is studying three combinations of drugs as treatments for breast cancer. The drugs involved in this study are:Fulvestrant Fulvestrant with Palbociclib Fulvestrant with Palbociclib and Avelumab
35	NCT04191135	2024	462	Triple Negative Breast Neoplasms	Pembrolizumab + Olaparib + Carboplatin + Gemcitabine	5.5 (4.2 to 8.3).	25.1 (18.3 to NA).	The purpose of this study is to compare the efficacy of olaparib (MK-7339) plus pembrolizumab (MK-3475) with chemotherapy plus pembrolizumab after induction with first-line chemotherapy plus pembrolizumab in triple negative breast cancer (TNBC). The primary hypotheses are: Olaparib plus pembrolizumab is superior to chemotherapy plus pembrolizumab with respect to progression-free survival (PFS). Olaparib plus pembrolizumab is superior to chemotherapy plus pembrolizumab with respect to overall survival (OS). As of Amendment 3, study enrollment was discontinued. Participants who were receiving benefit from the study intervention could continue treatment until criteria for discontinuation are met. Participants who are on study treatment or in follow-up phase will no longer have tumor response assessments by BICR.
36	NCT02981303	2024	64	Advanced Melanoma|Triple-Negative Breast Cancer	Imprime PGG + Pembrolizumab	RECISTv1.1 = 2.35 (1.35 to 3.98). irRECIST= 2.86 (1.81 to 4.11)	16.36(11.10 to 19.22)	Objective: To determine the Overall Response Rate (ORR) to Imprime PGG + pembrolizumab in subjects with advanced melanoma or metastatic TNBC Safety: To characterize the safety of Imprime PGG + pembrolizumab given in combination Hypothesis: Restore (for melanoma) or enhance (for TNBC) sensitivity to checkpoint inhibitors (CPI) by appropriate and effective stimulation of the subject's innate and adaptive immune systems in those subjects who have failed 1st line therapy The study will incorporate Simon's optimal 2-stage design with sample size fixed at 12 subjects each in Stage 1 for advanced melanoma and for Triple Negative Breast Cancer (TNBC) subjects. The safety criterion of ≤ 4 (or ≤ 33%) subjects with Grade 3/4 adverse events in Cycle 1 within either tumor type must be met in order to proceed to Stage 2. The starting dose is 4 mg/kg for Imprime PGG. In the event there are a total of > 4 (or > 33%) of subjects with Grade 3/4 adverse events in Cycle 1, the dose of Imprime PGG will be reduced to 2 mg/kg, and Stage 1 will be repeated at a dose of 2 mg/kg with an additional cohort of n=12 subjects. For the dose that meets the safety criterion in Stage 1, at least 1 response in melanoma subjects and 2 responses in TNBC subjects amongst the 12 subjects within each tumor type must be observed in order to proceed to Stage 2. Stage 2 will enroll an additional 17 subjects with melanoma, and 30 subjects with TNBC. For the dose that meets the Stage 1 safety criterion, success will be declared if at least 4

(Continued)

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TABLE 1 Continued

S. No.	Study ID	Year	Sample size	Type of breast cancer	Drug combination	Median; 95% CI interval	Median; 95% CI interval	Outcome
Clinical trial phase II in breast cancer								
								amongst the total of up to 29 subjects with melanoma, and 13 amongst the total of up to 42 subjects with TNBC achieve an objective response.
37	NCT03051659 PMID: 32880602)	2024	90	Breast Cancer	Eribulin Mesylate + Pembrolizumab	4.1(3.5 to 6.2)	13.4 (10.4 to NA)	Progression-free survival based on the Kaplan-Meier method is defined as the duration of time from study entry to documented disease progression (PD) or death. Progression Free Survival [Time Frame: 2 years] Median Overall Survival (OS) [Time Frame: Disease assessments is performed every 3 cycles (3 weeks/cycle) for the first 18 cycles. Median follow-up 10.5 months with range 0.43-19 months.]
clinical trial phase III in breast cancer								
1	NCT01160211	2022	442	hormone receptor positive, HER2+ metastatic Breast Cancer	lapatinib + Trastuzumab + Aromataseinhibitor	5.6(5.4 to 8.3)	0.60 (0.35 to 1.04)	PFS of Lapatinib+Trastuzumab+AI Combination vs. Trastuzumab+AI Combination [Time Frame: approximately 5 years]
2	NCT00876395	2017	719	HER2-overexpressing metastatic breast cancer	Everolimus + Placebo, Trastuzumab + Paclitaxel	14.49(12.29 to 17.08)	49.97 (40.84 to NA)	Progression-free Survival (PFS) Per Investigators' Assessment Based on Local Radiology Review - Full Population [Time Frame: date of randomization to the date of first documented tumor progression or death from any cause, whichever occurs first, reported between day of first patient randomized up to about 56 months]
3	NCT00545077	2014	380	HER-2 Negative Breast Cancer	Letrozole + Bevacizumab + Fulvestrant	19.3(16.5 to 22.1)	52.1 (35.79 to 68.49)	Progression-free Survival (PFS) [Time Frame: Up to 2 years] Overall Survival (OS) [Time Frame: Up to 2 years]
4	NCT01250379	2015	494	metastatic Breast Cancer	Bevacizumab [Avastin] + Chemotherapy	6.3(5.5 to 7.6)	19.7 (17.6 to 21.0)	Percentage of Participants Estimated to be Surviving at Months 6, 12, 18, and 24 [Time Frame: Months 6, 12, 18, and 24] Overall survival (OS) [Time Frame: approximately 42 months] Safety: Incidence of adverse events [Time Frame: approximately 42 months]
5	NCT01026142	2017	452	HER-2 Positive Breast Cancer	Capecitabine + Pertuzumab + Trastuzumab	11.1(9 to 13)	37.2 (33 to 42)	Progression Free Survival (Independent Assessment) [Time Frame: Tumor assessments every 9 weeks from randomization until Week 27, then every 12 weeks thereafter, until IRF-determined PD, initiation of alternative anticancer medication, or death (up to 5.5 years).]
6	NCT00391092	2014	424	HER-2 Positive Breast Cancer	Bevacizumab [Avastin] + Docetaxel, Herceptin	16.5(14.1 to 19.1)	38.5 (32.1 to NA)	Progression Free Survival (PFS) [Time Frame: Every 9 weeks up to Week 36, thereafter every 12 weeks until disease progression (up to the clinical cutoff of 30 June 2011, up to 4.75 years)] Overall Survival (OS) [Time Frame: Every 9 weeks up to Week 36, thereafter every 12 weeks until disease progression (up to the clinical cutoff of 30 June 2011, up to 4.75 years)]
7	NCT00333775	2013	736	HER-2 Negative Breast Cancer	Docetaxel + Placebo + bevacizumab	8.7(8.2 to 9.9)	NA (15.7 to NA)	Progression-free Survival [Time Frame: Baseline to the 15 Sep 2008 cut-off date (up to 2 years, 6 months) Overall Survival [Time Frame: Baseline to the 15 Sep 2008 cut-off date (up to 2 years, 6 months)]

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TABLE 1 Continued

S. No.	Study ID	Year	Sample size	Type of breast cancer	Drug combination	Median; 95% CI interval	Median; 95% CI interval	Outcome
clinical trial phase III in breast cancer								
8	NCT00553358	2019	455	HER2/ErbB2 over-expressing Breast Cancer	Lapatinib + Trastuzumab + Paclitaxel		9.70 (9.60 to 9.76)	Number of Participants With Pathological Complete Response (pCR) at the Time of Surgery [Time Frame: Weeks 20 to 22] Overall Survival (OS) - Median Survival Follow-up [Time Frame: From randomization up to approximately year 10]
9	NCT01663727	2017	481	HER-2 Negative Metastatic Breast Cancer	Bevacizumab + Paclitaxel + Placebo	11.0 (9.5 to 12.2)	28.8 (22.8 to 32.8)	Progression Free Survival (PFS) in ITT Population [Time Frame: Baseline, every 8 weeks until documented disease progression, death or clinical cut-off (up to 117.7 weeks)] Overall Survival (OS) - ITT Population [Time Frame: From randomization till death or clinical cut-off (up to 244 weeks)]
10	NCT01120184	2016	1095	HER-2 PositiveMetastatic Breast Cancer	Docetaxel + Paclitaxel + Pertuzumab	14.1 (10.9 to 16.8)	53.68 (48.36 to 64.36)	Progression-Free Survival (PFS) According to IRF Assessment [Time Frame: Up to 48 months from randomization until clinical cutoff of 16-Sept-2014 (at Screening, every 9 weeks for 81 weeks, then every 12 weeks thereafter and/or up to 42 days after last dose)] Overall Survival (OS) at Clinical Cutoff [Time Frame: Up to 70 months from randomization until clinical cutoff of 15-May-2016 (every 3 months until death, loss to follow-up, withdrawal, or study termination)]
11	NCT02019277	2024	242	HER2-positive Metastatic Breast Cancer	Enobosarm + Laboratory Biomarker Analysis + Pembrolizumab	17.02 (12.48 to 31.18)		This open-label, multicenter, Phase IIIb study will assess the safety, tolerability and efficacy of a combination therapy of intravenous (IV) pertuzumab (Perjeta), trastuzumab (Herceptin) SC, and taxane chemotherapy (docetaxel, paclitaxel or nab-paclitaxel) as first-line therapy in participants with HER2-positive metastatic breast cancer (mBC). All participants will be treated with 3-week cycles of pertuzumab IV (840 milligrams [mg] first dose; subsequent doses of 420 mg) and trastuzumab SC (600 milligrams [mg]). The taxane treatment regimen will be determined by the investigator. Participants will continue therapy until disease progression, unacceptable toxicity, or the participant withdraws consent, whichever occurs first.
12	NCT02819518	2023	882	Triple Negative Breast Cancer (TNBC)	Pembrolizumab + Nab-paclitaxel + Paclitaxel + Gemcitabine + Carboplatin + Normale Saline Solution	7.5 (6.3 to 7.7)	17.2 (15.3 to 19.0).	In Part 1, the safety of pembrolizumab (MK-3475) in combination with one of three different chemotherapies will be assessed in the treatment of locally recurrent inoperable or metastatic triple negative breast cancer (TNBC), which has not been previously treated with chemotherapy. the combination of pembrolizumab and chemotherapy prolongs Progression-Free Survival (PFS) compared to placebo and chemotherapy in:all participants, participants with programmed cell death-ligand 1 (PD-L1) combined positive score (CPS) ≥1 tumors, and participants with PD-L1 CPS ≥10 tumors, and the combination of pembrolizumab and chemotherapy prolongs Overall Survival (OS) compared to placebo and chemotherapy
13	NCT04177108	2024	242	Triple-Negative Breast Cancer	Atezolizumab + Ipatasertib + Paclitaxel + Placebo for Atezolizumab + Placebo for Ipatasertib	7.1 (5.1 to 9.3)	15.7 (12.5 to NA)	This study evaluated the efficacy and safety of ipatasertib in combination with atezolizumab and paclitaxel in locally advanced or metastatic Triple-Negative Breast Cancer (TNBC) previously untreated in this setting.

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TABLE 1 Continued

S. No.	Study ID	Year	Sample size	Type of breast cancer	Drug combination	Median; 95% CI interval	Outcome
clinical trial phase IV in breast cancer							
1	NCT01301729	2016	32	HER2-positive Breast Cancer	Docetaxel + Paclitaxel + Trastuzumab	9.9(6.28 to 13.63)	NA (22.64 to NA)	Progression-free survival, tumour assessments according to RECIST criteria [Time Frame: up to 4 years] Overall Response Rate [Time Frame: up to 28 months]
2	NCT02445586	2018	52	HER2-positive Breast Cancer	Docetaxel + Pertuzumab + Trastuzumab	23.0(13.0 to 29.0)	NA (26 to NA)	Overall Response Rate (ORR) [Time Frame: Up to 24 months after the last patient in] Progression-free survival (PFS) [Time Frame: Up to 24 months after the last patient in] Overall Survival (OS) [Time Frame: Up to 24 months after the last patient in]

and exclusion criteria, 143 were found eligible studies. After screening and sorting of studies, 55 were selected for OS and PFS in breast cancer, where phase I-03 (OS-03 and PFS-03) phase II-34,(OS-34 and PFS found in only 28 studies) phase III-14, (OS-13 and PFS found in all 14 studies) and phase IV-02 (PFS-02 and OS was not found) studies were included in the current study (Figure 2 and Table 1). Additionally, studies were excluded if the results did not have OS, PFS, and 95% confidence intervals.

3.2 Analysis of breast cancer in different phase clinical trials

3.2.1 Overall survival

In the current meta-analysis, we have analyzed the overall survival (OS) in selected studies in phase I/II/III/IV RCTs where patients receiving a combination of immunotherapy or immunotherapy with other molecules exhibited a significant difference compared to those receiving one immunotherapy alone.

The meta-analysis revealed a high level of heterogeneity in overall survival with an overall Risk Ratio of 16.17 [(CI 2.23,117.50 (overall significance P< 0.0001)] for clinical trial phase 1, 19.19 [CI 11.76,31.30.00 (overall significance P<0.00001)] for phase II, and 22.27 [CI 13.64,36.37 (with overall significance P<0.00001)] for phase III with 95% CI interval (Figures 3A–C). For phase IV trials, OS data was not found in selected studies. Results of OS suggest that combination immunotherapy is highly significant in comparison to monotherapy or single immunotherapy in improving breast cancer management.

3.2.2 Progression-free survival

We also analyzed progression-free survival in all four phases I, II, III, and IV RCTs. We observed Risk Ratio of 12.35 [CI 2.14, 71.26 (overall significance P<0.0001) for phase I, 6.10 (CI 4.31, 8.64 (overall significance P<0.00001)] for phase II, 8.95 [CI 6.09, 13.16 (overall significance P<0.00001)] for phase III and 14.82 [CI 6.49, 33.82 (overall significance P<0.00001)] for phase IV (Figures 4A–D).

In addition, funnel plots of overall survival (Supplementary Figures 1A–C) and progression-free survival (Supplementary Figures 2A–D) were also analyzed to check the publication biases of the study. Apart from this, we have also analyzed the risk of bias through the Cochrane risk of Bias (RoB) tool in Review Manager software (version 5.3) and found a low risk of bias for eligible included studies (Supplementary Figure 3). Overall, the findings of the current study suggest that combination immunotherapies significantly enhance both overall survival and progression-free survival outcomes compared to single immunotherapy and better disease outcomes were observed.

4 Discussion

Combinatorial therapies have enabled healthcare professionals to address the limitations of traditional treatments by integrating multiple treatment modalities, such as chemotherapy, targeted therapies, immunotherapies, and radiation, in a coordinated manner for improved outcomes. Prior evidence has shown how

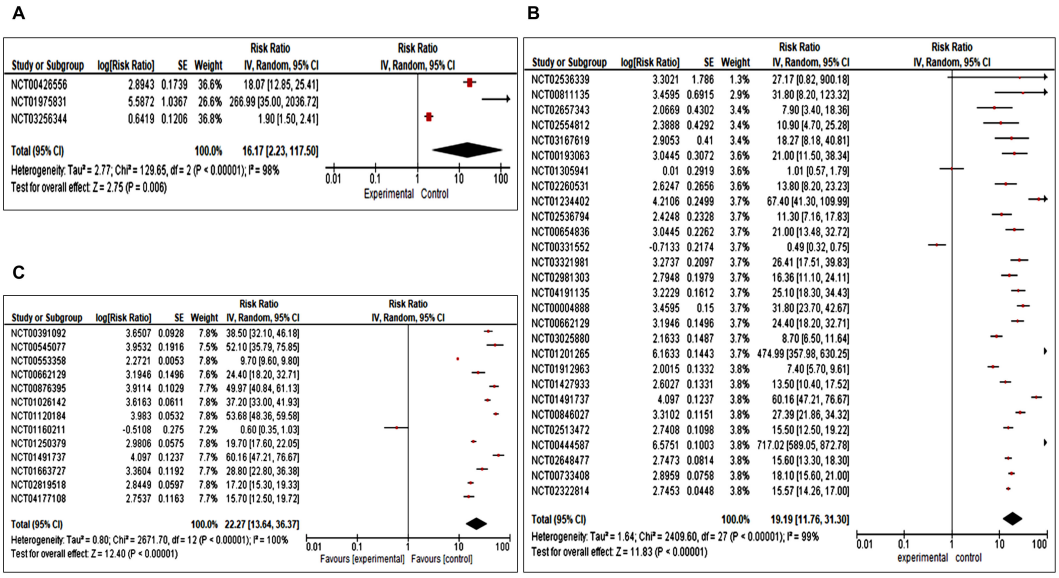

FIGURE 3
(A–C) Forest plot for a completed clinical trial comparing the effect of combination immunotherapies on overall survival **(A)** for phase I, **(B)** for phase II and **(C)** for phase III.

hypo-fractioned radiotherapy was utilized in conjunction with immunotherapy to induce cancer cell death (28). Additionally, Bashraheel et al. found that combining targeted therapies like immune checkpoint inhibitors (ICIs), ligand-targeted therapeutics (LTT) or tumor-targeted superantigens (TTS) have more profound effects in treating cancer (8). Further, several other studies have also explored the effect of trastuzumab deruxtecan in solid tumors (29). Pegram et al. (1999) observed that combining trastuzumab with cisplatin led to significantly higher response rates compared to each agent when used individually. Similarly, another study explored the impact of the combination of everolimus and endocrine therapy among postmenopausal women grappling with endocrine-resistant HR+, HER2− breast cancer. This combination showed notable enhancements in progression-free survival (PFS) and objective response rates, in comparison to endocrine therapy alone (30). Moreover, meta-analysis studies have determined the efficacy of PD-1/PD-L1 inhibitors in clinical trials, highlighting their potential as effective immunotherapeutic agents across various cancer types, drug combinations, stages of treatment, and therapeutic schedules (31).

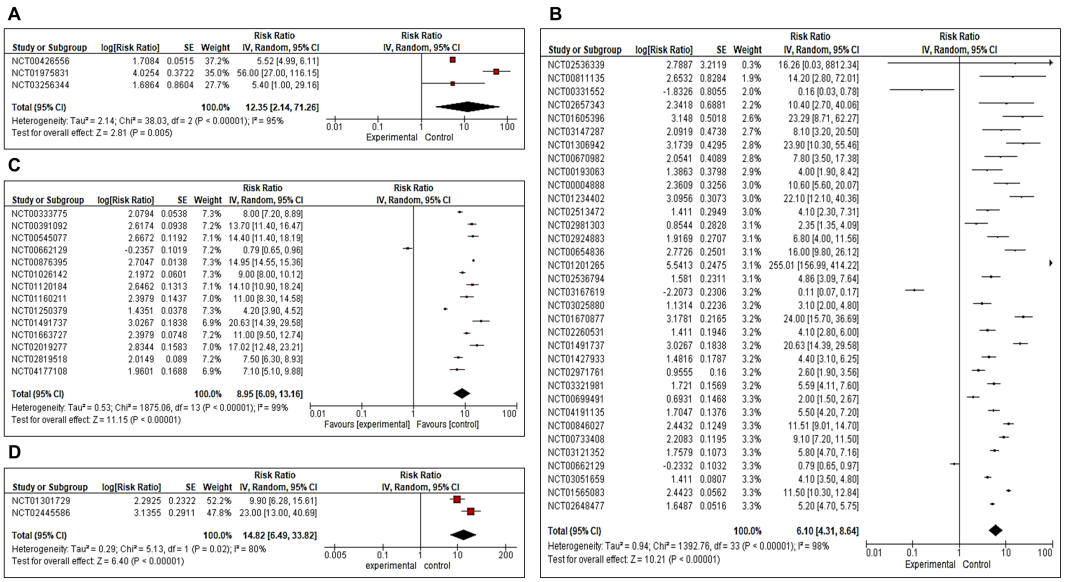

FIGURE 4
(A–D) Forest plot for a completed clinical trial comparing the effect of combination immunotherapies on progression-free survival **(A)** for phase II, **(B)** for phase II, **(C)** for phase III and **(D)** for Phase IV.

In order to evaluate the impact of combination immunotherapy vs single therapy, we performed a meta-analysis of the interventional studies with statistical data on survival outcomes in completed phase I/II/III/IV clinical trials in breast cancer. We focused on clinical trials that reported statistical interpretation of the trial in terms of Risk Ratio with 95% confidence intervals (CI) and observed that combination immunotherapies offered better overall survival (OS), and progression-free survival (PFS) outcomes to single immunotherapy. The studies were observed to be significant, with high heterogeneity in breast cancer ($p<0.005$) for OS and PFS. The strength of this study lies in the fact that it included only the completed phase I/II/III/IV clinical trials, providing a comprehensive assessment of the efficacy and specificity of the combination immunotherapies in breast cancer. This meta-analysis has provided us with evidence-based analysis of how combination immunotherapies are effective in overcoming the different challenges faced in cancer treatment, especially in breast cancer.

4.1 Limitations

Despite having 55 eligible studies for data analysis, there were limited number of studies in phase I and IV clinical trial and insufficient data for overall survival in phase IV. Additionally, data on various other survival outcome measures, such as recursion-free survival (RFS), time-to-time progression (TTP), and disease-free survival (DFS) was lacking. Further, randomized controlled trials will be necessary to validate these outcomes.

5 Conclusion and future prospects

Overall, our meta-analysis indicates that combinational immunotherapies involving two or more drugs or combining drugs with immune checkpoint inhibitors significantly increase overall survival (OS) and progression-free survival (PFS) in breast cancer as compared to single (one) immunotherapy. Notably, these findings provide valuable insights into the efficacy of combination immunotherapies, which can guide clinicians in making evidence-based decisions for improved breast cancer management. The future combination immunotherapies hold great potential, with numerous opportunities to enhance treatment efficacy, overcome drug resistance, and improve the quality of life in breast cancer patients particularly in complex and resistant cancer cases.

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

SS: Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing. VK: Data curation, Formal analysis, Writing – original draft. JR: Data curation, Formal analysis, Methodology, Writing – review & editing. NM: Supervision, Writing – review & editing. SK: Writing – review & editing. AK: Writing – review & editing. MA: Writing – review & editing. S: Writing – review & editing. PS: Writing – review & editing. EG: Writing – review & editing. PT: Writing – review & editing. SH: Conceptualization, Supervision, Writing – review & editing.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1469441/full#supplementary-material

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Effect of peripheral blood lymphocyte count on the efficacy of immunotherapy combined with TKI in the treatment of advanced liver cancer

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Background and aims: Compared with tyrosine kinase inhibitor (TKI) monotherapy, TKI combined with PD1 can improve the therapeutic effect of liver cancer and has been widely used in clinical practice. However, there is a lack of effective biomarkers to identify patients who would benefit more from this combination therapy. Therefore, this study aimed to evaluate whether baseline lymphocyte counts can identify patients with liver cancer who would benefit from targeted immune combination therapy.

Methods: Data from patients with hepatocellular carcinoma (HCC) who received TKIs or TKIs in combination with PD1 between June 2018 and June 2020 were retrospectively collected. The patients were divided into high and low groups based on the median absolute count of peripheral lymphocytes before systemic therapy and differences in overall survival (OS) and progression-free survival (PFS) between TKI and TKI+PD1 were compared between the two groups.

Results: In total, 72 patients were included in this study, with a median follow-up of 1.5 years. Both PFS and OS in the TKI+PD1 group showed a good prognostic trend ($p = 0.058$ and $p = 0.077$, respectively). Subgroup analyses based on peripheral blood lymphocyte counts showed that the combination regimen had a significant PFS and OS advantage only in patients with high peripheral blood lymphocyte counts ($p = 0.036$ and $p = 0.031$, respectively), but not in patients

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with low absolute peripheral blood lymphocyte counts (p = 0.819 and p = 0.913, respectively).

Conclusions: Peripheral blood lymphocyte count is a simple and effective biomarker that can be used to identify patients with liver cancer who will benefit more from TKI+PD-1 combination therapy.

KEYWORDS

hepatocellular carcinoma, TKI, PD1, peripheral blood lymphocyte count, combination therapy

Introduction

In recent years, new therapies such as targeted therapy with sorafenib/lenvatinib, and immunotherapy with PD-1/PD-L1 inhibitors have become the treatment of choice for liver cancer (1–3). Tumor vascular abnormalities lead to hypoxia and acidosis in the tumor microenvironment, which causes immunosuppression through a variety of mechanisms, and anti-angiogenic can normalize the blood vessels around the tumor and improve the microenvironment, thereby promoting the effect of immunotherapy (4, 5). Compared with the lower response rate of monotherapy, combination immunotherapy based on TKIs has shown promising efficacy in advanced liver cancer (6–9). For example, the Keynote524 studies showed that the objective response rate to lenvatinib in combination with PD1 reached 46% (8). However, many patients do not benefit from the combination regimen, which causes adverse reactions, such as hepatitis/pneumonia caused by immunotherapy, seriously reducing the patients' quality of life of patients and affecting subsequent antitumor therapy (1). Phase III clinical trial results showed that in the lenvatinib plus pembrolizumab group, 71 (18%) of 395 patients discontinued any study treatment because of treatment-related adverse events versus 42 (11%) of 395 patients in the lenvatinib plus placebo group. The treatment-related grade 3–4 adverse events were also higher in the combination group than alone lenvatinib (243 [62%] vs 224 [57%]) (7).

Considering the toxicity and increased treatment costs of the combination regimen, identifying which patients are more suitable for the two-drug combination is a clinically meaningful direction; however, current research in this area is very limited. Lymphopenia is associated with poor prognosis in multiple cancer types and can be used to predict the efficacy of tumor immunotherapy (10–12). Therefore, in this study, we aimed to investigate whether baseline lymphocyte counts could predict the probability of benefits from targeted immune combination therapy in patients with liver cancer.

Abbreviations: HCC, Hepatocellular carcinoma; TKI, tyrosine kinase inhibitor; KM, Kaplan–Meier; OS, overall survival; DFS, disease free survival; CI, confidence interval; HBV, hepatitis B virus.

Materials and methods

Patients

This retrospective study was conducted at Nanfang Hospital, Southern Medical University, and was approved by the Ethics Committee of Nanfang Hospital, Southern Medical University. We retrospectively analyzed patients with continuous liver cancer who received TKIs alone or TKIs in combination with PD1 at our hospital between June 2018 and June 2020. We included patients who met the following criteria: 1) age > 18 years; 2) diagnosis of liver cancer by clinical or pathological examination; 3) adequate recording of baseline blood routine tests; 4) PS score 0–2 points; and 5) the first systemic therapy was targeted therapy with lenvatinib or sorafenib, alone or in combination with PD-1 antibody. Patients were excluded if they had 1) history of organ transplantation, 2) immunodeficiency diseases, 3) incomplete medical data or loss to follow-up, 4) prior treatment with other systems, and 5) were administered immune checkpoints for second-and multiline therapy.

Systemic treatment

All patients provided written informed consent before undergoing systemic therapy. Sorafenib orally 400 mg 1/day. Lenvatinib 8 mg orally or at 12 mg 1/day. PD-1 inhibitor alone, camrelizumab (200 mg), toripalimab (240 mg), sintilimab(200 mg) or pembrolizumab(200 mg)once every 3 weeks as an intravenous infusion or nivolumab (3 mg/kg every 2 weeks). The reduction or discontinuation of treatment was determined by the clinician, depending on the disease status and adverse effects.

Data collection

Patient baseline characteristics, such as age, sex, ECOG Score and AFP, were obtained from their electronic medical records. Hematological parameters for all patients were concentrated in the 1 week before the first systemic therapy. Evaluation of patient efficacy was

based on imaging information using the mRESIST criterion, and patient survival information was collected via telephonic follow-up. OS was defined as the time from the first administration of systemic therapy to the patient's death or loss to follow-up. PFS was defined as the time from the first administration of systemic therapy to tumor progression.

Statistical analysis

Categorical or continuous variables were compared between groups using the chi-square test or t-test. Kaplan–Meier analysis was used for OS and PFS, and the log-rank test was used for comparisons between groups. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS software.

Results

Patient characteristics

In total, 72 patients were included in this study; their baseline characteristics are shown in Table 1. Most patients had hepatitis B virus (HBV) infection, BCLC stage C, Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, and Child–Pugh class A. A total of 36 (50.0%) patients received concomitant local therapy, including TACE/HAIC/radiotherapy/radiofrequency ablation. Among the 72 patients, 29 received targeted immune combination therapy as first-line treatment, whereas 43 received targeted therapy alone. A higher proportion of patients in the TKI+PD-1 group received lenvatinib than those in the TKI group (37.9% vs. 14.0%, p = 0.019).

Overall survival and progression-free survival

The median follow-up period was 1.5 years. By the end of follow-up time, 46 of the 72 patients had a death outcome and 64 had disease progression. Compared to the TKI treatment group, the PFS and OS of the TKI+PD1 group showed a better prognostic trend. The median PFS (mPFS) was longer in the TKI+PD1 group (3.5 months, 95%CI 1.5–5.5) than in the TKI group (2.7 months, 95%CI 2.2–3.2) [p = 0.058; Figure 1A]. The median OS was 10.2 months, (95%CI 5.7–14.6) in the TKI group and 19.9 months, (95% CI 7.3–32.5) in the TKI+PD1 group [p = 0.077; Figure 1B].

Survival analysis by absolute peripheral blood lymphocyte count

Stratified analysis was performed based on the absolute peripheral blood lymphocyte count before systemic therapy.

TABLE 1 Baseline characteristics of the patients.

Variables	All patients (n = 70)	TKI alone	TKI+PD1	P value
Age (yrs)	52.9 ± 12.1	54.2 ± 11.2	51.0 ± 13.4	0.283
Gender				
Male	66 (91.7%)	40 (93.0%)	26 (89.7%)	0.612
Female	6 (8.3%)	3 (7.0%)	3 (10.3%)	
Pathogeny				
HBV-related	69 (97.8%)	41 (95.3%)	28 (96.6%)	0.802
Others	3 (2.2%)	2 (4.7%)	1 (3.4%)	
ECOG Score				
0-1	49 (68.1%)	28 (65.1%)	21 (72.4%)	0.137
>1	23 (31.9%)	23 (34.9%)	8 (27.6%)	
Child-Pugh class				
A	45 (62.5%)	26 (60.5%)	19 (65.5%)	0.664
B	27 (37.5%)	17 (39.5%)	10 (34.5%)	
AFP (ng/mL)				
< 200	22 (30.6%)	14 (32.6%)	8 (27.6%)	0.653
≥ 200	50 (69.4%)	29 (67.4%)	21 (72.4%)	
BCLC stage				
B	3 (2.1%)	2 (4.7%)	1 (3.4%)	0.802
C	69 (97.8%)	41 (95.3%)	28 (96.6%)	

(Continued)

TABLE 1 Continued

Variables	All patients (n = 70)	TKI alone	TKI+PD1	P value
Types of TKIs				
Sorafanib	48 (66.7%)	37 (86.0%)	18 (62.1%)	0.019
Lenvatinib	24 (33.3%)	6 (14.0%)	11 (37.9%)	
MVT				
Yes	54 (75.0%)	32 (74.4%)	22 (75.9%)	0.89
No	18 (25.0%)	11 (25.6%)	7 (24.1%)	
EM				
Yes	38 (52.8%)	22 (51.2%)	16 (55.2%)	0.738
No	34 (47.2%)	21 (48.8%)	13 (44.8%)	
Local therapy				
Yes	36 (50.0%)	23 (53.5%)	13 (44.8%)	0.471
No	36 (50.0%)	20 (46.5%)	16 (55.2%)	

AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; EM, extrahepatic metastases; HBV, hepatitis B virus; MVT, macrovascular tumor thrombosis; TKIs, tyrosine kinase inhibitors.

Patients were divided into high- and low-L groups based on the median absolute count of peripheral blood lymphocytes. In the high-L group, patients in the TKI+PD1 group showed longer mPFS compared with those who received TKIs [3.5 months, (95%CI 0.1–14.1) versus 2.9 months (95%CI 1.6–4.2), p = 0.036], and mOS [22.9 months, (95%CI 1.4–44.5) versus 7.9 months, (95%CI 0.1–16.0) p = 0.031; Figures 2A, B].

No significant difference was found in mPFS [2.6 months, (95% CI 2.3–3.0) versus 3.2 months (95%CI 1.7–4.7), p = 0.819], and mOS [11.9 months, (95%CI 5.9–18.1) versus 15.3 months (95%CI 7.6–23.1), p = 0.913; Figures 2C, D] between TKI and TKI+PD1 use in the low-L group.

Moreover, we also used the lower limit of the normal value of lymphocytes to distinguish between people with high and low lymphocytes, and we found the same phenomenon (Additional File 1: Supplementary Figure S1).

Discussion

To date, there are no effective biomarkers to screen patients with cancer to identify those who are more suitable for TKI+PD1 rather than single-agent TKI use. In this study, we assessed whether peripheral blood lymphocyte count could be used as a prognostic marker for the combination regimen and found that patients with low lymphocyte counts did not receive additional benefit from the combination regimen compared with single-agent targeting. Thus, our results suggest that peripheral blood lymphocyte count can be used as a biomarker to identify patients with liver cancer who will benefit from TKI+PD-1 combination therapy.

The treatment of advanced liver cancer remains challenging, with molecularly targeted therapies such as sorafenib and lenvatinib having low response rates. Consequently, combination immunotherapy such as PD-1/PD-L1 monoclonal antibodies has

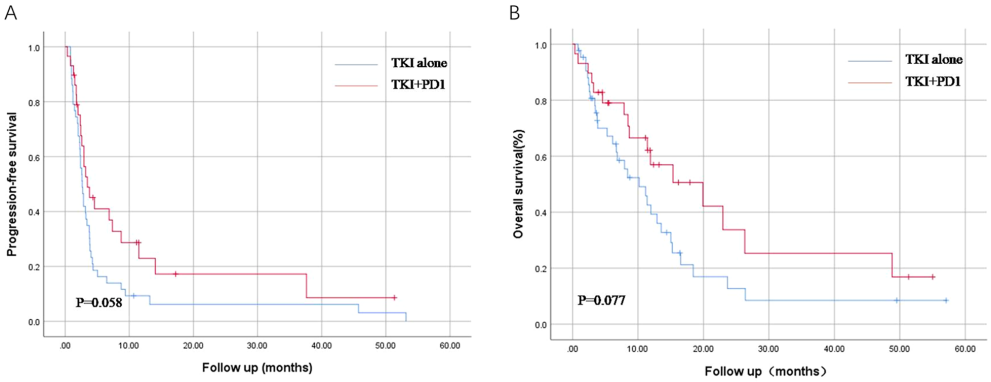

FIGURE 1
Kaplan–Meier curves for progression-free survival **(A)** and overall survival **(B)** in TKI alone and TKI+PD-1 group. TKIs, tyrosine Kinase Inhibitors. All statistical tests were two-sided.

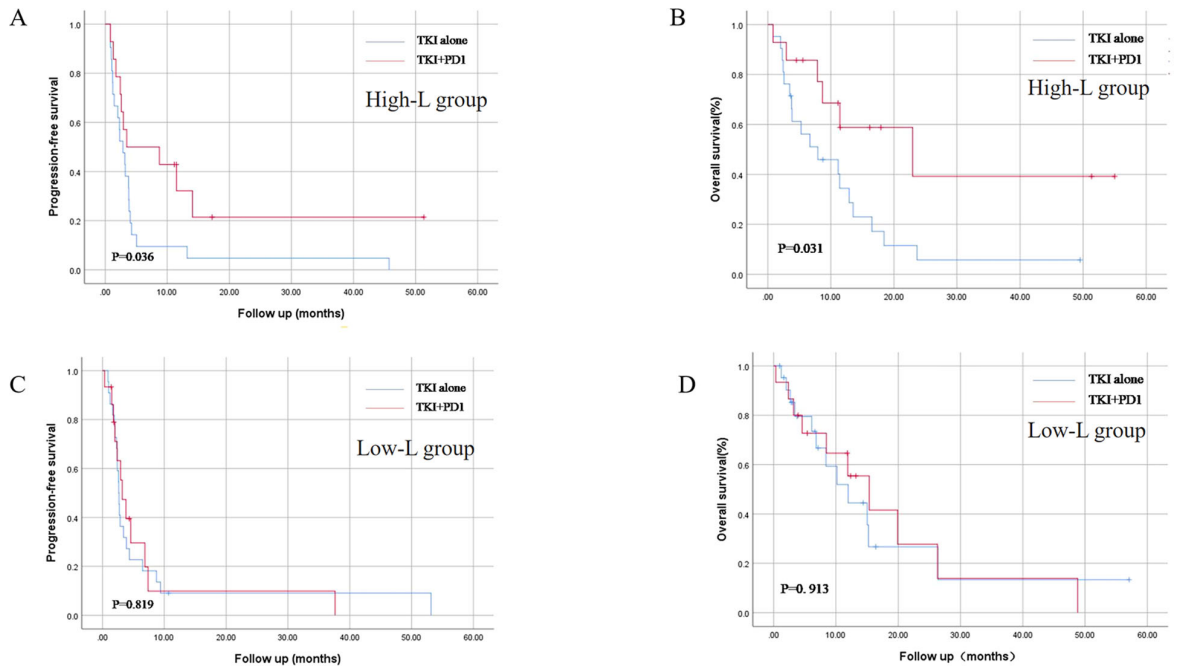

FIGURE 2
Kaplan–Meier curves for progression-free survival **(A, C)** and overall survival **(B, D)** in TKI alone and TKI+PD-1 group after stratification by peripheral blood lymphocyte count. TKIs, tyrosine Kinase Inhibitors. All statistical tests were two-sided.

become a trend in the treatment of liver cancer (1). Target-immune therapy has a higher objective response rate than single-agent targeting (6–9). Similarly, our data showed that targeted combined immunization can prolong overall patient survival, which supports the advantages of combination therapy. However, a significant proportion of patients do not benefit from additional combination therapy, and the overall toxicity of combination regimens is high. These issues have prompted clinicians to make granular treatment decisions.

Our study found that TKI+PD1did not improve the prognosis of patients with peripheral blood lymphocytopenia. Compared with the difficulty and heterogeneity of tissue biopsy, peripheral blood lymphocytes are a simple clinical test index, based only on a simple routine blood test. This can help roughly determine which patients do not require targeted immunotherapy drugs, especially considering the toxicity and cost of combination therapy.

Nonetheless, the mechanism of peripheral blood lymphopenia in targeted immune combination therapy remains unclear. It is speculated that the mechanism may be related to the key role of lymphocytes in tumor immunity. A low peripheral blood lymphocyte count suggests a preexisting immunosuppressive state, resulting in an inadequate tumor immune response (12–14).

In contrast, patients with advanced liver cancer in the context of hepatitis B often have cirrhosis, which contributes to the development of hypersplenism, which often manifests as a decrease in the number of peripheral blood cells, including peripheral blood lymphocytes (15). The results of our association analysis showed that patients with low peripheral blood lymphocytes are often accompanied by a decrease in platelets and leukocytes. Some studies have suggested that the cellular immune function of patients with hypersplenism is severely impaired (15, 16), which may be a possible reason why peripheral lymphocytes can predict target-immune combination therapy; further experiments are needed to verify this.

Our study had the following limitations. First, this was a single-center retrospective study and the small sample size limited further subgroup analyses. Moreover, patient data was mainly based on electronic medical records and telephone follow-up, and patients who were lost to follow-up may experience a certain degree of bias. Second, the study included a combination of systemic regimens, including lenvatinib and sorafenib, which may differ in prognostic outcomes depending on the choice of the drug. In addition, some patients received concomitant local therapies. These local treatments may have a certain impact on the interpretation of the results. Based on your suggestions, we further analyzed the situation of local treatments received during the same period in different groups and found that there was no statistical difference between the subgroups in whether or not local treatment was received, which weakened the impact of this factor to a certain extent (Additional File 2: Supplementary Table S1).

In conclusion, our study revealed that peripheral blood lymphocyte count is an objective and simple indicator to identify which patients with advanced HCC should receive TKI+PD1 as a first-line systemic therapy rather than TKI alone. Properly designed prospective studies are needed to further explore these interesting findings.

Data availability statement

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

Ethics statement

This study was approved by the Ethics Committees of Nanfang Hospital, Southern Medical University. The requirement for individual informed consent was waived by the committees.

Author contributions

QZ: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. LW: Data curation, Writing – review & editing. HF: Validation, Writing – original draft. YZ: Funding acquisition, Supervision, Writing – review & editing. QX: Funding acquisition, Supervision, Writing – review & editing, Conceptualization.

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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/fimmu.2024.1467429/full#supplementary-material

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Identification of beneficial populations for targeted-immunotherapy combinations: tailoring later-line care for patients with pMMR/MSS metastatic colorectal cancer

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Objective: This study explores the benefits of targeted-immunotherapy combination in third-line or beyond treatment for microsatellite stable (MSS) metastatic colorectal cancer (mCRC) in a real-world setting.

Methods: Patients with MSS mCRC who were treated with either a targeted-immunotherapy combination or targeted therapy alone in the third-line or beyond setting at our hospital from August 2018 to August 2022 were included in the study. Inclusion criteria comprised patients treated with targeted therapy alone or in combination with immunotherapy. Effectiveness was compared between treatments, and patients with the potential to benefit from targeted-immunotherapy combination were identified.

Results: Among 71 patients, 31 received targeted therapies alone (TT group) and 40 received a combination of targeted therapy and immunotherapy (TI group). The TI group had higher objective response rates (20% vs 3.2%) and disease control rates (82.5% vs 58.1%). The median progression-free survival was significantly better in the TI group (4.6 vs 4.1 months, P = 0.027). Liver metastasis was associated with poor prognosis, while patients with only lung metastases had the longest median progression-free survival of 12.3 months with combination therapy.

Conclusion: The study indicates that targeted-immunotherapy combination offers more benefits than targeted therapy alone for MSS mCRC in the third-line or beyond setting.

KEYWORDS

microsatellite stable metastatic colorectal cancer, third-line or beyond, real-world, targeted-immunotherapy combination, beneficial population

1 Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths worldwide (1). The incidence of CRC in China, although historically significantly lower than in Western countries, has increased rapidly in recent years, making it the most common malignant tumor of the digestive system. According to the latest 2022 data on the cancer burden in China, CRC ranks second in incidence and fourth in mortality in the country (2). For metastatic CRC (mCRC), treatment options are limited after progression following standard front-line treatments, resulting in limited survival benefits (3–6). Further, in contrast to front-line therapy, the main goals of third-line or beyond treatment for this population are to control tumor progression, prolong survival, and improve quality of life (7).

In recent years, immunotherapy has emerged as a promising new approach for treating mCRC, especially for tumors with high microsatellite instability (MSI-H), including as a third-line treatment for MSI-H mCRC (8–10). However, MSI-H tumors account for only about 5% of cases (11), while the remaining 95% are microsatellite stable (MSS) CRCs. MSI-H tumors are characterized by a high mutational burden, which increases the presentation of neoantigens and enhances the infiltration of immune cells, leading to an "inflamed" microenvironment. This feature makes MSI-H tumors more responsive to immune checkpoint inhibitors. In contrast, MSS tumors typically exhibit a "cold" immune microenvironment, with a low mutational burden and minimal immunity, rendering them representative "cold tumors" (12). Immunotherapy appears to be ineffective against MSS tumors, with many exploratory studies having failed (13–15).

Given the synergistic effects of immunotherapy with anti-angiogenic therapy, several studies have evaluated the addition of programmed cell death protein 1 (PD-1) inhibitors to the standard anti-angiogenic monotherapy in patients with MSS mCRC. The phase Ib REGONIVO study evaluating nivolumab combined with regorafenib as third-line or beyond treatment enrolled 25 patients in the CRC cohort, with 24 patients having MSS tumors, and showed the encouraging anti-tumor activity. Among the 25 patients, the objective response rate (ORR) was 36% (with an ORR of 33% in MSS patients), the median progression-free survival (PFS) was 7.9 months, and the median overall survival (OS) was not reached (16). On the contrary, the phase II REGOMUNE trial combining avelumab with regorafenib, patients achieved only stable disease as the best response (17). The inconsistent data indicated that only a small fraction of patients might benefit from targeted-immunotherapy combination. It is important to note that studies exploring combination therapies were all single-arm designs, and little is known about comparisons of targeted-immunotherapy combination with standard targeted monotherapy in this patient population. Also, the effectiveness of this combination therapy in routine clinical practice remains uncertain. Here, we designed this retrospective study to compare the effectiveness of targeted-immunotherapy combination with targeted therapy alone in the third-line or beyond setting for MSS mCRC patients and to identify the potential beneficial population of combined targeted-immunotherapy.

2 Materials and methods

2.1 Patient population

Data on MSS mCRC patients who received third-line or beyond treatment at the Fourth Hospital of Hebei Medical University between August 2018 to August 2023 were retrospectively collected by reviewing electronic medical records. Patients with MSS mCRC who were treated with targeted therapy alone or in combination with immunotherapy as third-line or beyond therapy were included. Immunohistochemistry (IHC) staining of four kinds of MMR protein (MLH1, MSH2, MSH6, PMS2) or polymerase chain reaction (PCR) analysis of five microsatellite markers (BAT25, BAT26, D5S346, D2S123, D17S250) were used to determine MSS status of colorectal cancer patients. Patients diagnosed with MSI-H/dMMR status were excluded from the study. The demographic data, clinicopathological information, treatment records, imaging examination results, and survival outcomes were collected in detail from electronic medical records. This study was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (approval number: 20230926) and was performed in accordance with the Declaration of Helsinki. This article is a retrospective study and has obtained ethical exemption.

2.2 Clinical data

The start date of third-line treatment was defined as the start date. The follow-up period was defined as the time from the date of initiation of third-line or beyond treatment until the data cut-off date (February 29, 2024), the last outpatient visit, or death. Baseline clinical characteristics were assessed either before or at the start of third-line or beyond treatment. After treatment, all patients underwent imaging examinations every two cycles (6 weeks) to evaluate clinical efficacy as per the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. The ORR was defined as the proportion of patients whose best response was either complete response (CR) or partial response (PR). Disease control rate (DCR) was defined as the proportion of patients who achieved CR, PR, or stable disease (SD). PFS was defined as the time from the start of third-line or beyond treatment to the first recorded disease progression or death, whichever occurred first. OS was defined as the time from the start of third-line or beyond treatment to death from any cause.

2.3 Statistical analysis

All statistical analyses in this study were conducted using IBM SPSS Statistics version 27.0 (New York, USA). Categorical variables

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were summarized as number (percentage) and compared using the chi-squared test or Fisher's exact test. Continuous variables were described with median and range. The OS and PFS were analyzed using the Kaplan-Meier method, and comparisons were made using the log-rank test. Additionally, univariate and multivariate Cox proportional hazards regression models were used to analyze potential risk characteristics. Hazard ratios (HRs) and the 95% confidence intervals (CIs) were estimated to quantify the strength of these associations. A p-value of <0.05 was considered statistically significant, and all tests were two-tailed.

3 Results

3.1 Baseline patient and clinical characteristics

Among the 71 patients included, 31 (43.7%) patients received targeted therapy (TT group) while 40 (56.3%) patients were treated with a combination of targeted therapy and immunotherapy (TI group) (Table 1). Both groups had a similar median age of 57 years, and the overall gender distribution showed more males (57.7%)

TABLE 1 Baseline characteristics of patients.

Characteristics	Total (*n* = 71)	TT group (*n* = 31)	TI group (*n* = 40)	*P* value
Age, years				0.912
< 60, *n* (%)	43 (60.6)	19 (61.3)	24 (60.0)	
≥ 60, *n* (%)	28 (39.4)	12 (38.7)	16 (40.0)	
Median	57	57	57.5	
Range	29-77	29-72	34-77	
Gender, *n* (%)				0.047
Male	41 (57.7)	22 (71.0)	19 (47.5)	
Female	30 (42.3)	9 (29.0)	21 (52.5)	
ECOG PS, *n* (%)				
0-1	62 (87.3)	26 (83.9)	36 (90.0)	0.127
2	9 (12.7)	5 (16.1)	4 (10.0)	
Primary tumor site, *n* (%)				0.265
Right colon	10 (14.1)	6 (19.4)	4 (10.0)	
center colon	25 (35.2)	8 (25.8)	17 (42.5)	
Rectum	36 (50.7)	17 (54.8)	19 (47.5)	
Stage at initial diagnosis, *n* (%)				0.686
Initial diagnosis of stage IV	34 (47.9)	14 (45.2)	20 (50.0)	
Postoperative recurrence	37 (52.1)	17 (54.8)	20 (50.0)	
Number of metastatic sites, *n* (%)				0.530
Single	18 (25.4)	9 (29.0)	9 (22.5)	
Multiple (≥ 2)	53 (74.6)	22 (71.0)	31 (77.5)	
Site of metastases, *n* (%)				
Lymph node	34 (47.9)	15 (48.4)	19 (47.5)	0.941
Liver	38 (53.5)	18 (58.1)	20 (50.0)	0.499
Lung	46 (64.8)	22 (71.0)	24 (60.0)	0.337
Bone	6 (8.5)	2 (6.5)	4 (10.0)	0.918
Peritoneum	17 (23.9)	5 (16.1)	12 (30.0)	0.174
RAS mutation status, *n* (%)				0.909
KRAS, NRAS all wild type	30 (42.3)	14 (45.2)	16	
KRAS or NRAS mutant	29 (40.8)	12 (38.7)	17 (42.5)	

(Continued)

TABLE 1 Continued

Characteristics	Total (*n* = 71)	TT group (*n* = 31)	TI group (*n* = 40)	*P* value
Unknown	12 (16.9)	5 (16.1)	7	
BRAF mutation status, *n* (%)				0.493
$BRAF^{V600E}$ wild type	45 (63.4)	21 (67.7)	24 (60.0)	
$BRAF^{V600E}$ mutant	5 (7.0)	1 (3.2)	4 (10.0)	
Unknown	21 (29.6)	9 (29.3)	12 (30.0)	

ECOG PS, Eastern Cooperative Oncology Group performance status; pMMR, mismatch repair proficient; dMMR, mismatch repair deficiency; MSI-H, high microsatellite instability; MSS, microsatellite stable; TT group, targeted therapy group; TI group, targeted-immunotherapy combination group.

than females (42.3%), with a notably higher percentage of males in the TT group (71.0%) than in the TI group (47.5%). Overall, most patients (87.3%) had an ECOG performance status of 0 or 1. The primary tumor site was distributed predominantly in the rectum (50.7%), followed by the left hemi-colon (35.2%) and the right hemi-colon (14.1%). Multiple metastatic sites were common (74.6%), with lung (64.8%), liver (53.5%), and lymph nodes (47.9%) being predominant. Regarding genetic mutations, KRAS or NRAS mutations were found in 40.8% of patients, and BRAF V600E mutations in 7%. There were no statistically significant differences between the TT and TI groups regarding baseline characteristics other than age.

As shown in Table 2, 25 (86.4%) and 31 (77.5%) patients in the TT and TI groups, respectively, received third-line treatment. In the third-line or beyond setting, regorafenib was the most commonly used targeted agent (64.5% in the TT group and 75.0% in the TT group), while in terms of immunotherapy in the TI group, camrelizumab was the dominant agent (65.0%).

3.2 Efficacy

A total of 71 patients were assessable for response. As shown in Table 3, there was a noticeable difference in the response to third-line or beyond treatment between the TT group and the TI group in patients with MSS mCRC. The ORR and DCR in the TI group were significantly higher than those in the TT group, with 20.0% vs 3.2% (odds ratio [OR] = 0.080, 95% CI: 0.023-0.275, $P = 0.000$) and 82.5% vs 58.1% (OR = 0.024, 95% CI: 0.008-0.074, $P = 0.000$), respectively. These findings suggest that the addition of immunotherapy to targeted therapy may improve the control of the disease in this patient population. For all the 71 patients regardless of treatment, the overall median PFS was 4.4 months (95% CI: 1.3-36.2) and the median OS was 13.8 months (95% CI: 1.6-38.8). Further, the median PFS was 4.1 months (95% CI: 2.7-5.5) in the TT group, while in the TI group, the corresponding value was 4.6 months (95% CI: 3.2-6.0), with a statistically significant difference between the two groups (HR = 0.561, 95% CI: 0.34-0.94, $P = 0.027$; Figure 1A). This demonstrates that in third-line or beyond setting, the combination of targeted therapy and immunotherapy may provide a longer PFS compared to monotherapy with targeted agents. In terms of OS, an improved trend was observed in the TI group as compared to that in the TT group (15.8 months [95% CI: 7.3-24.3] vs 13.2 months [95% CI: 9.9-16.4]), although with no statistically significant difference between the two groups (HR = 0.671, 95% CI: 0.37-1.21, $P = 0.189$; Figure 1B).

Cox proportional hazards univariate analysis showed that apart from the number of metastatic sites, lymph node metastasis and liver metastasis, none of the other factors showed a significant association with PFS (Table 4); similarly, lymph node involvement and liver metastasis were also significantly associated with OS (Table 5). Multivariate analysis further identified liver metastasis as an independent prognostic factor for both PFS (HR = 0.407, 95% CI: 0.217-0.761, $P = 0.005$) and OS (HR = 0.386, 95% CI: 0.179-0.832, $P = 0.015$).

TABLE 2 Prior systemic treatment regimens.

Treatment regimens	TT group (*n* = 31)	TI group (*n* = 40)	*P*
First line, *n* (%)			0.697
Chemotherapy	8(25.8)	12(30.0)	
Chemotherapy-targeted combination	23(74.2)	28(70.0)	
Second line, *n* (%)			0.146
Chemotherapy	7(22.6)	4(10.0)	
Chemotherapy-targeted combination	24(77.4)	36(90.0)	
Third-line or beyond, *n* (%)			0.747
Third-line therapy	25(80.6)	31(77.5)	
Beyond third-line therapy	6(19.4)	9(22.5)	
Targeted drugs for third-line or beyond, *n* (%)			0.337
Regorafenib	20(64.5)	30(75.0)	
Fruquintinib	11(35.5)	10(25.0)	
Immune checkpoint inhibitors for third-line or beyond, *n* (%)			–
Camrelizumab	–	26(65.0)	
Tislelizumab	–	5(12.5)	
Sintilimab	–	4(10.0)	
Others	–	5(12.5)	

TT group, targeted therapy group; TI group, targeted-immunotherapy combination group.

TABLE 3 Tumor response.

Tumor response, *n* (%)	Total (*n* = 71)	TT group (*n* = 31)	TI group (*n* = 40)	OR	95% CI		*P* value
					Lower	Upper	
CR	0 (0)	0 (0)	0 (0)				
PR	9 (12.7)	1 (3.2)	8 (20.0)				
SD	40 (56.3)	17 (54.8)	23 (57.5)				
PD	22 (31.0)	13 (41.9)	9 (22.5)				
ORR	9 (12.7)	1 (3.2)	8 (20.0)	0.080	0.023	0.275	0.000
DCR	51 (71.8)	18 (58.1)	33 (82.5)	0.024	0.008	0.074	0.000

CI, confidence interval; CR, complete response; DCR, disease control rate; EGFR, epidermal growth factor receptor; OR, odds ratio; ORR, overall response rate; PD, disease progression; PR, partial response; SD, stable disease; TT group, targeted therapy group; TI group, targeted-immunotherapy combination group.

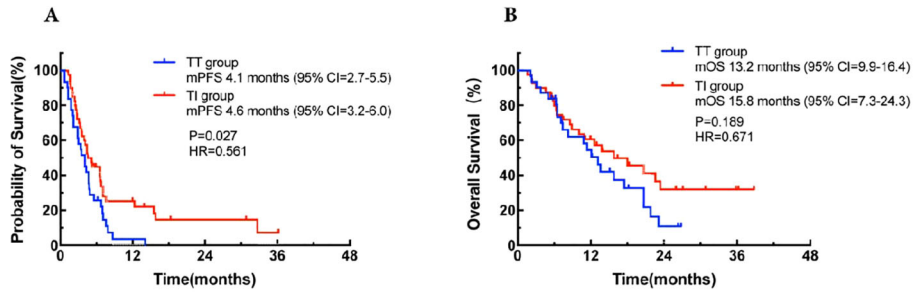

FIGURE 1
Kaplan-Meier curves for progression-free survival **(A)** and overall survival **(B)** in the TT group and the TI group and the TI group. CI, confidence interval; HR, hazard ration; mOS, median overall survival; mPFS, median progression-free survival; TT group, targeted therapy group; TI group, targeted-immunotherapy combination group.

TABLE 4 Univariate and multivariate analysis of factors predicting PFS.

Variable	Univariate analysis				Multivariate analysis			
	HR	95% CI		*P*	HR	95% CI		*P*
		Lower	Upper			Lower	Upper	
Age, years								
≥ 60 vs < 60	0.865	0.519	1.441	0.577				
Gender								
Female vs male	0.614	0.355	1.060	0.080	1.333	0.763	2.327	0.313
Primary tumor site								
center colon vs right colon	0.854	0.378	1.927	0.704				
Rectum vs right colon	0.888	0.407	1.936	0.765				
Stage at initial diagnosis								
Postoperative recurrence vs initial diagnosis of stage IV	0.803	0.486	1.326	0.391				
Number of metastatic sites								
Single vs multiple (≥ 2)	0.546	0.299	0.994	**0.048***	0.886	0.403	1.948	0.764

(Continued)

TABLE 4 Continued

Variable	Univariate analysis				Multivariate analysis			
	HR	95% CI		*P*	HR	95% CI		*P*
		Lower	Upper			Lower	Upper	
Lymph node metastasis								
No metastasis vs metastasis	0.569	0.340	0.950	**0.031***	0.638	0.356	1.142	0.130
Liver metastasis								
No metastasis vs metastasis	0.378	0.231	0.649	**0.000***	0.407	0.217	0.761	**0.005***
Lung metastasis								
Metastasis vs no metastasis	0.739	0.442	1.235	0.249				
Bone metastasis								
Metastasis vs no metastasis	0.964	0.409	2.272	0.934				
Peritoneum metastasis								
No metastasis vs metastasis	0.941	0.531	1.668	0.836				
RAS status								
Mutant vs wild	0.827	0.478	1.431	0.497				
Unknown vs wild	0.967	0.477	1.961	0.926				
BRAF status								
Mutant vs wild	0.651	0.230	1.843	0.419				
Unknown vs wild	0.757	0.433	1.325	0.330				

CI, confidence interval; HR, hazard ratio; PFS, progression-free survival; TT group, targeted therapy group; TI group, targeted-immunotherapy combination group. * indicates statistical significance.

TABLE 5 Univariate and multivariate analysis of factors predicting OS.

Variable	Univariate analysis				Multivariate analysis			
	HR	95% CI		*P*	HR	95% CI		*P*
		Lower	Upper			Lower	Upper	
Age, years								
≥ 60 vs < 60	0.818	0.445	1.505	0.519				
Gender								
Female vs male	0.563	0.299	1.059	0.075	0.536	0.276	1.039	0.065
Primary tumor site								
center colon vs right colon	0.704	0.273	1.813	0.467				
Rectum vs right colon	0.697	0.280	1.738	0.439				
Stage at initial diagnosis								
Postoperative recurrence vs initial diagnosis of stage IV	0.819	0.449	1.495	0.516				
Number of metastatic sites								
Single vs multiple (≥ 2)	0.469	0.217	1.012	0.054	1.121	0.408	3.079	0.824

(Continued)

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TABLE 5 Continued

Variable	Univariate analysis				Multivariate analysis			
	HR	95% CI		*P*	HR	95% CI		*P*
		Lower	Upper			Lower	Upper	
Lymph node metastasis								
No metastasis vs metastasis	0.435	0.235	0.807	**0.008***	0.563	0.262	1.207	0.140
Liver metastasis								
No metastasis vs metastasis	0.410	0.221	0.763	**0.005***	0.386	0.179	0.832	**0.015***
Lung metastasis								
Metastasis vs no metastasis	0.595	0.327	1.084	0.090	0.722	0.325	1.602	0.423
Bone metastasis								
No metastasis vs metastasis	0.965	0.345	2.701	0.946				
Peritoneum metastasis								
No metastasis vs metastasis	0.559	0.295	1.059	0.074	0.555	0.251	1.230	0.147
RAS status								
Mutant vs wild	1.074	0.568	2.031	0.826				
Unknown vs wild	0.658	0.261	1.655	0.373				
BRAF status								
Mutant vs wild	0.612	0.146	2.569	0.502				
Unknown vs wild	0.839	0.435	1.619	0.601				

CI, confidence interval; HR, hazard ratio; OS, overall survival; TT group, targeted therapy group; TI group, targeted-immunotherapy combination group. * indicates statistical significance.

Based on the risk factor liver metastasis, patients were stratified into liver metastasis group (n = 38) and non-liver metastasis group (n = 33) for further analysis. As shown in Figure 2A, patients in the non-liver metastasis group had a median PFS of 7 months (95% CI: 6.6-7.4), significantly better than the 3.2 months (95% CI: 2.0-4.2) observed in the liver metastasis group, with a statistically significant difference between the groups (HR = 0.39, 95% CI: 0.23-0.65, *P* = 0.0002). Similarly, a significant improved OS was observed in the non-liver metastasis group as compared to that in the liver metastasis group (median 20.7 months vs 10.8 months, HR = 0.41, 95% CI: 0.22-0.76, *P* = 0.005; Figure 2B).

Further stratified analysis of PFS and OS was performed in patients with and without liver metastases according to different treatment modalities. Among the 38 patients without liver metastases, the median PFS in the TI group (n = 20) was significantly superior to that in the TT group (n = 13) (7.1 months vs 5.6 months, HR = 0.42, 95% CI: 0.18-0.97, *P* = 0.034; Figure 3A), and an improvement trend in OS was observed in the TI

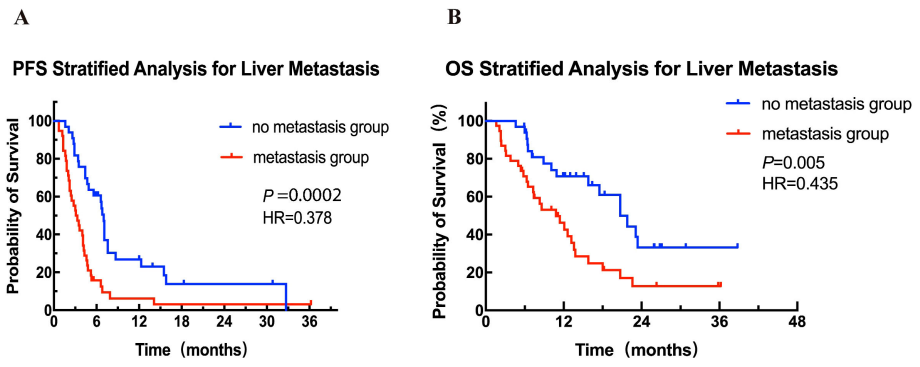

FIGURE 2
Kaplan-Meier curves for PFS **(A)** and OS **(B)** for patients with and without liver metastasis. HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

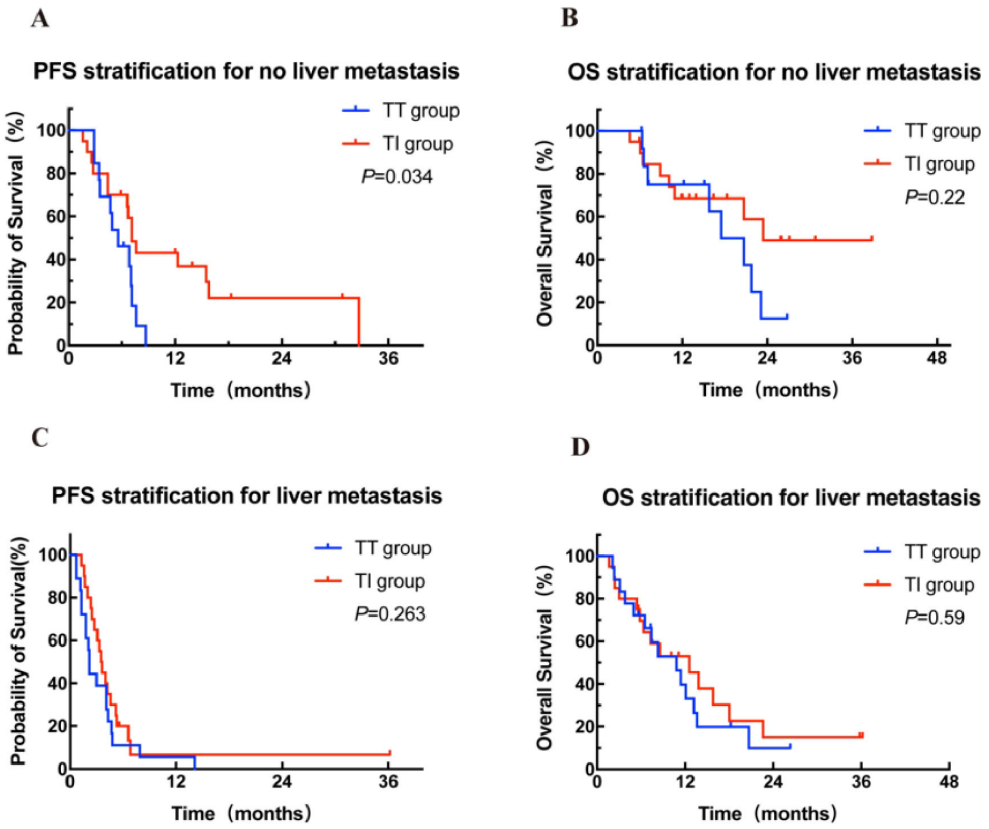

FIGURE 3
Kaplan-Meier curves for PFS **(A, C)** stratified by treatment modalities for patients with and without liver metastasis. Kaplan-Meier curves for OS **(B, D)** stratified by treatment modalities for patients with and without liver metastasis. OS, overall survival; PFS, progression-free survival; TT group, targeted therapy group; TI group, targeted-immunotherapy combination group.

group compared to the TT group (23.4 months vs 17.5 months, P = 0.22; Figure 3B). In contrast, in patients with liver metastases, there was no significant difference in either PFS or OS between the two treatment groups (Figures 3C, D).

In the 23 patients with only lung metastasis, there was a significant difference in PFS between the TT group (n = 10) and the TI group (n = 13) (4.7 months vs 12.3months, HR = 0.20, 95% CI: 9.8-25.3, P = 0.0013; Figure 4A). Patients in the TT group had a worse OS of 16.5 months compared to 31.1 months in the TI group (HR = 0.27, 95% CI: 11.8-21.2, P = 0.038; Figure 4B). Patients with only lung metastases may derive the greatest benefit from targeted-immunotherapy combination.

4 Discussion

Stratified therapy based on genetic testing is currently the main strategy for third-line treatment of mCRC. According to several large clinical trials, anti-PD-1 antibodies have been approved by the US Food and Drug Administration for the treatment of patients with MSI-H or dMMR mCRC (9, 18). However, for the vast majority of patients with MSS tumors, single-agent chemotherapy and immunotherapy are almost ineffective (8, 19). Currently, there are few trials on the efficacy and safety of targeted therapy combined with immune checkpoint inhibitors (ICIs) for MSS mCRC. Fruquintinib and regorafenib, both anti-angiogenic drugs, are third-line treatment options for mCRC (20, 21). Preclinical studies have shown synergistic effects of the combination of fruquintinib or regorafenib with PD-1 inhibitors in CRC models (22, 23). Meanwhile, some researchers believe that anti-angiogenic treatment may improve the immune condition of the tumor microenvironment, alleviate the immunosuppressive state, and thereby benefit immunotherapy (24). In this study, we conducted a retrospective analysis of the efficacy of targeted therapy alone versus targeted-immunotherapy combination in patients with MSS mCRC, identifying the potential beneficiary population for the targeted-immunotherapy combination.

In this study, the ORR was 12.7% and the DCR was 71.8% in the overall population. Among patients who received only targeted therapy, the ORR was 3.2% and the DCR was 58.1%; however, in those who received targeted therapy combined with immunotherapy, the ORR improved to 20% and the DCR to 82.5%. This indicates that the addition of immunotherapy enhances tumor response to regorafenib or fruquintinib in patients with MSS mCRC. The phase Ib REGONIVO trial (NCT03406871), which enrolled 24 patients with MSS mCRC, reported an ORR of 33% and a DCR of 88%, significantly surpassing our findings (16). This discrepancy might be attributed to the different types of ICIs used. The REGONIVO trial specifically explored the combination of nivolumab and regorafenib, whereas in real-world clinical practice, patients may receive a variety

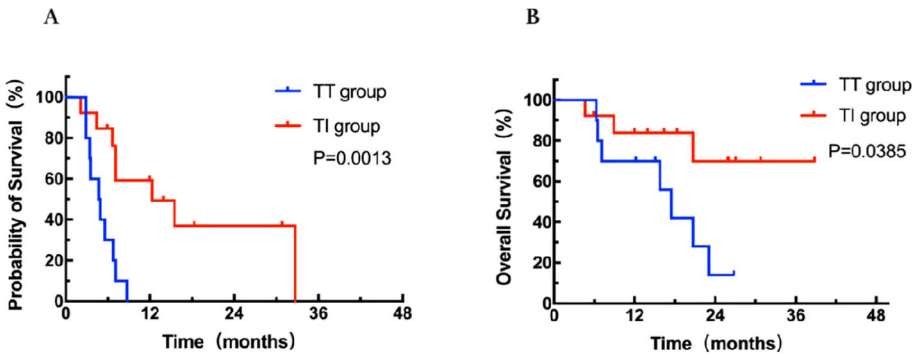

FIGURE 4
Kaplan-Meier curves for PFS **(A)** and OS **(B)** stratified by treatment modalities for patients with lung metastasis. OS, overall survival; PFS, progression-free survival; TT group, targeted therapy group; TI group, targeted-immunotherapy combination group.

of ICIs. Our study included additional ICIs beyond nivolumab, such as the homemade agent sintilimab.

In our study, median PFS was 4.6 months and median OS was 15.8 months for all patients receiving targeted-immunotherapy combination. Standard third-line treatment regimens included chemotherapy or targeted therapies such as irinotecan combined with cetuximab, regorafenib, fruquintinib, and trifluridine/tipiracil (TAS-102) (25). Patients with refractory mCRC who received anti-angiogenic treatment had a median PFS of approximately 2 months and a median OS of 7 months (26, 27). Our results suggest that the combination strategy of targeted therapy and immunotherapy may have certain advantages over traditional therapies. Previous small-scale studies have also evaluated the efficacy of combining ICIs with regorafenib in MSS CRC (28). Based on these, for refractory MSS CRC, a combined strategy of targeted therapy and immunotherapy may represent an effective treatment option.

Not all patients with MSS CRC responded well to combined therapies, suggesting the need for further stratification of patient populations to improve survival benefits. To assist in patient selection, we conducted Cox regression analyses for the discernment of prognostic-related risk factors, further identifying clinical characteristics associated with the effectiveness of targeted-immunotherapy combination. Multivariable analysis revealed significant correlations between liver metastasis and both PFS and OS. Clinical data indicated that patients with liver metastases responded less favorably to anti-PD-1 antibodies than those without liver metastases, a finding supported by basic research (29). Our results aligned with prior studies that the presence of liver metastases was an independent poor prognostic factor for various cancers, particularly in the context of ICI therapy (30, 31). The liver metastatic microenvironment is typically considered to be immunosuppressive, characterized by diminished infiltration of CD8+ T cells and enriched functionality of immune escape pathways (32, 33). Furthermore, recent studies have suggested that liver metastases could induce systemic resistance to ICIs mediated by macrophages and regulatory T cells (29). In the REGOTORI study, patients with liver metastases had a lower ORR compared to those without liver metastases (8.7% vs 30.0%). Indeed, various studies have shown that liver metastasis could reduce the effectiveness of anti-PD-1 antibodies. In patients with melanoma or non-small cell lung cancer treated with pembrolizumab, the response rates were 56.3% in those without liver metastasis and 30.6% in those with liver metastasis. Additionally, liver metastasis was also associated with significantly shorter PFS, with a median of 5.1 months vs 20.1 months (31). Our current study showed that patients without liver metastases responded better and derived greater benefit from the combination of targeted therapy and immunotherapy. In our previous study, we conducted a comprehensive analysis of MSS CRC cases with extrahepatic metastases. The results showed that, although MSS CRC is still referred to as a "cold tumor" in this field, patients with non-liver metastatic MSS mCRC could still benefit from targeted-immunotherapy combination (34). Therefore, effective management of liver metastases may be a key to overcoming resistance to ICIs.

This study found that in patients with only lung metastases, there were significant differences in both PFS and OS between targeted therapy alone and targeted-immunotherapy combination (HR = 0.20 for PFS and HR = 0.27 for OS). This suggests that patients with only lung metastases may benefit most from targeted-immunotherapy combination. Meanwhile, significant differences in PFS and OS were observed in patients with various distant metastasis conditions and treatment modalities. In the FRESCO trial, regorafenib was reported to yield a radiological CR in one case of multiple lung metastases from ascending colon cancer. Of note, regorafenib is primarily approved for third-line therapy of mCRC patients, and detailed reports on its effectiveness in lung metastases are limited. The case discussed demonstrated that in some instances, regorafenib could lead to significant tumor reduction, suggesting its potential efficacy in mCRC with lung metastases (21, 35). The results of this particular case from the FRESCO trial were consistent with the findings of this study. This evidence highlights the need for personalized treatment strategies in mCRC, particularly considering the organ-specific impacts of therapies.

This study has several limitations. Firstly, the study adopted a retrospective design, which restricted the applicability of the findings. Secondly, there was no restriction on the therapeutic drugs used in the study, affecting the consistency of the treatment regimen. Thirdly, the number of patients included was small. Fourth, not all patients underwent RAS and BRAF genetic testing, limiting the analysis of their impact on the efficacy of the drugs. To overcome the limitations of the retrospective design, we are planning to conduct a larger study

to improve statistical power, and will ensure that all patients undergo RAS and BRAF gene testing in order to comprehensively analyze the impact of genotype on drug efficacy.

5 Conclusion

Targeted-immunotherapy combination showed more benefit than targeted therapy alone in the third-line or beyond setting for MSS mCRC. Liver metastasis might be a key factor in the poor prognosis of this population. Patients with only lung metastasis were most likely to benefit from targeted-immunotherapy combination.

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 Ethics Committee of the Fourth Hospital of Hebei Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because This article is a retrospective study and has obtained ethical exemption. Patient's informed consent has been waived.

Author contributions

DL: Writing – original draft, Data curation. HJ: Writing – original draft, Formal analysis. YL: Writing – review & editing, Investigation. JL: Writing – review & editing, Conceptualization. XZ: Writing – review & editing, Methodology. LW: Writing – review & editing, Project administration. ZF: Writing – review & editing, Software. LF: Writing – review & editing, Supervision. JZ: Writing – review & editing, Validation. JH: Writing – review & editing. YW: Writing – review & editing.

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Conflict of interest

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

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Integrating immunotherapy with conventional treatment regime for breast cancer patients- an amalgamation of armamentarium

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Immunotherapy stands as the frontrunner in treatment strategies imparting efficient remission in various types of cancer. In fact, emerging breakthroughs with immune checkpoint inhibitors (ICI) in a spectrum of cancers have evoked interest in research related to the potential effects of immunotherapy in breast cancer patients. A major challenge with breast cancer is the molecular heterogeneity that limits the efficacy of many therapeutic regimes. Clinical trials have shown favorable clinical outcomes with immunotherapeutic options in some subtypes of breast cancer. However, ICI monotherapy may not be sufficient for all breast cancer patients, emphasizing the need for combinatorial approaches. Ongoing research is focused on untangling the interplay of ICI with established as well as novel anticancer therapeutic regimens in preclinical models of breast cancer. Our review will analyze the existing research regarding the mechanisms and clinical impact of immunotherapy for the treatment of breast cancer. We shall evaluate the role of immune cell modulation for improved therapeutic response in breast cancer patients. This review will provide collated evidences about the current clinical trials that are testing out the implications of immunotherapy in conjunction with traditional treatment modalities in breast cancer and summarize the potential future research directions in the field. In addition, we shall underline the recent findings related to microbiota modulation as a key regulator of immune therapy response in cancer patients and its plausible applications in breast cancer.

KEYWORDS

immunotherapy, breast cancer, triple-negative breast cancer, combination therapy, treatment

1 Introduction

Breast cancer persists as a global health menace, accounting for one-third of all new cancer cases and ranking as the second most prevalent malignancy among women (1). With more than 2.3 million cases worldwide, breast cancer incidence is currently on the rise. Depending on the type of hormone receptors being expressed on the breast carcinoma cells, there exist four primary molecular subtypes of breast cancer; estrogen receptor/progesterone receptor (ER/PR)-positive but HER2-negative (luminal A) that comprises of more than half of the breast cancer cases; ER/HER2-positive but PR-negative (luminal B)- hormone therapy as well as chemotherapy may be effective for treating both luminal A and luminal B subtypes; ER/PR-negative but HER2-positive (HER2 positive)- this group of tumors are likely to respond to HER2-targeted therapy; and triple-negative breast cancer (TNBC, basal-like) with too little to no expression of any of the receptors, making it the most challenging breast cancer subtype to target (2). Importantly, TNBC makes up about 10-15% of all breast cancers and is the most aggressive form of this malignancy. Given the complex heterogeneity with diverse molecular subtypes and various underlying genetic alterations, the choice of treatment and the therapeutic response varies greatly among breast cancer patients. At present, surgical resection, chemotherapy, and radiotherapy are the frontline treatment approaches for managing locally advanced breast cancer. Endocrine therapies such as SERM tamoxifen, SERD fulvestrant, or the aromatase inhibitors anastrozole and exemestane, are well-accepted forms of targeted therapy for ER-positive breast cancer (3–5). Small molecule inhibitors against CDK4/6 (palbociclib, abemaciclib, ribociclib), PARP (olaparib, talazoparib, rucaparib, niraparib), PI3K/AKT, mTOR, FGF receptors and VEGF also hold strong potential as precision medicines to mitigate breast cancer progression due to their intimate involvement in oncogenic signaling pathways (6, 7). *De novo* and acquired resistance to several therapies has also been noted in breast cancer leading to the development of newer regimens that may prove effective against resistant tumors. For e.g., everolimus (42-O-(2-hydroxyethyl) rapamycin), an mTOR inhibitor, is approved for advanced or metastatic ER-positive breast cancer that no longer responds to aromatase antagonist. Despite their clinical prowess, therapeutic resistance severely limits the efficacy of several drugs.

The emergence of immunotherapy as the fourth pillar of anticancer strategies has helped prolong the survival of several breast cancer patients. Immune checkpoint inhibitors (ICIs) encompassing CTLA-4, PD-1, and PD-L1 inhibitors have been authorized for treating solid tumors, including breast cancer. Multiple clinical trials, conducted over the years, led to the FDA approval of the first ICI-ipilimumab, a CTLA-4-blocking antibody in 2011 for metastatic melanoma (8). The ensuing investigations resulted in the development of several PD-1-targeting antibodies that were markedly effective in clinical settings, leading to the subsequent approval of nivolumab and pembrolizumab in 2014. These successes fueled further research endeavors helping the development of inhibitory antibodies against additional targets such as PDL1, LAG3 protein, hepatitis A virus cellular receptor 2 (also known as TIM3), and T cell immunoreceptor with Ig and ITIM domains (9). Moreover, efforts have been directed to engage T cell immune response *via* agonist antibodies that function primarily by activating receptors on the immune cells, like CD40, and TNF receptor superfamily member 9 and 4 (10). Immunotherapy repertoire has shown very promising responses in multiple cancer types while progress in breast cancer has been rather slow. Contrary to the older notion of a "poorly immunogenic" nature, current research strongly indicates that breast tumors are composed of a complex, heterogenous and dynamic network of untransformed epithelial cells, genetically modified cancer cells, fibroblasts, immune cells, and blood vessels. There exists an intricate communication among these different constituents. Also, these components interact with the surrounding microenvironment which changes with cancer progression and in response to therapy. Improved understanding of the dynamic breast cancer microenvironment has led to tremendous progress in the development of immunotherapy in breast cancer.

Abbreviations: ER, Estrogen Receptor; PR, Progesterone Receptor; HR, Hormone Receptor; HER2, Human Epidermal Growth Receptor 2; EGFR, Epidermal Growth Factor Receptor; IGF1R, Insulin-like Growth Factor 1 Receptor; AR, Androgen Receptor; SERM, Selective Estrogen Receptor Modulator; SERD, Selective Estrogen Receptor Degrader; PI3K, Phosphatidylinositol 3 Kinase; AKT, Protein Kinase B; CDK, Cyclin-Dependent Kinase; mTOR, mammalian Target of Rapamycin; FGF, Fibroblast Growth Factor; VEGF, Vascular Endothelial Growth Factor; PTEN, Phosphatase and tensin homolog; CTLA-4, Cytotoxic T Lymphocyte-associated Antigen-4; PD-1, Programmed cell Death receptor 1; PD-L1, Programmed cell Death 1 Ligand 1; MDSC, Myeloid Suppressor Cell; T_{reg}, Regulatory T cell; FDA, Food and Drug Administration; LAG3, Lymphocyte Activation Gene 3; CD40, Cluster of Differentiation 40; TNF, Tumor Necrosis Factor; TIL, Tumor Infiltrating Lymphocyte; CEA, Carcinoembryonic Antigen; MET, Mesenchymal-Epithelial Transition; PFS, Progression-free Survival; OS, Overall Survival; DFS, Disease-free Survival; pCR, Pathological Complete Response; ORR, Objective Response Rate; CAR, Chimeric Antigen Receptor; TAPUR, Targeted Agent and Profiling Utilization Registry; BRCA, BReast CAncer gene; PARP, Poly(Adenosine diphosphate-Ribose) Polymerase; HDAC, Histone deacetylase; Trop-2, Trophoblast cell-surface antigen 2; ALDH, Aldehyde dehydrogenase; EpCAM, Epithelial cell adhesion molecule; EMT, Epithelial Mesenchymal Transition.

2 Current status of immunotherapy for different subtypes of breast cancer

Multiple investigations have improved our current knowledge of immune evasion by tumor cells and enabled the development of specific immune checkpoint inhibitors as state-of-the-art therapeutic choice. Immunotherapy primarily entails boosting the host immune system so as to enable it to recognize cancer cells as a foreign invader and subsequently destroying them. The previously believed notion about the 'non-immunogenic environment of breast cancer' has been challenged with the discovery of tumor-infiltrating lymphocytes (TILs) in breast tumors (11). Of note, HER2-positive and TNBC subtypes demonstrate an elevated number of TILs compared to the other breast cancer subtypes (12, 13). Currently, there is an increasing interest in the application of immune checkpoint blockers to treat

breast cancer patients who are refractory to any other forms of treatment.

2.1 Immunotherapeutic approaches for the treatment of HER2-positive breast carcinoma

Clinical trials in patients with HER2-positive breast cancer have exhibited modest results with immunotherapy. The combination of anti-PD-1 mAb (pembrolizumab) and trastuzumab was assessed for the treatment of HER2-positive progressive metastatic breast cancer, wherein a partial response was achieved in 15% of the enrolled patients with PD-L1 positive tumors (14). Moreover, dendritic cell (DC) vaccines that were primed against the HER2 protein proved beneficial in mammary tumor regression through activation of anti-HER2 $CD4^+$ Th1 response in an early phase clinical trial (15). Preclinical investigations in immunocompetent mice suggest that PD-1 and CTLA-4 inhibition leads to a considerable increase in the immune-associated effects of HER2-based targeted therapies which is accomplished *via* synergistic activation of $CD8^+$ T cells (16, 17). The PANACEA trial revealed that 15% of trastuzumab-resistant HER2-positive breast cancer patients harboring PD-L1-positive tumors elicited a partial response when treated with pembrolizumab plus trastuzumab (14). In the "Proceedings of the 2018 San Antonio Breast Cancer Symposium", Emens et al. discussed the randomized phase II KATE2 trial, which revealed that patients with PD-L1-positive, HER2-positive, pre-treated metastatic breast cancer exhibited improved PFS following treatment with T-DM1 combined with atezolizumab relative to T-DM1 alone. Of note, CAR T-cell therapy, a popular example of adoptive T cell therapy, have proven successful in patients with hematological cancers and is currently being explored in solid tumors. Researchers have successfully expanded T cells specific for HER2 *ex vivo* in mice models and these were found to elicit antitumor activity (18). Administration of HER2 CAR T cells with CD28 costimulatory domain in the mice central nervous system resulted in the regression of HER2-positive metastatic breast carcinoma in the CNS (19). Nonetheless, clinical data regarding the application of adoptive T cell therapy for treating HER2-positive breast malignancy are still lacking. Preclinical and clinical observations, though limited, solidify the rationale for the clinical development of ICI for the treatment of HER2-positive breast carcinoma, and emphasize the need for more detailed research into the development of immunotherapeutic modules, especially in combination with HER2-targeted therapies.

2.2 Immunotherapeutic approaches for the treatment of triple-negative breast cancer

Reportedly, factors such as a heavier tumor mutation load, increased frequency of TILs and enhanced expression of PD-L1 may contribute to increased immunogenicity for TNBC, thus, TNBC patients are expected to benefit from ICIs (20). The efficacy of pembrolizumab monotherapy as a first-line of therapy for metastatic TNBC (mTNBC) patients was evaluated in the phase II KEYNOTE-086 study (21). The results from the study showed favorable anti-tumor activities with median PFS of 2.1 months whereas the median OS was improved to 18 months. Many investigations are additionally aimed at establishing pembrolizumab monotherapy as a second-line or later therapeutic strategy in pre-treated mTNBC patients, including the KEYNOTE-086 (21), the KEYNOTE-012 (22) and the TAPUR basket study (23). In the phase III KEYNOTE-119 trial, efficacy of pembrolizumab monotherapy was assessed in comparison to standard chemotherapy in second or third line of treatment for patients with mTNBC. However, there was no evident improvement on the prognosis of 622 TNBC patients, who had progressed on 1-2 cycles of either taxane or anthracycline (24). Such results indicate the immediate need for additional large-scale randomized controlled trials and the need for combination approaches, especially in earlier lines of treatment. Atezolizumab and durvalumab are two more anti-PD-L1 antibodies that are yielding promising results (22). A phase II trial, consisting of 199 patients with no disease progression after 6-8 cycles of chemotherapy, has evaluated the role of durvalumab for TNBC treatment. In the exploratory subgroup analysis of TNBC patients (n = 82), durvalumab dramatically increased the OS (25), suggesting the rationale for additional investigations into using durvalumab as a therapy for TNBC patients with advanced disease. Avelumab, another PD-L1 inhibitor, is currently being tested as second-line or posterior-line therapy at JAVELIN basket trial, which has shown some promising results (26). Findings from the phase II TONIC study have indicated that addition of cisplatin and doxorubicin may exert better tumor response to immunotherapy in TNBC patients (27). The detailed insight into the underlying molecular mechanisms is still not thoroughly understood and remains an imperative area of future research focus. Nonetheless these reports strongly support the improved and durable clinical efficacy of PD-1/PD-L1 inhibition as an effective treatment modality for patients with TNBC.

2.3 Attempts evaluating immunotherapeutic approaches for the treatment of luminal A/B breast cancer

It is important to note that not all subtypes of breast carcinoma equally respond to the effects of immunotherapy. For instance, in subjects bearing the luminal subtype of breast cancer, initial attempts used a combination of ICI with chemotherapy as a novel form of anti-cancer therapy- but that yielded disappointing results. One such study aimed at investigating the tumor suppressive effects of pembrolizumab combined with eribulin among patients harboring ER/PR-positive, HER2-negative metastatic breast carcinoma (28). However, the combination therapy did not lead to any noticeable improvement in the clinical outcome or prognosis of the metastatic luminal A-subtype breast cancer patients- the most possible reason underlying this pertains to the heavily-pretreated nature of the subjects in the study. A phase Ib non-randomized, open-label, multi-cohort study tried to evaluate the clinical impact of pembrolizumab plus abemaciclib in the presence or absence of anastrozole (endocrine therapy) in

metastatic breast cancer patients with ER-positive, HER2-negative subtype. Patients had no prior exposure to CDK4/6 inhibitor treatment (29). Unfortunately, the anti-tumor efficacy of the combination was mitigated by the appearance of toxicity in the liver and lungs following therapy. In contrast, administration of letrozole, palbociclib, and pembrolizumab as front-line therapy in HR-positive, HER2-negative metastatic breast carcinoma was associated with good tolerance and favorable clinical efficacy in a phase I/II trial (30). For luminal B- subtype, the neoadjuvant phase II GIADA trial subjected patients to sequential anthracycline-associated chemotherapy prior to the treatment with nivolumab and endocrine therapy (31). A 16.3% pCR rate was observed followed by the identification and characterization of immune-based gene signatures and immune sub-populations that were correlated with the achieved pCR. While previous studies have found no notable benefit from pembrolizumab in a metastatic setting of the luminal subtype, addition of pembrolizumab to a sequential cycle of chemotherapy in a neo-adjuvant setting was found to elevate the pCR rate from 13 to up to 30% amongst patients with luminal breast cancer (32).

3 Development of novel treatment modalities combining immunotherapy with existing and upcoming therapeutic agents to target breast cancer

Contemporary research is focused on exploring the synergistic effects of ICIs and commonly used chemotherapies for treating breast carcinoma patients as monotherapy approaches using ICIs exhibited modest activity. Chemotherapy is well-known to repress the actions of immunosuppressive cells, like MDSCs and T_{reg} cells, while simultaneously facilitating cancer cell apoptosis, promoting tumor antigen cross-presentation, and exacerbating recruitment and infiltration of $CD8^+$ T cells, NK cells and DCs *via* the secretion of pro-inflammatory cytokines in the macrophages. Preclinical evidences from animal models and clinical studies are already recognizing the intricate drug-dependent and dose-dependent interplay between chemotherapy and the immune system- thus, this interaction can be exploited for synergistic associations between cytotoxic drugs and immunotherapy (Figure 1).

3.1 Combination modalities involving immunotherapy and HER2-targeted therapies

Utomilumab is a receptor IgG2 mAb agonist against 4-1BB, a co-stimulatory receptor that is involved in immune cell proliferation once activated. For the treatment of patients with advanced HER2-positive breast cancer, a phase I dose-escalation trial is investigating the combination of utomilumab with either trastuzumab or T-DM1 (NCT03364348). The effects of utomilumab plus avelumab is also being studied in a phase II trial (AVIATOR, NCT03414658). The preclinical findings revealed that utomilumab, when combined with a mAb targeting the PD-1/PD-L1 axis, can aid in a strong immune response (33). Another component associated with the adaptive immune response is the toll-like receptor, and activation of TLR4 can stimulate antigen processing and cross-presentation *in vivo* (34). In pre-clinical models, oligodeoxynucleotides with CpG motifs that activate TLR9 have been shown to induce active immune cytotoxicity (35). Activation of TLR2 in HER2-positive breast cancer preclinical models augments trastuzumab-related cytotoxicity (36). Such results inspired research undertakings for testing the therapeutic efficacy of TLR agonists in combination with HER2-based vaccines (NCT02276300). Synergistic interactions between trastuzumab and docetaxel chemotherapy have yielded a 60% response rate compared to 11% in the case of monotherapy (37). Taking these observations forward, clinical trials inspecting the efficacy of combining atezolizumab with HER2 mAbs plus chemotherapy in patients who are receiving early first line therapy for HER2-positive breast cancer are in progress. The findings from such trials are likely to provide new avenues for the treatment modalities (NCT03125928, NCT03726879) (38). While approaches combining immunotherapy and HER2-targeted therapies are promising candidates for the treatment of HER2-positive breast cancer, they can also be useful for a wider patient population including tumors exhibiting a modest/low HER2 expression level which are not usually eligible for HER2 mAbs.

3.2 Integration of immunotherapy with chemotherapy regimens for improved outcomes

One-third of patients with TNBC experience distant recurrences, and eventually succumb to death within 5 years post-diagnosis. Therefore, TNBC has a dire need for superior treatment options and precision medicine. A phase III clinical study, IMpassion130, interrogated the impact of immunotherapy in 902 patients with advanced TNBC, and tested the efficacy and safety of the PD-L1 antibody atezolizumab in conjunction with chemotherapeutic drug nab-paclitaxel (39). The promising data showed a significant improvement in the mean OS from 15.5 to 25 months among PD-L1-positive patients (40). The findings led to the FDA approval of the combination of atezolizumab and nab-paclitaxel for therapy-naïve patients having PD-L1-positive advanced TNBC in 2019 (41). Follow up phase III trials IMpassion131 and Impassion 132 are delineating the clinical impacts of atezolizumab with paclitaxel or first-line chemotherapy (carboplatin, gemcitabine or capecitabine) in multiple settings of TNBC (42). The primary goal of the IMpassion131 trial was to test the efficacy of weekly administration of paclitaxel as the chemotherapy backbone plus atezolizumab in a group of patients constituting of similar inclusion criteria as IMpassion130. Unfortunately, the results from this trial were not in sync with the observations from Impassion130. Of note, in an abstract presented at the "2021 ASCO Annual Meeting", it was shown that several differences were present in the tumor

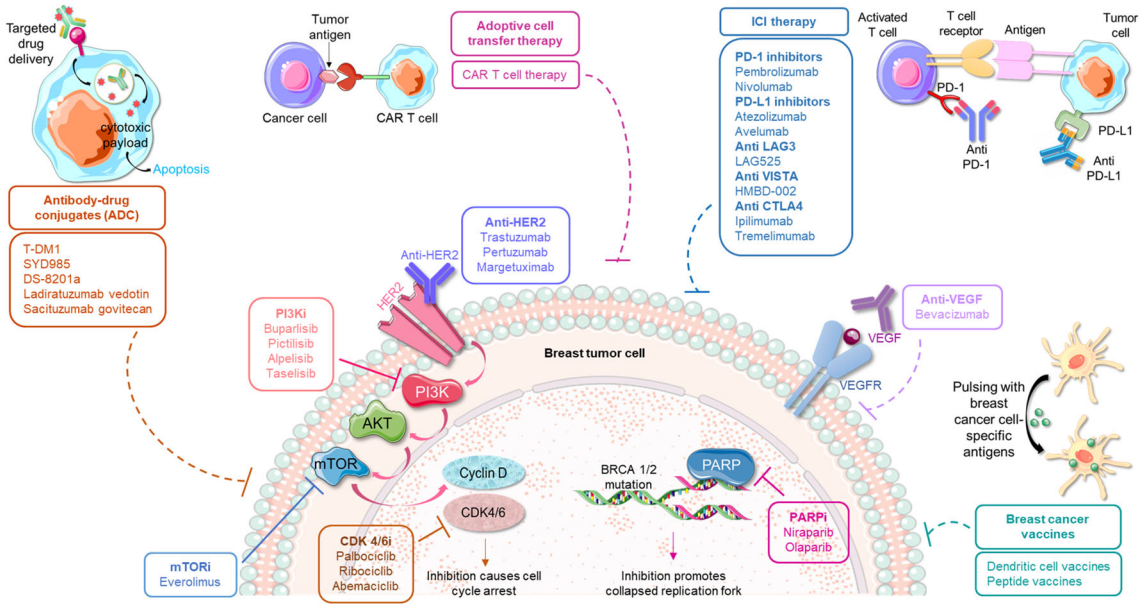

FIGURE 1
Immunotherapeutic strategies in conjunction with traditional modes of anticancer treatment approaches for the management of breast cancer. The predominant small molecule inhibitors used for treating breast cancer include inhibitors against PI3K, mTOR, CDK 4/6 and PARP. These agents can be potentially used in combination with various emerging immune-therapies, such as, anti-PD-1/anti-PD-L1 therapy, antibodies against CTLA-4 and other immune checkpoints, different antibody-drug conjugates, adoptive cell transfer therapy as well as specific vaccines. Such combinatorial regimes are currently being investigated for their safety and efficacy in breast cancer.

microenvironment of tumor samples from patients enrolled in the two trials. Additional reasons underlying the observed discrepancy in results may be attributed to the differences in the body mass index and gut microbiota composition among the enrolled patients (43). Another phase III trial, KEYNOTE-355, tested the effects of integrating pembrolizumab with chemotherapy (albumin-bound paclitaxel, paclitaxel or gemcitabine/carboplatin) for the treatment of locally recurrent, inoperable or mTNBC patients who have not undergone prior therapy. There was a considerable prolonged PFS among PD-L1 positive population in the pembrolizumab-based group (44), paving the way for the accelerated FDA approval of pembrolizumab in combination with chemotherapy for the treatment of patients with locally recurrent unresectable or metastatic PD-L1-positive TNBC. Final results of the KEYNOTE-355 trial reported that the combination considerably benefitted the patients with a mean OS of 23 and 16.1 months in the combination versus chemotherapy alone group, respectively. Intriguingly, 22% of the TNBC patients in the pembrolizumab arm boasted of a disease-free interval between 6 and 12 months. Results from a phase II randomized trial demonstrated that chemotherapy or radiotherapy promoted a more favorable tumor microenvironment in TNBC patients that boosted the response to PD-1 blockade through nivolumab. Patients subjected to the combination regimen that included immunotherapy experienced a clear improvement in their median DFS and OS, relative to the individuals treated with monotherapy alone (27). Of interest, Oleclumab, a mAb specific for CD73, is being studied in the phase Ib/II BEGONIA study, as a combination therapy with durvalumab, plus paclitaxel, as first-line treatment for mTNBC individuals (NCT03742102). In the ENHANCE 1 trial enrolling 104 patients with mTNBC, eribulin, a microtubule inhibitor, when administered with pembrolizumab, displayed attractive tumor-suppressive activity (45). An objective response was achieved in (i) 26% of the evaluable patients, (ii) 25% of the 48 patients who were not exposed to any prior chemotherapy and (iii) 27% of the 34 subjects who had previously received chemotherapy. Again, in agreement with observations from other trials, patients harboring PD-L1-positive breast cancer boasted a better response than those having PD-L1-negative tumors. The exciting findings from such trials have elicited investigations into various combination regimes among TNBC patients. Chemotherapy regimens continue to be the frontline treatment strategy for a majority of breast cancer patients; however, it is associated with adverse side effects affecting the quality of life as well as therapy resistance leading to suboptimal response. While immunotherapy regimens are still being investigated for their long-term impact on quality of life, the combination treatment strategies combining chemotherapy and immunotherapy are presenting improved responses than monotherapy alone.

3.3 Development of novel combinations using immunotherapy with antiangiogenic agents, HDAC inhibitors and topoisomerase inhibitors

Multiple studies are underway focusing on determining the efficacy of immunotherapy with anti-angiogenesis agents. The GINECO A-TaXel phase II trial in TNBC reported a significant activity and tolerable safety profile for the combination of paclitaxel, capecitabine and bevacizumab, a recombinant humanized mAb

against VEGF-A (46). In 2019, a single-arm trial investigated the effects of Nivolumab, paclitaxel and bevacizumab as first-line therapy in patients with HER2-negative metastatic breast carcinoma, consisting of both hormone-positive and TNBC populations. The treatment group exhibited a PFS of 8.1 months among the TNBC individuals and 19.1 months in the hormone receptor-positive subgroup (47). In addition, in heavily pretreated patients with advanced TNBC, a novel humanized mAb targeting PD-L1, TQB2450, was tested in combination with anlotinib, an anti-angiogenic small molecule inhibitor- the combination arm displayed an acceptable safety profile with potent activity (48). The capacity of HDAC inhibitors in upregulating antigen presentation genes and boosting tumor cell recognition by activated ICs suggest that they may act in augmenting the efficacy of ICIs. The cocktail of romidepsin, cisplatin and nivolumab indicated encouraging signs of efficacy in 34 pre-treated mTNBC patients, necessitating additional research in larger populations (NCT02393794). In contrast, evidences from a phase II study in people with advanced TNBC suggest that another HDAC inhibitor, entinostat, failed to improve PFS in combination with atezolizumab (NCT02708680), supporting the dire need for further investigation of the combination. With advances in research related to ADC, a randomized phase II trial is affirming the impact of pembrolizumab plus sacituzumab govitecan, composed of a topoisomerase I inhibitor (SN-38) and an anti-Trop2 monoclonal antibody, in patients with PD-L1 negative mTNBC (NCT04468061). Overexpression of Trop-2 is predictive of a more aggressive TNBC (49). Sacituzumab govitecan was found to serve as a potent immunomodulator, promoting antibody-driven cytotoxicity, depletion of T_{reg} cells and upregulation of MHC class I and PD-L1 expression in mice models, and it may overcome resistance to current immunotherapeutic strategies in PD-L1-negative tumors, which forms the rationale of the clinical trial. A recent presentation at the "2020 AACR annual meeting" demonstrated the potential medical application of another ADC with similar immunomodulatory features, ladiratuzumab-vedotin, an anti-LIV-1 ADC, in conjunction with pembrolizumab as first-line therapy in TNBC patients in a phase Ib/II study- the combination proved tolerable and exhibited promising anticancer activity. ICI therapy impedes the tumor-promoting signals that enable immune evasion by cancer cells. Combining this method with agents that function by potently targeting various other hallmarks of cancer, such as angiogenesis, epigenetics modulation and DNA damage response, can potentially results in synergistic effects that will ultimately lead to more successful response in breast carcinoma patients.

3.4 Examining combinations of immunotherapy with multiple kinase inhibitors

Various kinase inhibitors have been tested to target specific oncogenic pathways in breast cancer. Currently, many clinical studies are exploring combination regimens involving kinase inhibitors and immunotherapy. The phase II COLET study evaluated the therapeutic efficacy of atezolizumab, MEK1/2 inhibitor cobimetinib, and nab-paclitaxel or paclitaxel in locally advanced or mTNBC, wherein PD-L1-positive patients accomplished a visibly higher tumor ORR and PFS than PD-L1-negative individuals (50). The therapeutic outcome of combining PD-1 monoclonal antibody camrelizumab with apatinib for treating advanced TNBC was tested in a phase II study (51). The results were intriguing as they revealed an ORR as high as 32.5% compared to 18.5%, which is the highest recorded ORR for anti-PD-L1 monotherapy in TNBC, paving the groundwork for an effective alternative combinational approach for TNBC treatment. Moreover, a prospective phase II trial (FUTURE-C-PLUS) is ongoing that seeks to assess the efficacy and safety index of the combination of camrelizumab plus chemotherapy (nab-paclitaxel) and famitinib (multityrosine kinase inhibitor against VEGFR-2, PDGFR and c-kit) in mTNBCs. A major part (81.3%) of the population achieved objective responses with a 60.2% of 9-month PFS rate (52). These promising results inspired the ongoing phase II randomized trial FUTURE-SUPER (NCT04395989). A phase Ib/II study is presently determining the effects of tislelizumab, an anti-PD-1 IgG4-variant mAb, in combination with fruquintinib, a highly selective, oral tyrosine kinase inhibitor of VEGFR, in mTNBC, including patients who have been pretreated with immunotherapy in addition to naïve patients (NCT04579757). Meanwhile, patients suffering from AR-positive metastatic TNBC, when subjected to pembrolizumab combined with the AR regulator GTx-024 in a phase II clinical trial, demonstrated an ORR of 25% at 16 weeks (53). In another study, an AKT inhibitor ipatasertib was subsequently combined with the atezolizumab and paclitaxel or nab-paclitaxel cocktail as another candidate for front-line treatment. Irrespective of the expression of PD-L1 or alteration status of PIK3CA/AKT1/PTEN, 19 out of 26 patients showed a response accompanied with a significantly elevated ORR of 73%, thus advocating a novel therapeutic regime for treating TNBC (54). In addition to their traditional role in targeted inhibition of key proteins involved in cell survival and growth, kinase inhibitors eradicate tumors *via* certain immune-modulatory effects. Immunotherapy, when used in conjunction with such precision therapy, can suppress the toxicities associated with monotherapy and impart improved targeted anti-tumor functions even in breast cancer patients, who do not respond well to immunotherapy alone.

3.5 Combining PARP inhibitors and CDK inhibitors with immunotherapy as a new arsenal for targeting breast cancer

A more recent undertaking, which is currently recruiting patients with locally advanced or metastatic HER2-negative mammary carcinoma with homologous DNA repair deficiency, aims to uncover the efficacy of atezolizumab when incorporated with the PARP inhibitor, olaparib (NCT02849496). The TOPACIO/KEYNOTE-162 trial is a single-arm phase II study in advanced TNBC population that found a considerable anti-tumor activity and manageable safety profile for the combination of pembrolizumab and a PARP inhibitor, niraparib (55).

Importantly, this combination was especially beneficial for patients harboring tumors with BRCA mutation. Furthermore, niraparib synergistically potentiates the anticancer functions of the anti-PD-1 antibody, BioXCell RMP1-14, in TNBC models through activation of the interferon signaling (56). Furthermore, co-administration of anti-PD-L1 monoclonal antibody, durvalumab and olaparib in advanced breast carcinoma with genomic BRCA mutation exhibited better survival rates in a phase I/II MEDIOLA study (57), denoting the alluring prospect of integrating PARP inhibitors with immunosuppressants as an efficient anticancer module for TNBC patients. Moreover, the SGNLVA-002 study attempts to assess the effects of the novel combination of pembrolizumab with ladiratuzumab vedotin, an ADC with great potential, as a front-line treatment choice for locally advanced or mTNBC (NCT03310957). A phase II randomized controlled trial in 2019 unveiled that PD-L1-positive or TNBC patients demonstrated a pronounced benefit when subjected to treatment with durvalumab with the median OS of durvalumab-treated PD-L1 positive or TNBC patients being 21 months and 26 months, respectively (NCT02299999). The efficacy of durvalumab plus the PARP inhibitor, olaparib, as a maintenance strategy for patients with platinum-sensitive advanced TNBC is being determined in the DORA study (NCT03167619). Another category of agents that holds imminent interest in combination modules with ICIs are the CDK inhibitors. In preclinical models of TNBC, dinaciclib, an intravenous CDK inhibitor, potentiated the antitumor effects of ICI through heightened immune cell activation and tumor infiltration. Following this, a phase Ib, dose-escalation trial tested dinaciclib plus pembrolizumab in patients with advanced TNBC, which revealed manageable toxicities upon reduction and delayed administration of the specified dose while the dose expansion part is ongoing. Furthermore, the CDK4-6 inhibitor, palbociclib, is being interrogated in combination with avelumab in AR-positive TNBC (NCT04360941). DNA-damaging agents, such as PARP inhibitors, can potentiate immune response through enhanced tumor mutational burden and improved neoantigen release, thereby rendering the tumor more amenable to immunotherapy. CDK4/6 inhibition is known to impart transcriptional reprogramming of immune as well as tumor cells, resulting in higher immunogenicity of cancer cells and an immune-rich TME, which is, again, more susceptible to immune-based therapies. Therefore, combining these approaches with immunotherapy can lead to positive response rates in a number of breast cancer patients, including those who are originally less responsive to ICI therapy.

3.6 Combining multiple immunotherapy regimens to enhance the clinical efficacy

Recent studies are also investigating the utility of combining different ICI regimes. Multiple CTLA-4 inhibitors have shown the efficacy in combination therapy for solid tumors, including breast carcinoma. While ipilimumab was FDA approved for better survival among advanced metastatic melanoma patients, its anti-tumor effect was modest in TNBC (58). A single-arm clinical study in patients with metastatic breast cancer, including TNBC population, tested the efficacy of durvalumab in conjunction with tremelimumab but the trial did not meet a successful completion (59).Treatment with dual anti-PD-1 and anti-CTLA-4 plus cisplatin resulted in activation of DCs and $CD8^{+}CD4^{+}$ T cells along with suppression of $FOXP3^{+}$ T_{reg} cells and the effect was more pronounced in BRCA-1 deficient tumors (60). LAG3 is an immune checkpoint that blocks the activation of its host cell to facilitate further suppression in the immune response. LAG525 is an antibody raised against LAG3, which was tested in the setting of mTNBC in a phase II trial, in conjunction with PDR001, an anti-PD1 antibody in the presence or absence of carboplatin (61). The triplet arm showed an ORR of 32.4%. ICOS is a member of the CD28 superfamily that interacts with ligands expressed on B cells and phagocytes, thus promoting downstream signaling to regulate T cell proliferation and release of cytokines. In a phase I/II open-label study involving patients with advanced solid tumors, KY1044, a human anti-ICOS antibody, was tested as monotherapy and in combination with atezolizumab. KY1044 was well-tolerated in both the strategies and one complete response and four partial responses were noted in the TNBC cohort (NCT03829501). Results from the phase II part of the study are underway. Another immune-regulatory protein that suppresses T cell activation and cytokine production, VISTA, is capable of inducing an immunosuppressive environment. HMBD-002 is an antibody against VISTA, which is currently being studied in a phase I study among patients with advanced TNBC (NCT05082610). A summary of the clinical studies involving a combinatorial approach of immunotherapy plus some form of traditional anticancer therapeutic module that have demonstrated safe and favorable disease outcome so far is presented in Table 1.

3.7 The role of neoadjuvant immunotherapy in breast cancer

The application of immunotherapy in the neo-adjuvant setting, prior to any operative or adjuvant therapy, is expected to induce more beneficial results for cancer patients. This is based on preclinical evidences in animals that show improved immune responses and better survival rates when immunotherapy was administered before tumor resection or while the primary tumor plus the local lymph nodes were intact (62). This superior response is partly attributed to fact that immunotherapy, in a neoadjuvant environment, primes a stronger anti-tumor immune response prior to the changes in the tumor microenvironment or increased tumor antigen heterogeneity. Considering the attractive anticancer impacts of immunotherapy in treating patients with early-stage disease in the adjuvant setting, research now seeks to utilize ICI blockade for treating such patients in the pre-operative or neoadjuvant setup. A 2019 randomized phase II study, enrolling 174 patients with operable TNBC, administered durvalumab in addition to anthracycline/taxane-based chemotherapy in a

TABLE 1 Clinical trials in breast cancer subjects pertaining to the combination of immunotherapy with different forms of conventional anticancer treatment strategies.

Combined therapy	Anti-PD-1/PD-L1	Another agent	Phase	Number of patients	Conclusions	Disease setting	Clinical Trial number
HER-2-targeted	Pembrolizumab	Trastuzumab	I/II	58	Safe; with activity and durable clinical benefit in PD-L1/HER2, trastuzumab-resistant, advanced breast cancer patients; disease control achieved in 25% of patients in the PD-L1-positive subgroup.	Trastuzumab-resistant, advanced, HER2-positive breast cancer	NCT02129556
	Durvalumab	Trastuzumab	I	15	No objective response; best outcome was stable disease at week 6 in 4 (29%) of 14 patients with PD-L1-negative cancers.	HER-2 positive metastatic breast cancer progressing on prior anti HER-2 therapies	NCT02649686
PARPi	Pembrolizumab	Niraparib	I/II	55	Safe, promising antitumor activity; objective response was achieved by 13 (29%) of 45 evaluable patients overall, 8 (67%) of 12 patients with genomic BRCA mutations, 33% of patients with PD-L1-positive cancers and 15% of patients with PD-L1-negative cancers.	Advanced or metastatic TNBC	NCT02657889
	Durvalumab	Olaparib	I/II	288	Objective response was achieved by 67% of patients in the first-line setting group.	Germline BRCA-mutated, metastatic breast cancer	NCT02734004
Chemotherapy	Atezolizumab	Nabpaclitaxel	Ib	33	Safe, nab-paclitaxel did not impair systemic immune activation by atezolizumab; objective response from 13 (39%) patients overall and from 7 (54%) of 13 who received atezolizumab as first-line treatment.	Metastatic or locally advanced TNBC	NCT01633970
	Durvalumab	Anthracycline/taxane	II	117	Increases pCR rate in patients treated with durvalumab alone prior to start of chemotherapy; better response in PD-L1 tumors.	Early TNBC	NCT02685059
	Atezolizumab	Nabpaclitaxel	III	902	Median PFS was 7.2 months (95%) and median overall survival was 21.3 months (95%) for all patients; median overall survival in the PD-L1-positive subgroup was 25.0 months (95%).	Previously untreated, locally advanced or metastatic TNBC	NCT02425891
	Pembrolizumab	Chemotherapy	III	1372	Significantly longer overall survival than chemotherapy alone in patients with advanced TNBC with PD-L1-positive tumors.	Previously untreated, locally recurrent, inoperable or metastatic TNBC	NCT02819518
Tyrosine-kinase inhibitor	Camrelizumab	Apatinib	II	40	Median PFS of 8.1 (95%) months; grade 3/4 treatment-related adverse events occurred in 19 (41.3%) of 46 patients; combination showed promising efficacy with a measurable safety profile in patients with heavily pretreated advanced TNBC.	Metastatic or unresectable recurrent TNBC	NCT04303741
Cyclin-dependent kinase inhibitors	Pembrolizumab	Abemaciclib	Ib	28	Objective response was achieved in 14% of patients with HR-positive, HER-negative metastatic breast cancer.	HR-positive HER-negative breast cancer	NCT02779751
Microtubule inhibitor	Pembrolizumab	Eribulin	Ib/II	167	The combination was generally well tolerated and showed encouraging antitumor activity with an ORR of 23.4%, median OS of 15.5 months and median PFS of 4.1 months.	Metastatic TNBC	
Anti-PD-1 and anti-PD-L1 combinations	Durvalumab	Tremelimumab	I	17	Objective response was achieved by 3 (17%) patients overall; all 3 had TNBC, so objective response for this group was 43% (n=7).	Metastatic HER2-negative breast cancer	NCT02536794

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neoadjuvant setting- the durvalumab-treated arm demonstrated a superior pCR, particularly in the PD-L1-positive subgroup (63). In agreement, a phase Ib study involving 60 high-risk, early-stage TNBC patients displayed a pCR rate of 60% following a combination of pembrolizumab and chemotherapy as neoadjuvant therapy (64). Interestingly, the results from the study also reported a positive correlation between pCR and PD-L1 expression along with stromal TILs. In addition, the IMpassion 031 study recruiting 333 patients explored the outcome of atezolizumab in conjunction with chemotherapy as neoadjuvant therapy in early TNBC subjects (65). Their results suggested that the combination treatment led to a dramatic increase in pCR rate, implying the potential application of atezolizumab as an alternative therapy for patients with TNBC. Besides, an ongoing MIRINAE study is comparing the efficacy of atezolizumab plus capecitabine versus capecitabine alone among TNBC patients having residual tumors following neoadjuvant therapy (NCT03756298). On a similar note, a phase III study examining pembrolizumab plus chemotherapy as neoadjuvant treatment for early-stage TNBC revealed that pembrolizumab successfully increased the pCR rate. Also, data hinted that patients with a heavier tumor burden, advanced stage of the disease and with positive lymph nodes may especially benefit from pembrolizumab (66). Also, neoadjuvant chemotherapy plus pembrolizumab in 250 patients prior to surgery showed a significantly higher pCR rate among the TNBC population, a result that is consistent with the findings from the KEYNOTE-522 study (32). The I-SPY 2 trial (NCT01042379), involving early-stage TNBC patients, initially showed that pembrolizumab administered with neoadjuvant paclitaxel followed by chemotherapy (doxorubicin and cyclophosphamide) resulted in a notably enhanced pCR rate from 22% to 60% and, this was most probably due to the known immunostimulatory effects of anthracyclines. The efficacy of a treatment regimen constituted of pembrolizumab in combination with paclitaxel plus carboplatin followed by anthracycline plus cyclophosphamide as neoadjuvant therapy prior to surgery, and cycles of pembrolizumab administration as adjuvant therapy, was investigated in the KEYNOTE-522 trial (NCT03036488). The pCR rates escalated from 51.2% to 64.8% (NCT03036488). Extensive follow-up research and long-term immune-related adverse effects need to be thoroughly determined to strengthen the observations. Two ongoing key trials are addressing the effect of a year-long adjuvant anti-PD-1/PD-L1 therapy on the survival of TNBC patients- firstly, the SWOG S1418/BR006 trial (NCT02954874) involving pembrolizumab for patients with residual disease and, secondly, the A-brave trial (NCT02926196) examining avelumab for individuals with high-risk or residual disease. In accordance, two additional trials are inspecting the efficacy of atezolizumab in combination with both neoadjuvant and adjuvant therapy on patient survival outcomes- the placebo-controlled NSABP B-59 trial (NCT03281954) testing the efficacy of atezolizumab plus neoadjuvant chemotherapy prior to adjuvant atezolizumab for one year, and the IMpassion030 trial (NCT03498716) studying the standard adjuvant chemotherapy with or without atezolizumab before an annual regime of adjuvant atezolizumab.

4 Emerging concepts to further improve immunotherapy-involving cancer stem cells, tumor infiltrating lymphocytes and microbiota

Extensive research and clinical trials have enabled the slow but gradual integration of immunotherapy as a mainstream treatment strategy in conjunction to existing modules for breast cancer. In addition to the well-established regimes of immunotherapy, as discussed in earlier sections, there is increasing interest in the therapeutic efficacy of other components of the immune system, such as the TILs, as well as various oncogenic modifiers, including cancer stem cells and the microbiota.

4.1 Breast cancer stem cells as candidate for immunotherapy

Cancer stem cells, in contrast to other cancer cells, are slow-dividing with a repressed tendency to undergo apoptosis and more agile in terms of DNA repair. These features render the cancer stem cells exceptionally refractory to traditional methods of treatment, like irradiation or chemotherapy. Cancer stem cells are known to express ABC drug transporters, which may explain the underlying mechanism towards their resistant nature (67). Disease relapse and tumor metastasis commonly arise from cancer stem cells that are not affected by traditional anticancer therapy. Elimination of breast cancer stem cells (BCSCs) may be accomplished through immunotherapy, which is likely to improve treatment outcomes for breast cancer patients. Although numerous attempts have been made to target specific CSC markers using preclinical models employing various immunotherapeutic approaches, the biggest hurdle has been posed by the non-uniqueness of these markers since most of them are also expressed by normal stem cells. CSCs found in TNBC patients are highly heterogeneous and dynamic, demonstrating variable responses to chemotherapy. Again, HER2-positive BCSCs are characterized by $CD44^{high}/CD24^{low}$ phenotype and ALDH1 expression and they support resistance to anti-HER2 therapy, including trastuzumab. Importantly, this population of cells are frequently detected in recurrent breast cancer and not in primary tumors (68). Immunotherapeutic interventions seek to target BCSCs by utilizing immune cells such as NK cells, $CD8^+$ T cells and γδ T cells (69). Till date, many surface markers have been reported for BCSCs including CD90, CD49, CD44, CD24, ALDH and EpCAM (70). Elimination of CSCs was achieved *in vitro* in breast cancer cell lines with ALDH-specific $CD8^+$ T cells, which resulted in significant amelioration of mammary tumor development and metastases with prolonged survival (71, 72). Clinical trials are presently investigating CAR-T cells targeting CD44v6 (NCT04430595) and EpCAM (NCT02915445) surface antigens as an effective anticancer module for advanced breast carcinoma. Other studies recorded that BCSC-DCs can effectively inhibit BCSC proliferation when administered into the circulation of BCSC tumor-harboring rodents, suggesting the therapeutic

potential of BCSC antigen-primed DCs for elimination of BCSCs (73). These results were further strengthened in additional murine models, wherein BCSC-primed DCs had a positive effect on the survival time by 70% (74).

Vaccination strategies based on DCs encompass either antigen-defined vaccines or polyvalent vaccines (75). In a mouse model of spontaneous mammary tumorigenesis, a DC-based vaccine specifically targeting HER-2/neu led to the production of anti-neu antibodies along with T-cell mediated expression of interferon-γ, resulting in tumor regression (76). Encouraging results were also observed in patients with metastatic breast carcinoma, who were administered with lysate-pulsed DCs (NCT02063893). Nonetheless, immunotherapy approaches that target a single antigen often fail to eradicate the population of cells that contribute to tumor initiation or cancer metastasis; therefore, this significantly lowers the long-term success of this strategy. To circumvent this concern, emerging studies suggest targeting of multiple antigens for an effective response and one way to accomplish this is through polyvalent vaccines. In stage IV melanoma, a DC/irradiated tumor vaccine displayed complete tumor remission in 3 patients and a partial disease remission in an additional 3 out of 46 patients (77). In an interesting study, human heterokaryons were prepared that expressed both breast tumor-associated antigens and costimulatory molecules derived from DCs. These functionally active fusion cells could successfully induce autologous T cell proliferation and stimulate cytotoxic-T lymphocyte activity to fight against autologous breast cancer cells (78). Development of a polyvalent vaccine for BCSCs requires identification of as many antigens as possible that are unique to BCSCs. Determining the presence of mutations that facilitate the stem cell-like phenotype in BCSCs and the underlying mechanisms may unearth important avenues for immunotherapy. Moreover, chemokine receptors that promote migration of BCSCs can also be explored as future targets for immunotherapy. Overall, harnessing DC-based vaccines may be a viable option for targeted elimination of BCSCs (79). Immuno-targeting of BCSCs holds great clinical significance in an adjuvant setting as it can abrogate the BCSC population and can, therefore, improve the outcome of existing therapies.

4.2 The involvement of TILs in immunotherapy response in breast cancer

TILs are vital indicators of tumor immunogenicity (80), hence, the presence of TILs serves as a prognostic marker in many malignancies, including breast cancer (81). TILs collectively constitute of the T lymphocytes ($CD8^+$, $CD4^+$ and T_{reg} cells), B lymphocytes and natural killer cells present within the tumor. These lymphocytes impart crucial functions in breast carcinogenesis and immune recognition. The basic mode of action of $CD8^+$ T cells is the induction of direct cytotoxicity to the cancer cells whereas $CD4^+$ T cells primarily promote release of inflammatory cytokines to evoke anticancer immunity (82). On the other hand, the $CD4^+$ T_{reg} population promotes an immune-suppressive environment by restricting the activation and subsequent function of $CD8^+$ T cells (83). TILs are considered responsible for superior disease outcomes among breast cancer patients and are associated with relapse-free survival (84). However, we still do not entirely understand the T cell subtypes in breast carcinoma. One subset of the $CD8^+$ TILs is represented by the $CD8^+$ tissue-resident memory (TRM) cells that express cytotoxic molecules and immune checkpoint factors (85). These cells were found to positively correlate with higher relapse-free survival in TNBC patients (86). The presence of TRMs also favor improved prognosis among early-stage TNBC patients, denoted by better survival and reduced rates of tumor recurrence. Again, the presence of $CD39^+PD\text{-}1^+CD8^+$ T cells in the tumors is intimately connected with prolonged DFS of breast cancer patients (87). Importantly, the $FOXP3^+$ T_{reg} cells contribute to more aggressive outcomes in breast cancer, characterized by an enhanced likelihood of relapse and poor survival (88). A study found that the intra-tumoral infiltration of $CD8^+$ T cells led to a notable drop in the risk of death among 12,439 breast cancer patients. This was especially evident for TNBC and HER2+ tumors, who demonstrated a 28% reduction in mortality while ER+, HER2+ tumors had a 27% reduction in mortality (89). TIL therapy involves isolation of TILs from patients and expanding them in an *ex vivo* setup with considerable amounts of IL-2 and other necessary cytokines, followed by their re-infusion into the patient (90). Since TNBC patients express increased number of neoantigens relative to other subtypes, as revealed by whole genome sequencing of breast cancer tissues, TNBC patients may serve as possible candidates for TIL therapy (91). In accordance, preliminary data from an ongoing trial (NCT01174121) has reported tumor regression in a subset of patients in response to TIL therapy (92).

Despite the emerging studies, the effects of the intra-tumoral population of immune cells in dictating response of breast cancer patients to different modes of treatment, specifically immunotherapy, are not fully defined. Importantly, the proportion of the intra-tumoral immune infiltrates is an important factor that determines breast cancer patient response to therapy. In the SweBCG91RT trial, early-stage breast cancer patients possessing immune infiltrates with anti-tumor effects exhibited a lower risk of tumor recurrence (93). Limited benefits were observed in the test subjects following addition of radiotherapy. A high TIL count has been shown to promote sensitization of tumors to chemotherapy, resulting in a high pCR to pre-operative chemotherapy among primary breast cancer patients (94). Another study involving around 3,000 breast cancer patients found that increased TIL counts exerted a survival benefit with an improved response to neoadjuvant chemotherapy in TNBC and HER2-enriched mammary tumors (95). On the contrary, a high TIL count was associated with adverse prognosis in luminal breast cancer. Furthermore, DFS was sharply worse for TNBC patients with TIL^{low} tumors compared to patients with TIL^{high} tumors (96). TILs are, therefore, intimately involved in tumor prognosis, chemotherapeutic outcome and selection of immunotherapy or adoptive cell therapy in TNBC patients (97). Till date, most studies have focused on the prognostic relevance of TILs in breast cancer. However, attractive properties such as diversity of the

receptors, tumor specificity, and lack of toxicity have pushed TILs as a promising candidate for therapy (98).

4.3 A peek into the role of microbiota as a potent regulator of breast cancer development and response to immunotherapy

4.3.1 Microbiota in breast TME

Distinct differences in the composition of microbiota in the mammary tumor microenvironment of breast cancer patients compared to healthy subjects and, also, between tumor versus adjacent normal tissues have been observed (99). For instance, the abundance of Enterobacteriaceae, Staphylococcus, and Bacillus within the mammary tumor tissues among 71 breast cancer patients was noted (100). Another study found a significant enrichment of *Sphingomonas yanoikuyae* in normal tissues while *Methylobacterium radiotolerans* was abundant in the paired breast tumor tissues (101). *Sphingomonas* is known to regulate estrogen metabolism and activation of pathways associated with Toll-like receptor (TLR) 5, which can affect initiation of breast cancer (102) while colonization by *Methylobacterium* may be involved in ER modulation (101). In general, members of the phyla Proteobacteria, Firmicutes, and Actinobacteria were found to be particularly enriched in breast cancer. Other studies demonstrated reduced presence of the families, Alcaligenaceae, Clostridia, Pseudomonadaceae, Ruminococcaceae, and Sphingomonadaceae, in tumor-adjacent normal tissues relative to mammary tumor tissues whereas Caulobacteraceae, Methylobacteriaceae, Micrococcaceae, Nocardioidaceae, Propionicimonas, and Rhodobacteraceae were enriched in the breast carcinomas (103). The same study reported a decrease in the family Bacteroidaceae with an augmented presence of the genus Agrococcus with advancement of the disease, indicating that the microbiota dynamically changes with breast cancer progression. Furthermore, enrichment of several genera, such as, Fusobacterium, Atopobium, Gluconacetobacter, Hydrogenophaga, and Lactobacillus has been correlated with breast malignancy (99). Decreased breast cancer cell survival due to the presence of *Pseudomonas aeruginosa*, a pathogen found inside the breast was also observed (104). In addition, a study involving 668 breast tumor tissues from The Cancer Genome Atlas (TCGA) data set suggested a strong correlation between EMT-related genes with the presence of *Listeria fleischmannii*, while *Haemophilus influenzae* was associated with tumor growth, cell cycle progression, and mitotic spindle assembly (105). Again, *Staphylococcus epidermidis* facilitate a highly inflammatory tumor microenvironment, through induction of pro-inflammatory cytokines and complement activation, which favored tumor growth while treatment with antibiotic ameliorated these effects (106). Fu et al. further showed the presence of tumor-resident microbiota in a spontaneous murine breast cancer model that stimulated metastatic progression (107). However, the precise effects of these breast tumor-residing microbes on response to immunotherapy remain to be investigated and validated.

4.3.2 Pleiotropic effects of microbial metabolites on breast carcinogenesis

The intestinal microbiota is responsible for the production of short-chain fatty acids (SCFAs), such as acetate, butyrate, lactate, and propionate, which are important constituents of the tumor microenvironment. Microbial metabolites enter the circulation and exert pleiotropic anticancer effects in target cells. Interestingly, the presence of SCFA-producing bacteria was found to be considerably decreased among premenopausal patients with breast cancer in comparison to healthy premenopausal women (108). Microbial dysbiosis alters the bacterial metabolites to favor multiple hallmarks of cancer, including cell proliferation, apoptosis, metabolism, invasion, inflammation and immune regulation (109, 110). Sodium butyrate, for example, enhances oxygen consumption in breast cancer cells (111). Increased breast cancer cell death is observed following treatment with butyrate or inhibition of lactate metabolism (112, 113). SCFAs reportedly crosstalk with the immune environment as well, and are known to stimulate secretion of cytokines, such as IL-17, IFN-γ, IL-10 among others, and promote T cell differentiation. Butyrate has been shown to metabolically rewire activated $CD8^+$ T cells that influences the transition of $CD8^+$ T cells to memory cells (114). A recent study in a cohort of TNBC patients demonstrated a correlation between enrichment of Clostridiales in tumor tissues with an activated immune microenvironment. This bacterium is responsible for the production of metabolite, trimethylamine N-oxide, which imparts activation of M1 macrophages and $CD8^+$ T cell-mediated antitumor response, thus opening avenues for understanding its effect on immunotherapy (115). In melanoma patients, responders to immunotherapy usually exhibit an abundance of butyrate-producing microbiota relative to non-responders (116). Additionally, butyrate improved the efficacy of anti-PD-1 therapy *via* enhanced T cell infiltration in the tumor microenvironment in colorectal carcinoma murine model (117). Although such evidence clearly point towards the close interactions between microbial metabolites and the immune system, there is a lack of understanding regarding the mechanisms of metabolite-induced changes in immunotherapeutic response across breast cancer patients. Future studies should also focus on the implications of supplementation with such metabolites as an adjunct regime for immunotherapy in breast cancer.

4.3.3 Modulation of the microbiota as a strategy for overcoming resistance to immunotherapy

Multiple studies have confirmed the involvement of host microbiota in oncogenesis and therapeutic response (118). A seminal study showed the attenuated effects of anticancer treatment in mice with depleted gut microbiota (either due to treatment with an antibiotic or housing in germ-free conditions), suggesting that the host microbiota is a critical determinant of therapeutic response (119–121). Study examining the effects of gut microbiota on tumor suppressive efficacy of trastuzumab in HER2-positive breast cancer revealed that antibiotic exposure or FMT from antibiotic-treated mice greatly impair the antitumor activity of trastuzumab (122). In fact, HER2-positive breast carcinoma

patients refractory to trastuzumab treatment, demonstrated a lower α-diversity and reduced abundance of *Bifidobacteriaceae*, *Prevotellaceae*, *Lachnospiraceae*, and *Turicibacteraceae* compared to individuals who achieved pCR (122). A direct interaction between the gut microbiota and patient responsiveness to therapy implies that modulation of the gut microbiota may be explored to achieve optimal ICI efficacy. As microbial dysbiosis strongly influences local and systemic antitumor immune response (119), an intricate connection between ICIs' efficacy and host microbiota has also been observed. Gut microbial community strongly influences the antitumor immune responses through modulation of $CD8^+$ T cells, T helper 1 (T_h1) and tumor-associated myeloid cells (120, 121, 123). Multiple landmark efforts, subsequently, in murine models recognized the association between the gut microbiota and ICI effectiveness. Responses to anti-PD-L1 therapy alter based on the gut microbiota composition which can be modulated with fecal microbial transfer (FMT) or co-housing approach. Of note, oral administration of *Bifidobacterium* augmented the maturation of DCs and $CD8^+$ T cells priming and tumor infiltration, which restored the antitumor efficacy of PD-L1 therapy (124). In agreement, supplementation with *Bacteroides fragilis* along with *Bacteroides thetaiotaomicron* or *Burkholderia cepacian* enhanced the anti-tumor effects of anti-CTLA-4 blockade in microbiota-depleted mice (125). Other studies also showed enhanced efficacy of ICIs *in vivo* following treatment with several bacterial strains such as *Lactobacillus johnsonii*, *Bifidobacterium pseudolongum*, and *Olsenella* species (126). Interventions such as FMT, probiotic and prebiotic supplementation are presently being interrogated to determine the impact of restoration of the gut microbiota on therapeutic efficacy of various modes of immunotherapy (Figure 2). For instance, a clinical trial in patients with breast cancer is delineating the outcome of probiotics administration (13 strains of beneficial bacteria) on $CD8^+$ T cell infiltration in the tumor microenvironment (NCT03358511). The collated evidence, thus, points to the need for future clinical research to test if manipulation of the host microbiota may aid in improving immunotherapy outcomes in patients with breast carcinoma.

5 Perspectives of combined therapy modules in breast cancer and avenues for future research

Currently, despite its immense potential, the efficacy of immunotherapy as monotherapy is quite limited in solid tumors. Emerging results clearly point towards the benefits of the combinatorial approaches involving immunotherapy and conventional treatment modules but there are certain aspects that

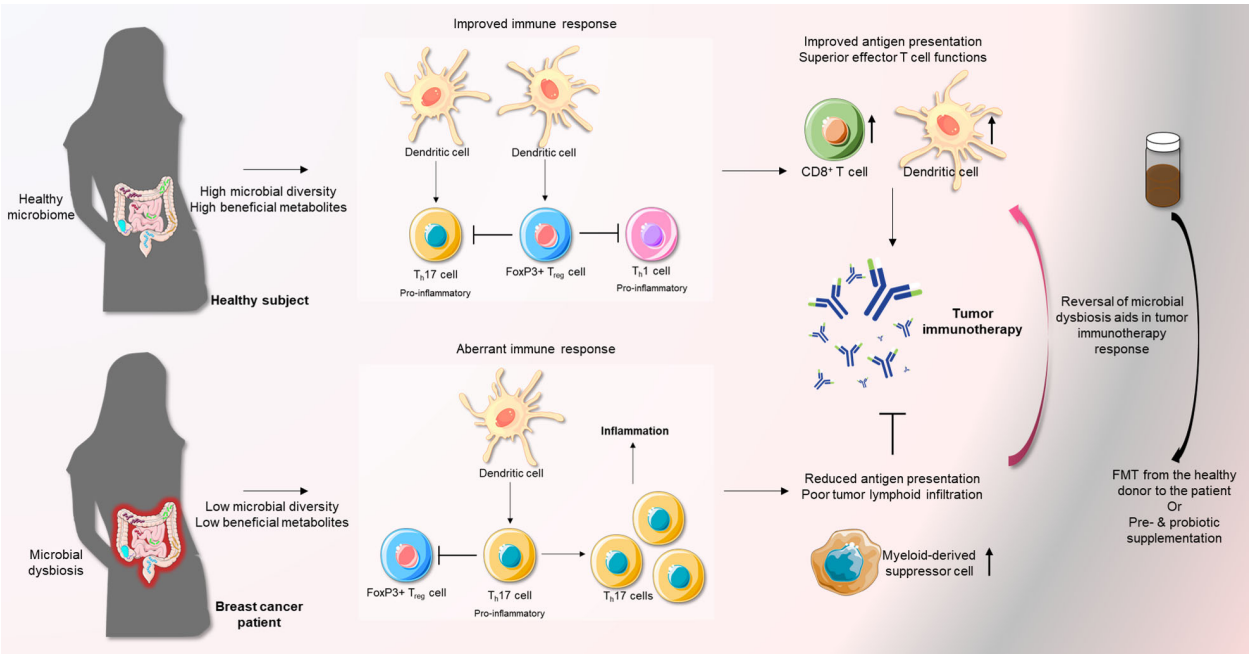

FIGURE 2
A favorable microbiota strengthens the anti-tumor immune response mediated by immunotherapy. Normally, a healthy microbiota maintains a state of equilibrium of T_h17 cells and FoxP3+ T_{reg} cells. Overall, this inhibits pro-inflammatory immune responses. However, microbial dysbiosis, an important risk factor of breast cancer, can abrogate this regulation and induce a state of inflammation by favoring T_h17 pro-inflammatory T cells over regulatory FoxP3+ T cells. This can severely impede the effects of immune-therapy. Remodeling of the microbiota in breast cancer patients through the use of FMT or supplementation with pre-/probiotics can facilitate a more favorable state of immune response, especially in the presence of immunotherapy.

demand additional in-depth research, such as the precise timing of intervention, optimal drug combinations, and the order of administration of drug combinations. Identifying potentially responsive tumors is also extremely important as the efficacy of ICIs varies among all tumor types and, in certain cases, there is the occurrence of immune-related adverse events (irAEs). In addition to PD-1/PD-L1 inhibitors, other immunotherapy modalities, such as CTLA-4 inhibitors, CAR T cell therapy and tumor vaccines are also being investigated in combination strategies. Development of vaccines to enhance anticancer immunity is another upcoming strategy to target breast cancer. Presentation of breast cancer peptides to T cells through these vaccines can stimulate T cell priming and activation in addition to boosting immune recognition of cancer cells. At present, several clinical trials with a goal to identify the efficacy of breast cancer vaccines in combination with PD-1/PD-L1 inhibitors in TNBC setting are ongoing. Interestingly, neoantigen vaccines are designed to target the peptides procured from tumor-specific mutations, absent in normal cells, and unique to the tumor of the patient for minimizing self-tolerance (127). A randomized phase I study will determine the impact of a neoantigen vaccine plus durvalumab among patients with residual TNBC following neoadjuvant therapy (NCT03199040). Another phase II trial is enrolling mTNBC patients, who have not been exposed to any form of treatment, in addition to those mTNBC subjects, who have been treated with chemotherapy (gemcitabine and carboplatin) for 18 weeks, to examine the effects of nab-paclitaxel plus durvalumab in conjunction with a neoantigen vaccine (NCT03606967). Another advance in immunotherapy repertoire is the CAR T cell therapy engineered for specific targeting of tumor antigens. Albeit preliminary, studies have determined that intra-tumoral administration of engineered CAR T cells does not elicit any serious adverse effects in patients with metastatic breast cancer (NCT01837602) (128). These upcoming promising immunotherapies warrant additional preclinical, translational and clinical studies to improve the existing treatment regime for breast cancer patients.

Results from the current trials suggest that TNBC patients at earlier stages of the disease responded better to combination therapy but the prognosis of advanced TNBC has scopes for considerable improvement. More elaborate studies need to be designed for assessing the long-term synergistic interactions between immunotherapies with chemotherapies. Efforts are required to consider the plausible toxicity profile that may be associated with such new treatment modalities. Since the immune system is highly variable from person to person, studies need to focus on the differential tolerance to such combination therapies amongst different cohorts of patients. Customization of precision immunotherapies assisted through predictive biomarkers is expected to enhance the clinical efficacy and responsiveness to therapy among patients, thus making this an important and interesting area of further research. Emerging studies have pointed that race may be a contributing factor to dictating the responsiveness of breast cancer patients to therapy- a recent study showed the role of racial disparity in response to immunotherapy among Asian breast cancer patients (129). This underscores the importance of conducting investigations to ensure the effectiveness of the combination approaches for immunotherapy among breast cancer patients. For TNBC patients, we need to explore predictive markers to identify the responders versus non-responders across TNBC subtypes, such as basal-like, mesenchymal stem cell-like, etc. which promotes the observed heterogeneity in clinical efficacy of the combinatorial immunotherapy-based strategies. Despite the promising potential of the combination strategies for breast cancer patients, the extremely high cost of this type of treatment makes it relatively hard to pursue, especially for a long duration. Consequently, future studies should try to implement better ways to make this form of therapy reasonably feasible and accessible for all compliant patients.

Clinical and preclinical data indicate the presence of complex and dynamic interactions between various components of the immune system that need to be further comprehended to achieve improved treatment outcomes. Although immune-based treatment modalities have gained momentum in the last few years as key therapy in multiple cancers, more rigorous clinical trials are required to prove the clinical efficacy of these agents in breast carcinoma. Modulation of the tumor microenvironment represents an unexplored area of increasing interest as this can be altered to facilitate drug delivery and improve cytotoxicity. For instance, antiangiogenic therapy has not yielded significant results for the treatment of HER2-positive breast cancer patients (130), but immune evasion through $CD8^+$ T cell suppression or other mechanisms brought upon by proangiogenic stimuli, such as increased VEGF production, supports the idea of developing antiangiogenic agents in conjunction with ICI as a novel therapeutic approach (131). The innovation of immunotherapies to target HER2-positive breast carcinoma requires close attention to the concerns of favorable efficacy to toxicity ratio. Notably, contemporary evidences suggest that HER2-directed vaccines exhibit favorable toxicity profiles with minor side-effects while adoptive T cell-based therapies have, unfortunately, been associated with greater side-effects (132). Multiple small studies established that a decrease in TIL counts and PD-L1 expression is mostly more common in metastatic lesions relative to primary breast tumors (133, 134). In agreement, one study with paired primary and metastatic breast cancer samples unveiled that metastatic breast cancer tissues were characterized by the downregulation of immunotherapy drug targets, pro-inflammatory cytokines and antigen presentation, along with upregulation of molecules that support immunosuppression (135). Such results hint at the immune-depleted nature of metastatic breast cancers compared to primary tumors. Therefore, a combinatorial approach to enhance the immune response of metastatic breast cancer may prove more beneficial for such immunologically inert tumors. In addition, a more thorough and intensive understanding of the tumor microenvironment may successfully enable a durable and potent anti-tumor response from the combination therapies. Despite these hurdles, activation of the immune system is closely related to self-sustaining and prolonged tumor suppressive actions and numerous patients are likely to benefit from well-designed immunotherapies with limited side-effects.

Author contributions

DN: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Writing – original draft. DS: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

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

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Efficacy and safety of anlotinib combined with immune checkpoint inhibitors and platinum-containing chemotherapy for later-line advanced non-small cell lung cancer: a retrospective three-arm real-world study using propensity-score matching

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Objective: To assess the efficacy and safety of anlotinib combined with immune checkpoint inhibitors (ICIs) in patients with advanced non-small-cell lung cancer (NSCLC).

Methods: Clinical data on patients with advanced NSCLC were collected from June 2019 to October 2022 at Hebei General Hospital, China. The efficacy and safety of anlotinib combined with ICIs and platinum-containing chemotherapy were retrospectively analyzed. The primary endpoint was progression-free survival (PFS). The secondary endpoint was the disease control rate (DCR) and overall survival (OS). Survival curves were created using the Kaplan–Meier method. The efficacy and adverse reactions were evaluated according to the RECIST 1.1 and CTCAE 5.0 standards.

Results: A total of 54 patients were enrolled in this study after propensity score matching (PSM), including 27 men and 17 women, with a median age of 59. A total of 26 patients received anlotinib + ICIs + platinum-containing chemotherapy (AIC), 15 patients received anlotinib + platinum-containing chemotherapy (AC), and 13 patients received ICIs + platinum-containing chemotherapy (IC). The PFS of the AIC group was 7.76 months (95% CI: 3.71–NC). The DCR was 65.38%. The OS endpoint had not been reached, The AIC combination regimen group had a significantly longer PFS than the IC group (mPFS, 7.76 vs. 2.33 months, p=0.012, HR=0.23, 95% CI: 0.06–0.8). There was no

significant difference in the DCR between the two groups (65.38% vs. 53.85%, p=0.326). There was a statistically significant difference in PFS between the AC group and the IC group (mPFS, 9.2 vs. 2.33 months, p=0.02, HR=0.14, 95% CI: 0.03–0.65). There was no significant difference in the DCR between the two groups (40% vs. 53.85%, p=0.445). The common adverse reactions of the combination of anti-angiogenic agents, ICIs, and platinum-containing chemotherapy were anemia (34.62%), allergic reactions (19.23%), thrombocytopenia (11.54%), gastrointestinal reactions (15.38%), and hepatobiliary disorders (11.54%). Most of them were manageable.

Conclusions: Anlotinib combined with immune checkpoint inhibitors and platinum-containing chemotherapy regimens offers a good survival benefit for patients with advanced non-small-cell lung cancer who fail to respond to standard therapy. When both efficacy and safety are considered, a combination of anti-angiogenic agents, ICIs, and platinum-containing chemotherapy can be used as a choice for the treatment of advanced NSCLC.

KEYWORDS

anlotinib, immune checkpoint inhibitors, PD-1/PD-L1, angiogenesis inhibitors, combination therapy

1 Introduction

Globally, NSCLC represents the most common cancer in men and the third most common cancer in women (1). In China, the age-standardized incidence rates of lung cancer for male and female populations are 48.87 and 23.52 per 100,000, respectively (2).

Vascular endothelial growth factor (VEGFR)-associated multi-targeted tyrosine kinase inhibitors (TKIs) and ICIs have achieved commendable success in treating both NSCLC and SCLC. Angiogenesis inhibitors can effectively inhibit tumor proliferation and metastasis. Anlotinib is an orally administered small-molecule kinase inhibitor that blocks the activity of several protein kinases, including those involved in tumor pathogenesis, such as VEGFR, fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), and c-kit (3–5). In large randomized placebo-controlled trials, such as the ALTER series (6, 7), anlotinib was associated with a survival benefit in patients with NSCLC who progressed on standard therapies. Therefore, anlotinib represents a potential further line of therapy in this otherwise treatment-refractory population. Anlotinib has been approved as a third-line therapy for NSCLC in China.

ICIs have revolutionized the treatment of NSCLC by harnessing the power of the immune system to target cancer cells (8). These agents block the immune checkpoints that tumors use to evade detection by the immune system, thereby enhancing the immune response against cancer (9). ICIs such as nivolumab and pembrolizumab have been approved for treating advanced NSCLC, demonstrating improved survival outcomes compared to traditional chemotherapy (10). The potential of ICIs in combination with other therapies, including antiangiogenic drugs, is an area of active investigation (11).

Some clinical studies have explored the efficacy of antiangiogenic therapy plus chemotherapy or ICIs in treating NSCLC. Currently, the IMpower150 study (NCT02366143), an open-label phase III randomized controlled trial (RCT), has explored the efficacy of the first-line treatment with chemotherapy plus angiogenesis inhibitors and ICIs in advanced non-squamous NSCLC. The results showed that the combined regimen had favorable clinical effects compared to the non-combined treatment regimen (12). Some scholars have started to study combination therapy with anlotinib and ICIs for advanced solid tumors (13, 14). The combination of anti-angiogenic agents, ICIs, and platinum-containing chemotherapy is effective and well tolerated in the second- or later-line treatment of advanced solid tumors.

In this retrospective study, we conducted a three-arm retrospective real-world analysis of patients receiving anlotinib, ICIs, and platinum-containing chemotherapy who had progressed on more than two lines of therapy at our institution.

Abbreviations: NSCLC, non-small cell lung cancer; ICIs, immune checkpoint inhibitors; VEGFR, Vascular Endothelial Growth Factor; FGFR, fibroblast growth factor receptor; PDGFR, platelet-derived growth factor receptor; RCT, randomized controlled trial. TKI, tyrosine kinase inhibitors; PFS, progression-free survival; OS, overall survival; DCR, disease control rate; PR, partial response; SD, stable disease; AIC, Anlotinib + ICIs + platinum-containing chemotherapy; IC, ICIs + platinum-containing chemotherapy; AC, anlotinib + platinum-containing chemotherapy; PSM, propensity score matching; AEs, adverse events.

2 Materials and methods

2.1 Study design

This was a retrospective, single-center, real-world study to evaluate the effectiveness and safety of anlotinib, ICIs, and platinum-containing chemotherapy for patients with advanced NSCLC. A total of 67 patients with advanced lung cancer were included between June 2019 and October 2022. The study was conducted following the Declaration of Helsinki and was approved by the ethics committee institutional review board of Hebei General Hospital. Informed consent from patients was exempted from the ethical review.

2.2 Patients

All patients were aged 18–80 years, had histopathologically or cytologically confirmed advanced primary NSCLC according to the Guidelines for the Diagnosis and Treatment of Primary Lung Cancer (2022 edition) in China, and received at least two cycles of combined therapy for the study.

The inclusion criteria were as follows (1): age ≥ 18 years (2), primary non-small-cell lung cancer diagnosed by cytology or histology, and (3) hospitalization ≥2 times.

The exclusion criteria were as follows (1): patients who did not receive the combination of anlotinib + ICIs + platinum-containing chemotherapy, anlotinib + platinum-containing chemotherapy, or ICIs + platinum-containing chemotherapy regimens (2); TNM staging for patients in stages I and II; and (3) patients with missing key data (such as organizational credit type).

Patients were also required to have survival data, adverse events (AEs), and at least one follow-up radiological information (computed tomography). Patients who underwent pregnancy or lactation were excluded from this study. The flowchart of the retrospective study is shown in Figure 1.

2.3 Procedures and treatment

The study patients were divided into three groups according to the medication plan: AIC, AC, or IC regimen. Anlotinib was given orally once daily with an initial dose of 8–12 mg (day 1–14, every 3 weeks per cycle; Chia-tai Tianqing Pharmaceutical Co., Ltd., Nanjing, China). The ICIs, including Sintilimab (200 mg every 3 weeks; Jiangsu Hengrui Pharmaceutical Co., Ltd., Lianyungang, China), Camrelizumab (200 mg every 3 weeks; Jiangsu Hengrui Pharmaceutical Co., Ltd., Lianyungang, China), Tislelizumab (200 mg every 3 weeks; BeiGene Co., Ltd., Shanghai, China), Nivolumab (3 mg/kg every 3 weeks; Bristol-Myers Squibb), and Pembrolizumab (200 mg every 3 weeks; MSD R&D (China) Co., Ltd., Shanghai, China), were administered via an intravenous drip. The intravenous platinum chemotherapy consisted of 40 mg/m^2 infusions of Cisplatin (Qilu Medicine Co., Ltd., China) or AUC 4–6 infusions of Carboplatin (Qilu Medicine Co., Ltd., China) for 1 h. Discontinuation, suspension, and dose modification were allowed according to disease progression or AEs.

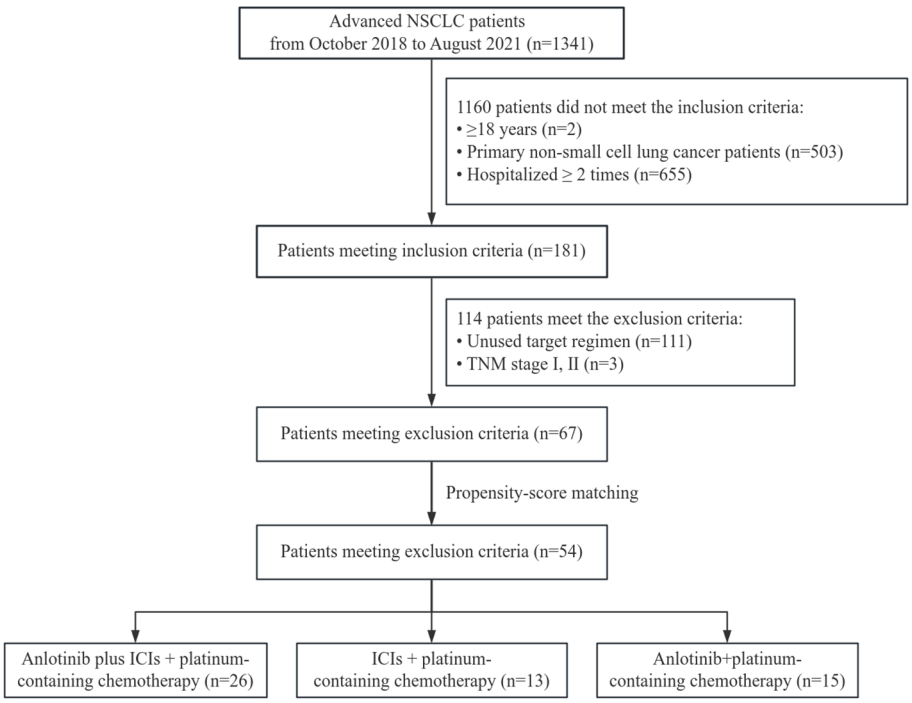

FIGURE 1
Flow chart of the retrospective study.

2.4 Treatment evaluation

Information on the patient's demographic characteristics, laboratory test results, radiological information, survival data, and AEs was collected retrospectively. The tumor response was assessed by the investigator according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 using computed tomography scans.

The primary endpoint was PFS, defined as the time from the first medication to the occurrence of disease progression or death from any cause. The secondary endpoints included the DCR, OS, and safety. The DCR was defined as the proportion of patients with confirmed complete response, partial response (PR), or stable disease (SD) at the best response. Progressive disease was defined radiographically based on the radiologist's interpretation. Disease control was defined radiographically as stable disease or partial response, based on the radiologist's interpretation. The OS was defined as the time from the first medication to the occurrence of death. The Kaplan–Meier method was used to estimate PFS and OS, and Cox proportional hazards modeling was used to evaluate predictors of those outcomes.

Safety was assessed by AEs according to the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE 5.0). If the patient acquired disease progression, serious AEs, or drug toxicity, the drug should be discontinued immediately.

2.5 Statistical analysis

To eliminate confounding factors, the control test and control groups were matched by the PSM method. The control variables include gender, age, TNM stage, and histological types.

Quantitative data are statistically described using the number of cases, mean, standard deviation, median, minimum and maximum, and upper and lower quartiles. Categorical indicators are statistically described using the number and percentage of patients in each category. All data are described as median (quartile 2) and quartiles 1 and 3 (Q1–Q3). For multiple-choice categorical indicators, the number and proportion of cases in each category are listed separately.

When describing qualitative or hierarchical indicators, we list the frequency and percentage. For comparisons of unordered categorical indicators, we use the chi-square test or exact probability method (Fisher's method).

All the statistical tests were performed using two-sided tests, with the test statistics and corresponding p-values given. When using the exact probability method (Fisher's method), the p-value was directly given. A p-value ≤ 0.05 was considered statistically significant for the difference tested.

The SPSS software (version 21.0, SPSS Institute. IL., USA) was used for statistical analysis. PFS and OS were calculated by the Kaplan–Meier method and compared using a stratified log-rank test. The analysis of ORR and DCR was based on the best overall response. $p<0.05$ was considered significant.

3 Results

3.1 Demographic characteristics

A total of 1,341 patients with NSCLC were admitted to the oncology department during the study period (2019–2022). According to the inclusion and exclusion criteria, 67 patients were included. Among them, 28 patients received anlotinib + ICIs + platinum-containing chemotherapy, 26 patients received anlotinib + platinum-containing chemotherapy, and 13 patients received ICIs + platinum-containing chemotherapy. There were significant imbalances between the three groups in terms of gender and TNM stage, which were recognized as strong risk factors for the outcome and were addressed through PSM. To ensure the balance of the baseline in pairwise comparison and to increase precision, we adopted a consistent matching ratio of 2:1, which was achieved by calculating the difference within each matched set between the patients' outcome in the intra-group and the mean outcome among the inter-group (15).

After matching according to the ratio of 2:1, 54 samples remained: 26 patients received anlotinib + ICIs + platinum-containing chemotherapy, 15 patients received anlotinib + platinum-containing chemotherapy, and 13 patients received ICIs + platinum-containing chemotherapy. The baseline characteristics of the patients are shown in Tables 1, 2.

3.2 Analysis of the efficacy of different treatment regimens

3.2.1 AIC group vs. IC group

The Wilcoxon rank sum test was used to determine if there is a significant difference in age between the AIC group and the IC group. There was no statistically significant difference in age between the two groups. Fisher's exact probability method was used to compare gender, TNM stage, and histological types between the AIC group and the IC group. There were no statistically significant differences in gender, TNM stage, or histological types between the two groups (Table 1).

The Wilcoxon rank sum test was used to determine if there is a significant difference in PFS between the AIC group and the IC group. There was a statistically significant difference in PFS between the two groups, and the median (Q1–Q3) PFS in the AIC chemotherapy group was 3.68 (2.38–7.65), >1.25 (0.99–1.97) in the IC group (p=0.001).

There was a statistically significant difference in PFS between the two groups, and the median PFS of the AIC group was 7.76 months (3.71–NC) and >2.33 months (0.99–6.03) in the IC group (p=0.012). The hazard of progression was 0.23 (95% CI, 0.06–0.8). Furthermore, there was a statistically significant difference in OS between the two groups. The OS endpoint of the AIC group had not been reached, and the median OS of the IC group was 11.67 months (5.59–NC). The Kaplan–Meier survival curve is shown in Figure 2.

In this study, the AIC group shows good anti-tumor activity with a favorable response rate and a tolerable toxicity profile in patients with advanced NSCLC, of which 5 (19.25%) achieved PR, 12 (36.15%) achieved SD, and 9 (34.62%) achieved PD. The DCR was 65.38%, which was not obviously different from that of the IC group (53.85%, p=0.326).

3.2.2 AC group vs. IC group

The t-test was used to compare the age difference between the AC group and the IC group, and there was no statistically significant difference in age between the two groups. Fisher's exact probability method was used to compare gender, TNM stage, and histological types between the AC group and the IC group. There were no statistically significant differences in gender, TNM stage, or histological type between the two groups (Table 2).

The Wilcoxon rank sum test was used to compare the differences in PFS between the AC group and the IC group. There was a statistically significant difference in PFS between the two groups, and the median (Q1–Q3) PFS in the AC group was 3.62 (2.12–8.71) months and >1.25 (0.99–1.97) months in the IC group (p=0.001). There was no significant difference in OS between the two groups [median OS (Q1–Q3): 15.94 (8.05–29.54) vs. 11.67 (5.92–34.81), p=0.548].

There was a statistically significant difference in PFS between the AC group and the IC group, and the median PFS of the AC group was 9.2 months (2.14–11.8), >2.33 months (0.99–6.03) in the IC group (p=0.02). The hazard of progression was 0.14 (95% CI, 0.03–0.65). The Kaplan–Meier survival curve is shown in Figure 2.

The AC group showed similar anti-tumor activity to the IC group, of which one (6.67%) achieved PR, five (33.33%) achieved SD, and nine (60%) achieved PD. The DCR was 40%, which was not different from that of the IC group (53.85%, p=0.445).

4 Safety of different treatment regimens

In this study, we also evaluated the safety of anlotinib for the treatment of patients. AEs observed in these groups are summarized. The most common AEs are shown in Table 3. Among the three groups, myelosuppression was the most common adverse event. The AIC group had the highest incidence

TABLE 1 Baseline characteristics of study population before and after PSM with the anlotinib + ICIs + platinum-containing chemotherapy group or the ICIs+ platinum-containing chemotherapy group.

	Baseline comparison before matching				Baseline comparison after matching			
	Anlotinib plus ICIs + platinum-containing chemotherapy (N=28)	ICIs + platinum-containing chemotherapy (N=13)	Statistics	P-value	Anlotinib + ICIs + platinum-containing chemotherapy group (N=26)	ICIs + platinum-containing chemotherapy group (N=13)	Statistics	p-value
Gender								
Male	23 (82.14%)	8 (61.54%)	χ^2=1.079	0.299	21 (80.77%)	8 (61.54%)	Fisher	0.253
Female	5 (17.86%)	5 (38.46%)			5 (19.23%)	5 (38.46%)		
Age								
Mean (SD)	60.93 (10.46)	57.00 (13.02)	W=125.5	0.116	60.19 (10.48)	57.00 (13.02)	W=122.0	0.165
Median (Q1–Q3)	62.00 (56.00–65.75)	55.00 (50.00–65.00)			62.00 (56.00–64.75)	55.00 (50.00–65.00)		
TNM stage								
III	11 (39.29%)	6 (46.15%)	χ^2=0.173	0.678	11 (42.31%)	6 (46.15%)	Fisher	1.000
IV	17 (60.71%)	7 (53.85%)			15 (57.69%)	7 (53.85%)		
Histological types								
Adenocarcinoma	14 (50.0%)	9 (69.23%)	Fisher	0.302	14 (53.85%)	9 (69.23%)	Fisher	0.371
Squamous carcinoma	13 (46.43%)	3 (23.08%)			11 (42.31%)	3 (23.08%)		
Adenosquamous carcinoma	1 (3.57%)	1 (7.69%)			1 (3.85%)	1 (7.69%)		

Before PSM, gender, age, TNM stage, and histological types were statistically different between the anlotinib + ICIs + platinum-containing chemotherapy group and the ICIs+ platinum-containing chemotherapy group. After PSM, all baseline characteristics were balanced between two groups: gender (proportion of men, 80.77% vs. 61.54%, p = 0.253), age [60.19 (10.48) vs. 57.00 (13.02), p = 0.165], TNM stage (proportion of III, 42.31% vs. 46.15%, p = 1.000), histological types [adenocarcinoma (53.85% vs. 69.23%, p = 0.371)]. After matching, 39 cases were included in the PSM model. All covariates were all well matched, there were no statistical difference (p > 0.05).

TABLE 2 Baseline characteristics of study population before and after PSM with the anlotinib + platinum-containing chemotherapy or the ICIs plus platinum-containing chemotherapy.

	Baseline Comparison before Matching				Baseline Comparison after Matching			
	Anlotinib plus platinum-containing chemotherapy (N=26)	ICIs plus platinum-containing chemotherapy (N=13)	Statistics	P-value	Anlotinib plus platinum-containing chemotherapy (N=15)	ICIs plus platinum-containing chemotherapy (N=13)	Statistics	P-value
Gender								
Male	14 (53.85%)	8 (61.54%)	Fisher	0.740	8 (53.33%)	8 (61.54%)	Fisher	0.718
Female	12 (46.15%)	5 (38.46%)			7 (46.67%)	5 (38.46%)		
Age								
Mean (SD)	61.96 (9.98)	57.00 (13.02)	t=−1.283	0.207	60.33 (10.51)	57.00 (13.02)	t=0.722	0.477
Median (Q1–Q3)	62.00 (55.25–68.25)	55.00 (50.00–65.00)			60.00 (55.00–65.00)	55.00 (50.00–65.00)		
TNM stage								
III	8 (30.77%)	6 (46.15%)	Fisher	0.482	5 (33.33%)	6 (46.15%)	Fisher	0.700
IV	18 (69.23%)	7 (53.85%)			10 (66.67%)	7 (53.85%)		
Histological types								
Adenocarcinoma	18 (69.23%)	9 (69.23%)	Fisher	1.000	10 (66.67%)	9 (69.23%)	Fisher	0.37
Squamous carcinoma	6 (23.08%)	3 (23.08%)			4 (26.67%)	3 (23.08%)		
Adenosquamous carcinoma	2 (7.69%)	1 (7.69%)			1 (6.67%)	1 (7.69%)		

Before PSM, gender, age, TNM stage, and histological types were statistically different between the anlotinib + ICIs + platinum-containing chemotherapy group and the ICIs+ platinum-containing chemotherapy group. After PSM, all baseline characteristics were balanced between two groups: gender (proportion of men, 53.33% vs. 61.54%, p = 0.718), age [60.33 (10.51) vs. 57.00 (13.02), p = 0.477], TNM stage (proportion of III, 33.33% vs. 46.15%, p = 0.700), histological types [adenocarcinoma (66.67% vs. 69.23%, p = 0.370)]. After matching, 28 cases were included in the PSM model. All covariates were all well matched; there were no statistical difference (p > 0.05).

rate: 11 (42.31%) patients had neutropenia, 9 (34.62%) patients had anemia, and 3 (11.54%) patients had thrombocytopenia. Other adverse reactions with an incidence >10% were allergic reactions (19.23%), hypertension (15.38%), other gastrointestinal reactions (15.38%), and hepatobiliary disorders (11.54%). In the AIC group and AC group, four (15.38%) patients and five (33.33%) patients had hypertension, respectively. No adverse reactions of hypertension occurred in the IC group without anlotinib. Patients in the AIC and IC groups with ICIs had hepatobiliary disorders, hypothyroidism, pulmonary infection, and other immune-related AEs. The AC group without ICIs did not experience any of the above AEs.

5 Discussion

Due to the aggressive nature of non-small-cell lung cancer, patients with advanced disease who have undergone multiple chemotherapy treatments often do not respond well to treatment. Immune checkpoint inhibitors or antiangiogenic monotherapy have had only a limited response. For those patients with better performance status, a more intense combination of therapies is expected to result in a better response and prognosis.

This study is intended to evaluate the efficacy and safety of the combination of anti-angiogenic agents, ICIs, and platinum-containing chemotherapy in advanced NSCLC. We explored this issue through a retrospective analysis of clinical data. In the present study, we conducted a three-arm retrospective real-world analysis of patients taking anlotinib and ICIs and platinum-containing chemotherapy who had progressed on prior lines of therapy at our institution. Our results demonstrated the efficacy of anlotinib + ICIs + platinum-containing chemotherapy, as shown by the DCR of 65.38% with a median PFS of 7.76 months (95% CI, 3.71–NC), and the median OS has not been reached (Figure 2). The median PFS in our cohort was superior to that in patients in the earlier real-world cohort (PFS, 6.9 months; DCR, 86.6%) (16). This may be because more patients in our cohort started treatment at earlier TMN stages (stage III, 37.31% vs. 16%) (Table 1).

Moreover, compared with the ICI combined with the platinum-containing chemotherapy group, the combination of anti-angiogenic agents, ICIs, and platinum-containing chemotherapy combination regimen group had a significantly longer PFS (mPFS, 7.76 vs. 2.33 months, p=0.012, HR=0.23, 95% CI: 0.06–0.8). The DCR of the anlotinib + ICIs + platinum-containing chemotherapy group was 65.38%, which was not different from that of the ICIs + platinum-containing chemotherapy group (53.85%, p=0.326).

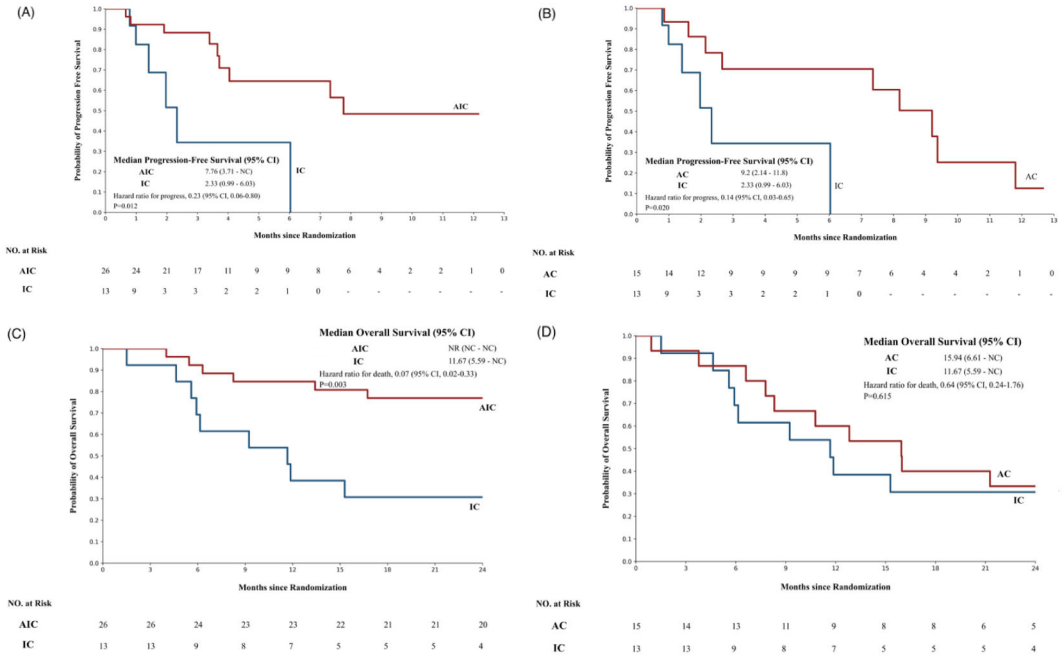

FIGURE 2
The Kaplan–Meier survival curve of different groups. **(A)** The progression-free survival of patients treated with the anlotinib + ICIs + platinum-containing chemotherapy group or the ICIs+ platinum-containing chemotherapy group. **(B)** The progression free survival of patients treated with the anlotinib + platinum-containing chemotherapy or the ICIs plus platinum-containing chemotherapy. **(C)** The overall survival of patients treated with the anlotinib +ICIs+ platinum-containing chemotherapy group or the ICIs+ platinum-containing chemotherapy group. **(D)** The overall survival of patients treated with the anlotinib + platinum-containing chemotherapy or the ICIs + platinum-containing chemotherapy. AIC, anlotinib + ICIs + platinum-containing chemotherapy; IC, ICIs + platinum-containing chemotherapy; AC, anlotinib + platinum-containing chemotherapy. In this study, the patients were divided into three groups according to the medication situation and were compared by pairwise control. The analysis was conducted after PSM of the 2:1 matching ratio(N = 54).

TABLE 3 Comparison of safety between different groups.

	Anlotinib plus ICIs + plus platinum-containing chemotherapy (N=26)	ICIs plus platinum-containing chemotherapy (N=13)	Anlotinib plus platinum-containing chemotherapy (N=15)
Neutropenia	11 (42.31%)	9 (69.23%)	6 (40.0%)
Anemia	9 (34.62%)	4 (30.77%)	5 (33.33%)
Thrombocytopenia	3 (11.54%)	2 (15.38%)	4 (26.67%)
Hypertension	4 (15.38%)	0 (0)	5 (33.33%)
Gastrointestinal reactions	4 (15.38%)	6 (46.15%)	2 (13.33%)
Allergic reactions	5 (19.23%)	1 (7.69%)	3 (20.0%)
Cough	2 (7.69%)	3 (23.08%)	1 (6.67%)
Hepatobiliary disorders	3 (11.54%)	3 (23.08%)	0 (0)
Vomit	1 (3.85%)	2 (15.38%)	2 (13.33%)
Nausea	1 (3.85%)	2 (15.38%)	2 (13.33%)
Hypothyrea	2 (7.69%)	3 (23.08)	0 (0)
Fatigue	1 (3.85%)	2 (15.38%)	0 (0)
Dyspnea	2 (7.69%)	1 (7.69%)	0 (0)
Localized edema	1 (3.85%)	2 (15.38%)	0 (0)

(Continued)

TABLE 3 Continued

	Anlotinib plus ICIs + plus platinum-containing chemotherapy (N=26)	ICIs plus platinum-containing chemotherapy (N=13)	Anlotinib plus platinum-containing chemotherapy (N=15)
Rash	0 (0)	1 (7.69%)	2 (13.33%)
Pulmonary infection	1 (3.85%)	2 (15.38%)	0 (0)
Immune-related adverse events	1 (3.85%)	1 (7.69%)	0 (0)
Proteinuria	1 (3.85%)	1 (7.69%)	1 (6.67%)
Fever	0 (0)	1 (7.69%)	0 (0)
Chest distress	1 (3.85%)	0 (0)	0 (0)
Diarrhea	0 (0)	1 (7.69%)	0 (0)

Furthermore, there was a statistically significant difference in PFS between the anlotinib + platinum-containing chemotherapy group and the ICIs + platinum-containing chemotherapy group. The median PFS of the anlotinib + platinum-containing chemotherapy group was 9.2 months (range, 2.14–11.8 months), which was longer than 2.33 months (range, 0.99–6.03 months) in the ICIs+ platinum-containing chemotherapy group ($p = 0.02$). The hazard of progression was 0.14 (95% CI, 0.03–0.65). Immune checkpoint inhibitors plus chemotherapy have not shown better efficacy for non-small-cell lung cancer (Figure 2).

Combination therapy involving anti-angiogenic agents, ICIs, and platinum-containing chemotherapy could be a treatment strategy. All the phase III clinical trials and subsequent updated data analysis support that ICIs plus chemotherapy continued to improve treatment efficacy. The addition of ICIs to standard chemotherapy continued to improve treatment efficacy compared to those in the chemotherapy group. The IMpower150 trial explored the combination of the anti-PD-L1 agent atezolizumab, the angiogenic inhibitor bevacizumab, and chemotherapy (carboplatin and paclitaxel) in the first-line treatment of advanced non-small-cell lung cancer. The addition of atezolizumab to bevacizumab plus chemotherapy significantly improved progression-free survival and overall survival, regardless of PD-L1 expression and EGFR or ALK genetic alteration status (12, 17–20).

For patients with end-stage disease progression who have undergone multiple lines of chemotherapy, ICIs often fail to fully utilize the effects of immunotherapy. Single ICIs are not effective for non-small-cell lung cancer. This finding is similar to that of a previous study (21). The combination therapy of PD-1/PD-L1 has also been shown to improve survival compared to platinum-based chemotherapy in advanced NSCLC, particularly in people with a high tumor mutational burden (TMB). The time to response (TTR) of ICIs is generally >2 months. In previous clinical trials, the average time to respond to immune checkpoint inhibitors was 1.9–3.5 months, depending on factors such as CPS status, disease, and duration of medication (22–24). Further CR cases were detected after 8 months of pembrolizumab treatment, and the results of the KEYNOTE-189 trial also showed that the health status/quality of life began to improve globally at week 21 in the pembrolizumab plus chemotherapy group compared to the placebo plus chemotherapy group (25).

In terms of safety, myelosuppression remains the most common adverse reaction of the AIC regimen. Then, there are allergic reactions, hypertension, and gastrointestinal adverse reactions in that order. The platinum-containing chemotherapy is the foundation of the treatment of non-small-cell lung cancer. The combination of anti-angiogenic agents, ICIs, and platinum-containing chemotherapy can lead to more severe bone marrow suppression, which is consistent with previous research findings (16). In the AIC group and IC group, there were a total of four patients with elevated IL-6 levels. Two of them developed pulmonary infections, one had jaundice, and one had myocarditis. These four patients, in addition to the above adverse reactions, also had elevated CRP levels and decreased blood cell counts, which may be related to immune checkpoint inhibitor-induced cytokine release syndrome (CRS). CRS refers to the phenomenon where ICIs can cause self-targeted immune toxicity by overactivating the immune system, ultimately leading to immune-related adverse reactions (26). IL-6 plays an important role in CRS immunopathogenesis, and the overexpression of IL-6 often signifies CRS (27).

Our cohort experienced a longer PFS than that reported in the ALTER0303 trial (PFS, 5.4 months) (6). This difference might be attributable to the more stringent enrolment criteria and differences in baseline demographics between patients treated with therapies containing ICIs and platinum agents, which are more or less effective for patients with metastatic cancer than chemotherapy treatments not containing ICIs or platinum agents. Our results suggest that the combination of anti-angiogenic agents, ICIs, and platinum-containing chemotherapy may offer better survival outcomes for patients with metastatic cancer compared to the two other therapeutic schedules. However, it is important to note that the optimal treatment strategy for individual patients may vary depending on their specific characteristics and disease status.

This study inevitably has limitations. First, this study was retrospective and involved only one hospital. In addition, this was a small sample study, and although we used propensity scoring to reduce bias, the statistical results were not very convincing, and the optimal patient populations for the combination therapy were not identified. Given the above limitations, our conclusions may require a larger sample size for further confirmation.

6 Conclusions

In the real-world setting, the combination of anti-angiogenic agents, ICIs, and platinum-containing chemotherapy is effective and well tolerated in the later-line treatment of advanced NSCLC, and this combination can be used as a treatment choice for advanced NSCLC. The addition of ICIs and anlotinib to the traditional chemotherapy has led to a shift in the approach to treating advanced NSCLC. However, randomized controlled studies are still needed to confirm their efficacy and safety.

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 Ethics Committee of Hebei General Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this is a retrospective study. This study used the hospital electronic medical record research system to obtain treatment related data, which had no risk for patients who met the inclusion criteria, and objectively could not obtain the informed consent of patients.

Author contributions

ZW: Writing – original draft, Writing – review & editing. BR: Writing – original draft, Writing – review & editing. HY: Formal analysis, Investigation, Conceptualization, Writing – original draft. XQ: Investigation, Project administration, Writing – review & editing. YW: Formal analysis, Investigation, Writing – review & editing. CX: Formal analysis, Investigation, Project administration, Writing – review & editing. YZ: Formal analysis, Investigation, Writing – review & editing. XL: Writing – review & editing. ZY: Data curation, Formal analysis, Methodology, Software, Writing – review & editing. JZ: Data curation, Formal analysis, Methodology, Software, Writing – review & editing.

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Conflict of interest

Authors ZY and JZ were employed by Beijing Medicinovo Technology Co., Ltd.

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.

Publisher's note

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Efficacy and safety of adding immune checkpoint inhibitors to first-line standard therapy for recurrent or advanced cervical cancer: a meta-analysis of phase 3 clinical trials

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Background: Immune checkpoint inhibitors (ICIs) combined with standard therapy (ST) have emerged as a novel treatment strategy for recurrent or advanced cervical cancer (r/a CC). However, the available data from phase 3 clinical trials have yielded mixed results. This study aims to evaluate the therapeutic efficacy and safety of adding ICIs to ST in the treatment of r/a CC.

Methods: Data from four phase 3 clinical trials (KEYNOTE-826, CALLA, BEATcc, and ENGOT-cx11/GOG-3047/KEYNOTE-A18), involving 2,857 patients, were analyzed. Meta-analyses were conducted to combine hazard ratios (HRs) for overall survival (OS) and progression-free survival (PFS), odds ratios (ORs) for the objective response rate (ORR), and relative risks (RRs) for adverse events (AEs).

Results: The addition of ICIs to ST significantly improved PFS (HR, 0.67; 95% CI, 0.60-0.75), OS (HR, 0.66; 95% CI, 0.58-0.75), and ORR (OR, 1.48; 95% CI, 1.13-1.94) compared to ST alone. However, there was a modest increase in grade 3-5 AEs (RR, 1.08; 95% CI, 1.03-1.13) with the combined therapy.

Conclusion: This meta-analysis indicates that the combination of ICIs with ST in the treatment of r/a CC not only demonstrates superior efficacy over ST alone but also maintains a comparable toxicity profile, offering strong evidence for an effective and relatively safe treatment approach for managing this disease.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/, identifier CRD42024593895.

KEYWORDS

immune checkpoint inhibitors, cervical cancer, efficacy, safety, meta-analysis

Introduction

Cervical cancer (CC) remains a significant global health issue, being the fourth most common cancer in women worldwide (1). For patients with recurrent or advanced cervical cancer (r/a CC), current standard therapies (ST), such as chemotherapy (CT), targeted therapy, and concurrent chemoradiotherapy (CCRT), offer limited benefits, underscoring the need for innovative treatments (2–4). Immunotherapy, especially immune checkpoint inhibitors (ICIs), has shown promise in various cancers and is increasingly being explored for CC treatment (5–8).

Recently, four phase 3 clinical trials have assessed the incorporation of ICIs into first-line ST for r/a CC. The KEYNOTE-826 trial evaluated the efficacy of pembrolizumab in combination with CT as a first-line treatment for r/a CC (9, 10). The results demonstrated a significant improvement in both progression-free survival (PFS) and overall survival (OS) compared to CT alone. This finding underscores the potential role of immunotherapy in the treatment of CC and supports its consideration in clinical practice. The CALLA trial investigated the addition of durvalumab to CCRT for the treatment of locally advanced cervical cancer (la CC) (11). Unfortunately, the study did not meet its primary endpoints of improving PFS or OS compared to CCRT alone. These results indicate that further research is necessary to determine the role of immunotherapy in the context of la CC treatment. The BEATcc trial assessed the efficacy of atezolizumab combined with platinum-based CT and bevacizumab as a first-line treatment for metastatic (stage IVB), persistent, or recurrent CC (12). The results showed a significant improvement in PFS and OS compared to CT and bevacizumab alone. This outcome highlights the potential of immunotherapy, in conjunction with traditional CT and targeted therapy, as a valuable treatment option for r/a CC, supporting its integration into clinical practice. The ENGOT-cx11/GOG-3047/KEYNOTE-A18 trial evaluated the therapeutic potential of combining pembrolizumab with CCRT as a first-line treatment for newly diagnosed, high-risk la CC (13, 14). The findings revealed a notable enhancement in both PFS and OS for patients receiving combination therapy compared to those treated with CCRT alone. These results reinforce the idea that immunotherapy plays a crucial role in the management of la CC and suggest its integration into standard clinical care. While KEYNOTE-826, BEATcc, and ENGOT-cx11/GOG-3047/KEYNOTE-A18 yielded positive results regarding their design, CALLA failed to meet its primary endpoint in the intention-to-treat (ITT) population. Additionally, the individual studies lacked sufficient power to analyze various clinically relevant subgroups.

Given the need in the treatment landscape of r/a CC, we conducted a meta-analysis of phase 3 clinical trials comparing the combination of ICIs with ST *versus* ST alone in patients with r/a CC. This study aims to provide insights into the clinical benefits and risks associated with the addition of ICIs to ST, empowering clinicians with robust data to inform their treatment decisions and patient management strategies.

Methods

Data sources

For this meta-analysis, data were sourced from four published phase 3 clinical trials: KEYNOTE-826, CALLA, BEATcc, and ENGOT-cx11/GOG-3047/KEYNOTE-A18. We extracted necessary data directly from the original publications of these trials and cross-referenced it with information available in clinical trial registries such as *ClinicalTrials.gov* to ensure consistency in trial design and reporting of outcomes. To guarantee the accuracy and completeness of the data, we also reviewed associated conference abstracts and supplementary materials. The data included primary and secondary endpoint results, along with key metrics for assessing treatment efficacy and safety.

Data extraction and assessment of risk of bias

Data pertinent to the study objectives were extracted by a primary investigator and subsequently verified for accuracy by an independent secondary reviewer. The information extracted included, where available, the title of the clinical trial, the date of publication, sample size, study design, therapeutic regimens for both the experimental and control groups, characteristics of the participants, hazard ratios (HRs) along with their corresponding 95% confidence intervals (CIs) for OS and PFS, odds ratios (ORs) with associated 95% CIs for objective response rate (ORR), and the incidence of any adverse events (AEs), as well as the number of patients experiencing grade 3-5 AEs. The assessment of risk of bias was conducted using the Cochrane Collaboration's Risk of Bias assessment tool (15).

Statistical analysis

For the efficacy analysis, HRs with 95% CIs for OS and PFS, as well as ORs with 95% CIs for ORR, were computed for each study to derive an overall estimate. In the context of safety analysis, relative risks (RRs) with 95% CIs for AEs were calculated for each study to obtain a comprehensive estimation. The I^2 statistic and the Cochrane Q test were utilized to evaluate between-study heterogeneity. An I^2 value exceeding 50% and a p-value below 0.1 from the Q test signified considerable heterogeneity, necessitating the use of a random-effects model. In contrast, a fixed-effect model was applied when these criteria were not satisfied. A funnel plot was created, and Egger's test was conducted to evaluate publication bias. All statistical analyses were conducted using R (v4.2.2), with statistical significance set at a two-tailed p-value < 0.05.

Results

Characteristics of the four phase 3 trials

Among the four included studies, one employed an open-label design, while the remaining three utilized a double-blind design. A cumulative total of 2,857 patients diagnosed with r/a CC were analyzed. Of these, 1,428 patients were treated with ICIs in combination with ST, and 1,429 patients received ST alone. The experimental treatment regimens comprised pembrolizumab, durvalumab, and atezolizumab, each in conjunction with ST. The control arm regimens consisted of placebo plus ST, which included platinum-based CT ± bevacizumab, CCRT, and bevacizumab plus CT. The main characteristics of the four trials are summarized in Table 1.

Efficacy analysis

PFS in ITT population

In the absence of significant between-study heterogeneity (I^2 = 31%), a fixed-effect model was utilized to derive the pooled estimate of PFS. The combined analysis showed that adding ICIs to ST significantly improved PFS compared to ST alone (HR, 0.67; 95% CI, 0.60-0.75; Figure 1A).

OS in ITT population

Similarly, no heterogeneity (I^2 = 0) was observed across these studies. The meta-analysis suggested that combining ICIs with ST led to a significant extension of OS compared to ST alone (HR, 0.66; 95% CI, 0.58-0.75; Figure 1B).

ORR in ITT population

Given the significant heterogeneity observed among studies (I^2 = 57%), a random-effects model was used to compute the combined OR with 95% CI. The meta-analysis suggested that the addition of ICIs to ST significantly enhanced the ORR compared to ST alone (OR, 1.48; 95% CI, 1.13-1.94; Figure 1C).

Safety analysis

Grade 3-5 AEs

Among the 1,425 patients treated with ICIs plus ST, 1,029 (72.2%) experienced grade 3-5 AEs, compared to 949 out of 1,422 patients (66.7%) in the ST alone group. The meta-analysis showed that adding ICIs to ST was linked to a small rise in the risk of grade 3-5 AEs (RR, 1.08; 95% CI, 1.03-1.13; Figure 2).

Subgroup analysis

To achieve a more profound understanding of the efficacy of ICIs combined with ST in patients with r/a CC, we conducted several stratified analyses based on patient characteristics and treatment regimens.

In light of the observed heterogeneity within the r/a CC cohort and significant variations based on PD-L1 status, we performed a targeted subgroup analysis to ascertain whether PD-L1 status could serve as a biomarker for the efficacy of ICIs plus ST. The analyses revealed that the combination of ICIs with ST significantly improved PFS (HR, 0.68; 95% CI, 0.56-0.84; Figure 3A), OS (HR, 0.66; 95% CI, 0.57-0.77; Figure 3B), and ORR (OR, 1.73; 95% CI, 1.15-2.59; Figure 3C) in the

TABLE 1 Main characteristics of the four phase 3 trials.

Study	Year	ITT population	Design	Age median, range, (IQR), years	Regimens		Population characteristics	Cancer stage at diagnosis
					Experimental arm	Control arm		
KEYNOTE-826 (9, 10)	2021 2023	617 ICIs + ST: 308 ST: 309	Phase III double-blind RCT randomization 1: 1	ICIs + ST: 51, 25-82 ST: 50, 22-79	Pembrolizumab plus platinum-based CT ± bevacizumab	Placebo plus platinum-based CT ± bevacizumab	Persistent, recurrent, or metastatic	I-IVB
CALLA (11)	2023	770 ICIs + ST: 385 ST: 385	Phase III double-blind RCT randomization 1: 1	ICIs + ST: 50 (41-57) ST: 48 (40-57)	Durvalumab plus CCRT	Placebo plus CCRT	Locally advanced	IB2-IVA
BEATcc (12)	2024	410 ICIs + ST: 206 ST: 204	Phase III open-label RCT randomization 1: 1	ICIs + ST: 51, 24-90, (43-60) ST: 52.5, 21-79, (43.5-61)	Atezolizumab plus bevacizumab and CT	Bevacizumab and CT	Metastatic, persistent, or recurrent	I-IVB
KEYNOTE-A18 (13, 14)	2024	1060 ICIs + ST: 529 ST: 531	Phase III double-blind RCT randomization 1: 1	ICIs + ST: 49 (40-57) ST: 50 (41-59)	Pembrolizumab plus CCRT	Placebo plus CCRT	Newly diagnosed, high-risk, locally advanced	IB2-IVA

ITT, intention-to-treat; IQR, inter-quartile range; ICIs, immune checkpoint inhibitors; ST, standard therapy; RCT, randomized clinical trial; CT, chemotherapy; CCRT, concurrent chemoradiotherapy.

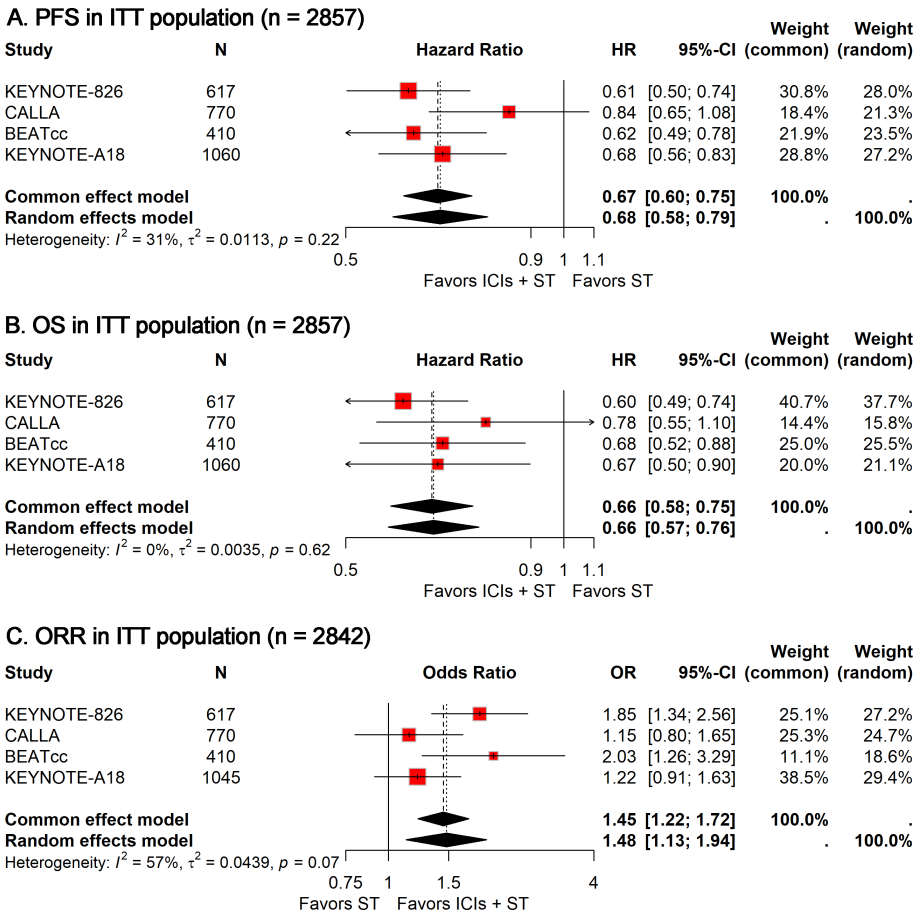

FIGURE 1
Forest plots comparing ICIs plus ST to ST alone in the ITT population for PFS **(A)**, OS **(B)**, and ORR **(C)**. PFS, progression-free survival; OS, overall survival; ORR, objective response rate; ICIs, immune checkpoint inhibitors; ST, standard therapy; ITT, intention-to-treat.

PD-L1-positive population. Conversely, no significant statistical disparities were detected in the PD-L1-negative population (Figure 3). However, it is crucial to acknowledge the limited number of PD-L1-negative patients, which necessitates cautious interpretation of these data.

To examine the impact of clinical characteristic variations on the efficacy of ICIs plus ST in patients with r/a CC, we conducted multiple subgroup analyses based on patient attributes, including age, race, Eastern Cooperative Oncology Group (ECOG) Performance Status, and disease status. In the subgroup of patients under 65 years of age, the addition of ICIs to ST significantly improved PFS (HR, 0.68; 95% CI, 0.62-0.75; Figure 4A) and OS (HR, 0.62; 95% CI, 0.55-0.70; Figure 4B). In contrast, for patients aged 65 years and older, the addition of ICIs to ST significantly improved PFS (HR, 0.63; 95% CI, 0.46-0.87; Figure 4A), whereas no significant differences in OS were observed (Figure 4B). Subgroup analyses based on race and ECOG status indicated that the addition of ICIs to ST

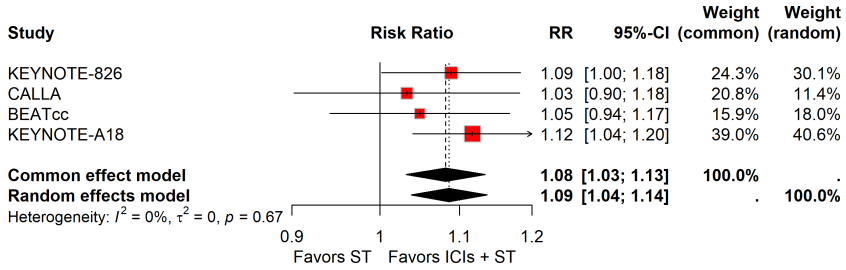

FIGURE 2
Forest plot of grade 3-5 AEs comparing ICIs plus ST to ST alone in the ITT population. AEs, adverse events; ICIs, immune checkpoint inhibitors; ST, standard therapy; ITT, intention-to-treat.

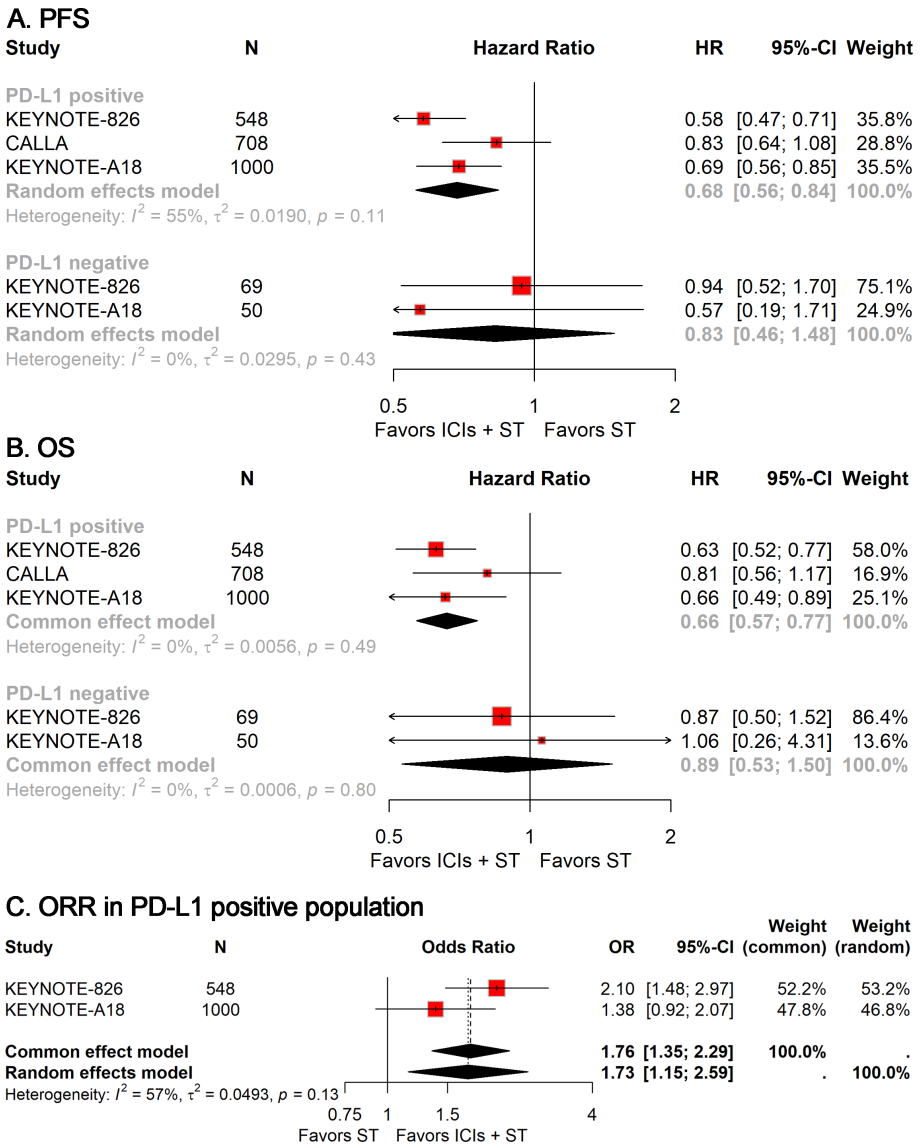

FIGURE 3
Forest plots of subgroup analysis stratified by PD-L1 status, showing results for PFS **(A)**, OS **(B)**, and ORR **(C)**. PFS, progression-free survival; OS, overall survival; ORR, objective response rate.

significantly improved both PFS and OS irrespective of patient race (White and others) (Figures 4C, D) and ECOG status (0 and 1) (Figures 4E, F). Among patients with metastatic disease, the addition of ICIs to ST did not significantly improve either PFS or OS (Figures 4G, H). However, in patients with non-metastatic disease, the addition of ICIs to ST significantly enhanced both PFS (HR, 0.59; 95% CI, 0.48-0.71; Figure 4G) and OS (HR, 0.58; 95% CI, 0.48-0.70; Figure 4H).

To gain further insights into the treatment modalities, we performed stratified analyses according to the type of ICIs and ST employed in the treatment regimens. The addition of either anti-PD-1 or anti-PD-L1 to ST was associated with significant improvements in PFS (anti-PD-1: HR, 0.64; 95% CI, 0.55-0.75; anti-PD-L1: HR, 0.72; 95% CI, 0.54-0.96; Figure 5A) and OS (anti-PD-1: HR, 0.62; 95% CI, 0.53-0.74; anti-PD-L1: HR, 0.71; 95% CI, 0.58-0.88; Figure 5B). Similarly, the inclusion of ICIs in ST was linked to superior PFS (CT ± bevacizumab: HR, 0.61; 95% CI, 0.53-0.71; CCRT: HR, 0.74; 95% CI, 0.63-0.87; Figure 5C) and OS (CT ± bevacizumab: HR, 0.63; 95% CI, 0.54-0.74; CCRT: HR, 0.71; 95% CI, 0.57-0.89; Figure 5D), regardless of the specific ST regimen used.

Risk of bias and sensitivity analysis

Among the four trials, three were conducted as double-blind trials, whereas one was conducted as an open-label trial. Consequently, the open-label trial was assessed as having a high risk of performance bias, an unclear risk of detection bias, and a low risk of selection, attrition, and reporting biases. The remaining studies were all evaluated as having a low risk of bias across all assessed criteria. The risk of bias assessment is graphically summarized in Supplementary Figure S1. The funnel plot, along

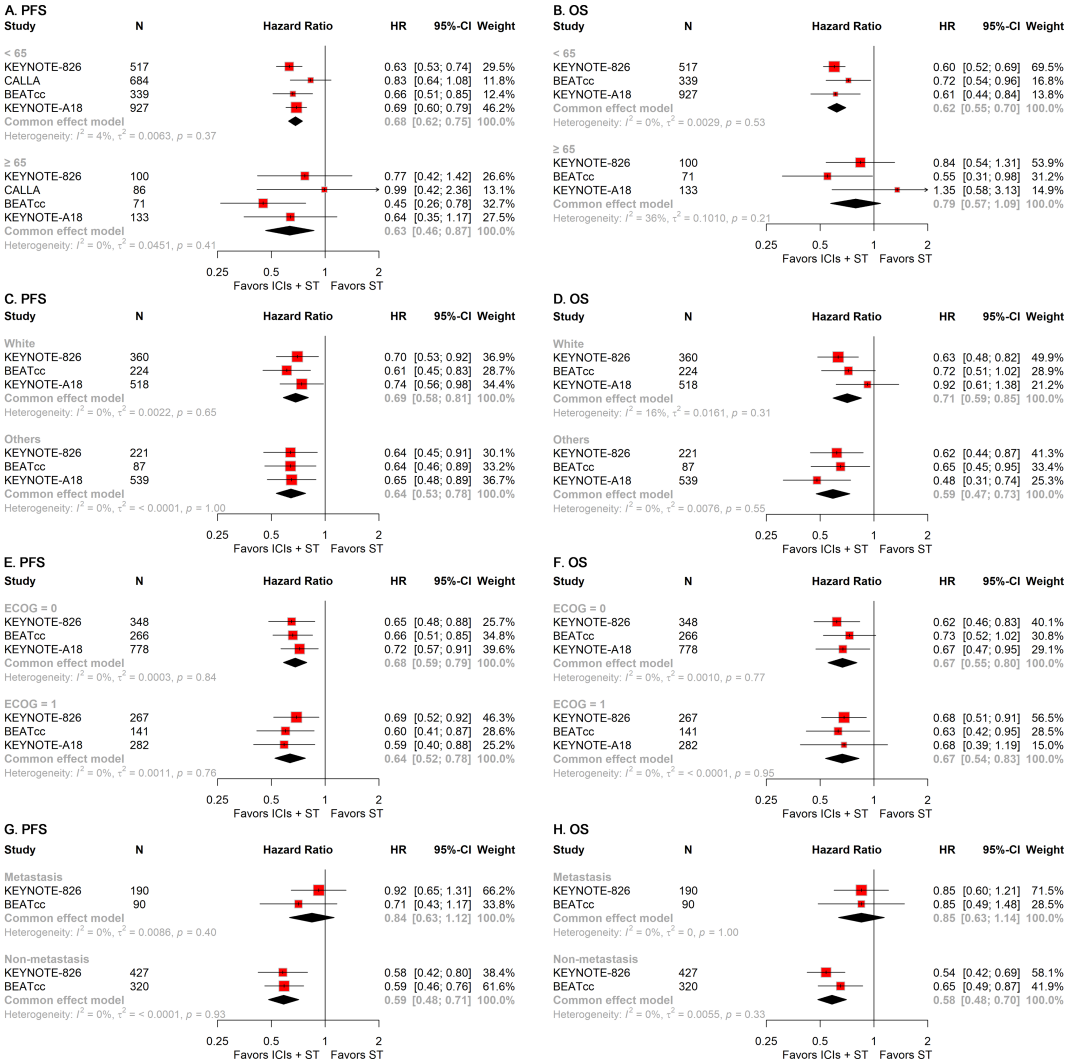

FIGURE 4
Forest plots of subgroup analysis stratified by clinical characteristics. Results for age on PFS **(A)** and OS **(B)**. Results for race on PFS **(C)** and OS **(D)**. Results for ECOG performance status on PFS **(E)** and OS **(F)**. Results for disease status on PFS **(G)** and OS **(H)**. PFS, progression-free survival; OS, overall survival; ECOG, Eastern Cooperative Oncology Group.

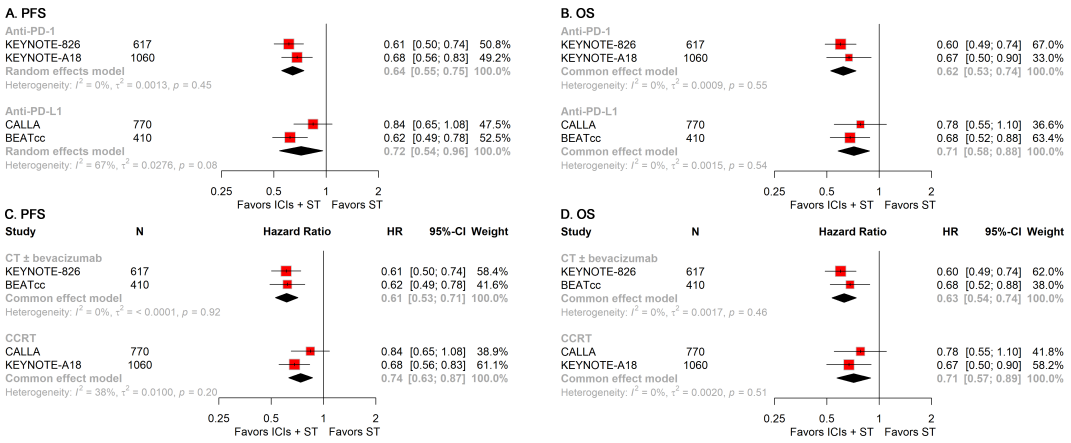

FIGURE 5
Forest plots of subgroup analysis stratified by treatment regimens. Results for the type of ICIs used on PFS **(A)** and OS **(B)**. Results for the regimens of ST employed on PFS **(C)** and OS **(D)**. PFS, progression-free survival; OS, overall survival; ICIs, immune checkpoint inhibitors; ST, standard therapy.

with Egger's test (P = 0.2984), did not indicate significant publication bias (Supplementary Figure S2). Sensitivity analysis for PFS, OS, and ORR confirmed the robustness of the pooled results (Supplementary Figure S3).

Discussion

Currently, the integration of ICIs into ST has emerged as a predominant area of research for patients diagnosed with r/a CC. However, published phase 3 trials have yielded conflicting results, leading to ongoing debate regarding the efficacy of ICIs combined with ST in treating r/a CC. The KEYNOTE-826 trial is a landmark study that evaluated pembrolizumab in conjunction with platinum-based CT and/or bevacizumab. The results demonstrated a significant improvement in both PFS and OS compared to CT alone. The CALLA trial explored the addition of durvalumab to CCRT; unfortunately, the study did not meet its primary endpoints compared to CCRT alone. The BEATcc trial investigated the incorporation of atezolizumab into a regimen of bevacizumab plus CT. The results showed a significant improvement in PFS and OS compared to CT and bevacizumab alone. In parallel, the ENGOT-cx11/GOG-3047/KEYNOTE-A18 trial assessed the efficacy of adding pembrolizumab to CCRT for r/a CC. The findings revealed a notable enhancement in both PFS and OS for patients receiving the combination therapy compared to those treated with CCRT alone. Therefore, we included these four phase 3 trials encompassing a total of 2,857 participants to evaluate the efficacy and safety of ICIs in combination with ST as a first-line treatment for patients with r/a CC. Our findings provide high-quality, evidence-based recommendations for the clinical management of patients with r/a CC and highlight crucial considerations for treating patients with different PD-L1 statuses.

While ICIs plus ST offer promising efficacy, it is important to acknowledge the increased toxicity associated with this treatment approach. Our meta-analysis revealed a slight increase in grade 3-5 AEs with ICIs plus ST compared to ST alone. These findings highlight the importance of carefully monitoring and managing AEs in patients undergoing ICIs plus ST. Clinicians should weigh the potential benefits of ICIs plus ST against the risk of increased toxicity when considering this treatment option for r/a CC patients.

To gain deeper insights into the efficacy of ICIs plus ST in the treatment of r/a CC, we conducted several specific subgroup analyses focusing on PD-L1 status, clinical characteristics, and treatment regimens. These subgroup analyses suggest that the combination of ICIs and ST holds particular therapeutic promise in the following patient populations: i) PD-L1-positive patients. The combination therapy of ICIs and ST demonstrated a significant improvement in patients with PD-L1-positive r/a CC, indicating that PD-L1 status is a critical biomarker for identifying patients who are likely to benefit the most from this treatment approach. ii) Patients aged less than 65 years. Our data revealed that the benefits of ICIs plus ST in terms of PFS and OS were more pronounced in patients under the age of 65. This suggests that younger patients may have a more favorable response to this combination therapy. iii) Non-metastatic patients. The combination of ICIs and ST appeared to be more effective in patients with non-metastatic disease compared to those with metastatic disease. This finding highlights the potential for ICIs to improve outcomes in patients with less advanced forms of the disease. These findings have significant therapeutic implications, as they can guide clinicians in personalizing treatment strategies for r/a CC patients. By focusing on these specific populations, clinicians can optimize the use of ICIs and ST, potentially leading to improved patient outcomes and minimizing the risk of unnecessary treatment-related AEs in those who may not benefit as much.

The potential of CCRT to enhance anticancer immune responses by promoting the release of cancer antigens is widely recognized (16). However, the optimal CCRT regimen to synergize with ICIs remains an open question, prompting ongoing research to identify treatment strategies that effectively mobilize and activate tumor-specific T cells while mitigating immune suppression. The results from the KEYNOTE-A18 study did not align with those observed in the CALLA study, highlighting the need for further investigation into the interplay between CCRT and ICIs in r/a CC. Three possibilities may explain the differences observed between CALLA and KEYNOTE-A18: i) Differences in drugs. Durvalumab is a PD-L1 inhibitor, while pembrolizumab is a PD-1 inhibitor. This raises the question of whether a PD-1 antibody targeting the T-cell surface has a more direct regulatory effect on the immune system compared to a PD-L1 antibody targeting the tumor cell surface, potentially leading to better treatment outcomes (17). ii) Patient population. The CALLA study enrolled a relatively high proportion of patients with early-stage disease (IB2-IIB). Patients with early-stage disease generally experience favorable outcomes with CCRT alone, which could narrow the survival gap between experimental and control groups, making it difficult to observe significant statistical differences in PFS between the two study arms (18). iii) Radiation dose regimen. The radiation dose regimen specified in the CALLA study might be more conservative compared to KEYNOTE-A18. Clinically, achieving a tumor-killing dose (radical dose) with radiotherapy is crucial for local control and patient prognosis (19). Unlike CT, immunotherapy may not achieve satisfactory tumor control as monotherapy (20). Therefore, adding immunotherapy to a regimen with an insufficient tumor-killing dose may not fully exploit the long-lasting benefits of immunotherapy. These considerations underscore the complexity of integrating CCRT with ICIs and highlight the necessity for further research to optimize treatment strategies for r/a CC.

There are some limitations in this meta-analysis. Firstly, a discrepancy exists in the techniques utilized to assess PD-L1 across the trials. Specifically, two trials employed the PD-L1 IHC 22C3 pharmDx assay, characterizing PD-L1 expression by a combined positive score (CPS) ≥ 1 (9, 10, 13). In contrast, the CALLA trial assessed PD-L1 expression according to the tumor area positivity (TAP) score using the VENTANA PD-L1 (SP263) assay, with TAP ≥ 1% serving as the criterion (11). This discrepancy in PD-L1 assessment poses a significant challenge in clinical studies exploring immunotherapy for r/a CC. To address this issue, there is a need for better harmonization of PD-L1 testing across clinical

trials to ensure consistency and comparability of results. Secondly, the reporting of AEs was inconsistent across the included studies, and only those AEs reported in all trials were included in this meta-analysis. This limits our comprehensive understanding of potential AEs associated with the treatment regimens.

Conclusion

This meta-analysis is the inaugural study to elucidate that the integration of ICIs into ST represents a potent and relatively low-risk therapeutic strategy for individuals with r/a CC, offering robust support for the management of this malignancy. The synergistic effect of ICIs and ST is particularly pronounced within certain subsets of patients, including those with high PD-L1 expression, those younger than 65 years, and those with a non-metastatic disease state. The assessment of PD-L1 expression serves as a valuable biomarker in identifying patients likely to experience enhanced therapeutic gains from the combined regimen of ICIs and ST. The implications of these findings for clinical decision-making are significant, highlighting the need for further research to optimize the integration of ICIs with ST in the treatment of r/a CC. As such, these data have the potential to inform future clinical guideline development, particularly with regard to the incorporation of ICIs into standard ST protocols.

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

XZ: Writing – original draft, Methodology, Software, Visualization. JS: Methodology, Software, Visualization, Writing – original draft. MH: Data curation, Software, Writing – review & editing. RL: Funding acquisition, Project administration, Resources, Software, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1507977/full#supplementary-material

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Establishment and validation of a survival prediction model for stage IV non-small cell lung cancer: a real-world study

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Objective: The aim of this study is to develop and validate a predictive model for predicting survival in individual advanced non-small cell lung cancer patients by integrating basic patient information and clinical data.

Methods: A total of 462 patients with advanced non-small cell lung cancer collected from Shanxi Cancer Hospital were randomly assigned (in a 7:3 ratio) to a training cohort and an internal validation cohort. Independent factors affecting patients' 3-year survival were screened and predictive models were created by using a single-factor followed by multifactor Cox regression analysis. Evaluate the performance of the model using the consistency index (C-index), calibration curves, receiver operating characteristic curves (ROC) and decision curve analysis (DCA). The collected patients who received chemotherapy alone and those who received chemotherapy combined with immunotherapy were statistically paired using propensity score matching between the two groups, and subgroup analyses were performed among the screened variables.

Results: A better prognostic model was created and a nomogram chart visualizing the model was drawn. Based on the median risk score of the training cohort, all individuals were categorized into high- and low-risk groups, with the high-risk group having worse OS in both cohorts (P<0.05). The results of subgroup analysis showed that chemotherapy alone versus chemotherapy combined with immunotherapy in patients with advanced NSCLC affected OS.

Conclusion: A clinical predictive model was developed to predict 3-year survival in patients with advanced non-small cell lung cancer. The study demonstrated that chemotherapy combined with immunotherapy is superior to chemotherapy alone.

KEYWORDS

clinical predictive modeling, advanced non-small cell lung cancer, three-year survival, chemotherapy, immunotherapy

Introduction

Lung cancer, as the leading cause of cancer-related deaths worldwide, poses a great threat to human health (1). Based on the size and type of cancer cells, lung cancer can be categorized into two types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), of which NSCLC accounts for about 85% of lung cancers (2, 3). Lung cancer is subdivided into three types according to pathology: squamous cell carcinoma, lung adenocarcinoma, and large cell lung cancer (4). Due to the lack of obvious early symptoms, most NSCLC patients are in advanced stages upon diagnosis and have a poor prognosis (5). Faced with the high risk of surgical treatment for advanced NSCLC patients, radiotherapy and drug therapy are mostly used in clinical treatment (6). There are many drugs to choose from when receiving drug therapy programs, and the current main drug programs include traditional cytotoxic drug therapy, targeted drug therapy for tumor gene mutations, and emerging immune monoclonal antibody therapy (7, 8). Due to the long drug treatment cycle, it is also difficult to accurately assess the survival benefit of patients in clinical practice. In order to improve the therapeutic effect and the quality of patient survival, there is an urgent clinical need for a model that can predict the prognosis of patients with advanced NSCLC. Such a model can help physicians assess patients' risk of disease progression, response to treatment, and survival expectations, and thus develop an individualized treatment plan for each patient. In this study, we constructed a survival model for advanced NSCLC patients treated with antitumor drugs can be used to assess the prognosis of advanced NSCLC patients and provide a reference for clinical treatment decisions.

Method

Patient selection

This retrospective study followed the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of Shanxi Cancer Hospital. The study was exempted from informed consent requirements. A total of 2005 cases of patients treated with antitumor drugs between December 2018 and May 2020 were queried for this study, and 462 patients diagnosed with advanced primary non-small cell lung cancer were finally included. Inclusion criteria: (1) Primary non-small cell lung cancer diagnosed at stage IV on initial admission. (2) Received anti-tumor drugs. (3) Clinical characterization and follow-up data can be used. Exclusion criteria:

(1) With other cancers or having had other cancers. (2) Underwent surgical treatment. (3) Missing clinical data. All patients were restaged according to AJCC 8th edition staging principles (9). Overall survival (OS) was defined as the time between the date of diagnosis and the date of death from any cause or the last follow-up. OS was the primary endpoint of this study.

The 462 patients who met the criteria were randomly assigned (ratio 7:3) to the training cohort and the internal validation cohort. Follow-up was performed via telephone communication with patients, with a final follow-up date of December 31, 2023. This was a retrospective study based on clinical data and did not require informed patient consent.

Clinical parameter collection

We collected baseline clinical parameters as well as treatment regimens of patients with primary advanced non-small cell lung cancer prior to treatment. These included gender, age, weight, height, Eastern Cooperative oncology Group Performance Status (ECOG PS), smoking history, alcohol consumption history, complication (Hypertension, hyperglycemia), family history, pathology, TNM staging, chest radiotherapy, liver metastases, bone metastases, brain metastases, absolute neutrophil counts (NEUT#)(normal range: 1.80~6.30*10^9/L, platelet counts (PLT) (normal range: 125~350*10^9/L), absolute lymphocyte counts (LYMPH) (normal range:1.10~3.20*10^9/L), absolute monocyte counts (MONO) (normal range: 0.10~0.60*10^9/L), fibrinogen (FIB) (normal range: 2.00~4.00g/L), lactate dehydrogenase (LDH) (normal range:120.0~250.0U/L), D-dimer (normal range: 0~0.256mg/L), carcinoembryonic antigen (CEA) (normal range: <3.00ug/L), neuron-specific enolase (NSE) ((normal range: <12.00ug/L), squamous cell carcinoma-associated antigen (SCC) ((normal range: <1.00ng/mL), glycan antigen CA-125 ((normal range: <35.00U/mL), glycan antigen CA19-9 ((normal range: <37.00U/mL), cell proliferation index (Ki67(%)), tumor driver mutations (EGFR, MET, KRAS, ALK, ROS1, HER2, BRAF, RET, PIK3CA), treatment options. The tumor marker indicators included in this study are significant for the diagnosis of tumors and the detection of efficacy after treatment, but the effect on prognosis is not clear enough, thus we also included them in the influencing factors and tried to explore their correlation with prognosis. Elevated D-dimer may imply an increased risk of thrombosis or is associated with malignant tumors, so does it affect the patient's prognosis, and we considered to include it in the analysis of the factors. Ki67 suggests the degree of malignancy of the tumor and is important in clinical diagnosis and prognosis, so it was included in variable selection in our study in the expectation of a more accurate determination of prognosis.

Data analysis

All statistical analyses for this study were performed on R version 4.3.3. Patient characteristics were compared between cohorts using chi-square tests. Clinicopathological characteristics significantly associated with survival were screened using univariate and multivariate Cox regression analyses, and variables were further screened using stepwise inverse regression to select the model with the smallest Akaike Information Criterion (AIC) score as the ideal

model. Finally, nomogram pre-models were constructed using the screened variables to predict the incidence of OS at 1, 2, and 3 years in patients with advanced NSCLC. We then evaluated the performance in terms of model discrimination, accuracy, and clinical application. Discriminative power was assessed using the consistency index (C-index) and the area under the subject operating characteristic curve; calibration curves measured the agreement of the probabilities generated by the nomogram plots with the actual probabilities observed. Decision curve analysis to assess the clinical utility of models. Risk scores were available for each individual in the nomogram, and risk stratification was performed for all patients using the median risk score of patients in the training cohort as a threshold. Kaplan-Meier survival analyses were performed to determine whether there were significant differences in the incidence of OS across risk groups. The flowchart for patient screening and study design is shown in Figure 1.

Subgroup analysis of treatment programs

The collected patients who received chemotherapy alone and chemotherapy combined with immunotherapy were statistically paired using two-group propensity score matching, and subgroup analyses were performed among screened independent risk factors. Cox proportional risk models were used to analyze the relationship between treatment and prognosis for each subgroup. Finally, the results are displayed in a forest map.

Analysis of the importance of variables

Importance analysis of the final variables of the model was performed using the XGBoost machine learning method. To understand the importance of the influences included in the model in the prognosis of advanced non-small cell lung cancer and to perform survival analysis on the most important variables.

Result

Participant characteristics

A total of 462 patients with advanced NSCLC were collected and randomly assigned to a training cohort (n = 323) and an internal validation cohort (n = 139), and there were no differences in clinicopathological and demographic characteristics between the two cohorts.

Introduction to data characterization

Most of the 462 patients collected from Shanxi Provincial Tumor Hospital were middle-aged and elderly, with most of them concentrated between the ages of 53-85 years (75.8%), 298 (64.5%) patients were male and 164 (35.5%) were female. Epithelioid was the type of squamous carcinoma pathology in patients with a definite histologic diagnosis (17.1%). AJCC staging showed that

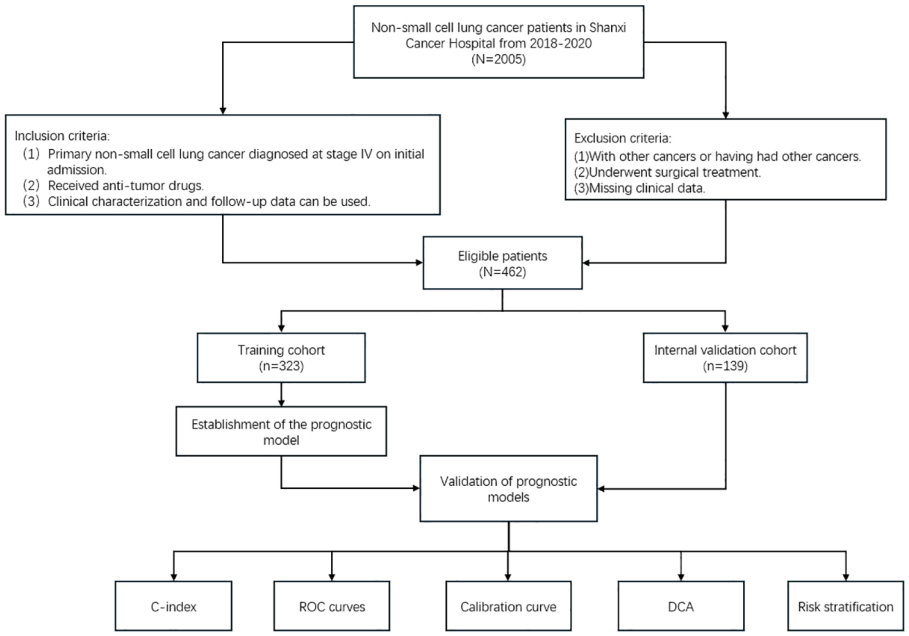

FIGURE 1
Flowchart of participant inclusion and exclusion.

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most of the patients were T4 (32.6%) or N2 (45.2%), as it was to explore the prognosis of patients with advanced NSCLC, here we enrolled all patients with Stage IV and removed the M staging.

Laboratory data had been classified as dichotomous variables according to the reference index.

63.8% of the patients had tumor-associated mutations. 37.7% of the patients received chemotherapy alone, 29.4% of the patients received targeted therapy alone, 15.6% of the patients received chemotherapy in combination with targeted, and 17.3% of the patients received chemotherapy in combination with

TABLE 1 Demographics and clinicopathologic characteristics of the training and validation cohort.

Characteristics	Training cohort *(N=323)*	Validation cohort *(N=139)*	*P*.value
Sex			0.858
female	116 (35.9%)	48 (34.5%)	
male	207 (64.1%)	91 (65.5%)	
Age	61.0 [53.0;67.0]	61.0 [55.0;67.0]	0.538
Weight	63.0 [55.5;70.0]	62.0 [54.0;68.5]	0.678
Height	165 [158,170]	165 [158;170]	0.469
ECOG PS			0.220
0	2 (0.62%)	4 (2.88%)	
1	270 (83.6%)	112 (80.6%)	
2	48 (14.9%)	21 (15.1%)	
3	3 (0.93%)	2 (1.44%)	
Smoke			0.599
No	152 (47.1%)	61 (43.9%)	
Yes	171 (52.9%)	78 (56.1%)	
Drink			0.270
No	209 (64.7%)	98 (70.5%)	
Yes	114 (35.3%)	41 (29.5%)	
Complication			1.000
No	200 (61.9%)	86 (61.9%)	
Yes	123 (38.1%)	53 (38.1%)	
History			0.214
No	303 (93.8%)	135 (97.1%)	
Yes	20 (6.19%)	4 (2.88%)	
Pathology			0.844
Non-squamous carcinoma	269 (83.3%)	114 (82.0%)	
Squamous carcinoma	54 (16.7%)	25 (18.0%)	
AJCC T			0.958
1	70 (21.6%)	28 (20.1%)	
2	97 (30.0%)	45 (32.4%)	
3	51 (15.9%)	20 (14.4%)	
4	105 (32.5%)	46 (33.1%)	
AJCC N			0.712
0	49 (15.2%)	17 (12.2%)	
1	15 (4.64%)	8 (5.76%)	
2	142 (44.0%)	67 (48.2%)	
3	117 (36.2%)	47 (33.8%)	
Chest radiation			0.366
No	279 (86.4%)	125 (89.9%)	
Yes	44 (13.6%)	14 (10.1%)	
Liver metastasis			0.922
No	277 (85.8%)	118 (84.9%)	
Yes	46 (14.2%)	21 (15.1%)	
Bone metastasis			0.968
No	165 (51.1%)	72 (51.8%)	
Yes	158 (48.9%)	67 (48.2%)	
Brain metastasis			0.049
No	220 (68.1%)	108 (77.7%)	
Yes	103 (31.9%)	31 (22.3%)	
NEUT			0.305
Abnormal	81 (25.1%)	28 (20.1%)	
Normal	242 (74.9%)	111 (79.9%)	
PLT			0.905
Abnormal	73 (22.6%)	30 (21.6%)	
Normal	250 (77.4%)	109 (78.4%)	
LYMPH			0.215
Abnormal	36 (11.1%)	22 (15.8%)	
Normal	287 (88.9%)	117 (84.2%)	
MONO			0.402
Abnormal	117 (36.2%)	44 (31.7%)	
Normal	206 (63.8%)	95 (68.3%)	
FIB			0.489
Abnormal	171 (52.9%)	68 (48.9%)	
Normal	152 (47.1%)	71 (51.1%)	
LDH			0.585
Abnormal	110 (34.1%)	43 (30.9%)	
Normal	213 (65.9%)	96 (69.1%)	

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TABLE 1 Continued

Characteristics	Training cohort (N=323)	Validation cohort (N=139)	P.value
D-dimer			0.847
Abnormal	193 (59.8%)	81 (58.3%)	
Normal	130 (40.2%)	58 (41.7%)	
CEA			1.000
Abnormal	203 (62.8%)	88 (63.3%)	
Normal	120 (37.2%)	51 (36.7%)	
NSE			0.552
Abnormal	17 (5.26%)	10 (7.19%)	
Normal	306 (94.7%)	129 (92.8%)	
SCC			0.521
Abnormal	41 (12.7%)	14 (10.1%)	
Normal	282 (87.3%)	125 (89.9%)	
CA199			0.642
Abnormal	80 (24.8%)	38 (27.3%)	
Normal	243 (75.2%)	101 (72.7%)	
CA125			0.167
Abnormal	150 (46.4%)	75 (54.0%)	
Normal	173 (53.6%)	64 (46.0%)	
Ki67(%)	40.0[30.0;70.0]	50.0 [30.0;60.0]	0.986
Mutation			0.369
No	112 (34.7%)	55 (39.6%)	
Yes	211 (65.3%)	84 (60.4%)	
Treatment			0.757
Alone targeted	97 (30.0%)	39 (28.1%)	
Chemotherapeutics	120 (37.2%)	54 (38.8%)	
Plus immunotherapy	53 (16.4%)	27 (19.4%)	
Plus targeted	53 (16.4%)	19 (13.7%)	

immunotherapy. The clinicopathologic characteristics of all patients are shown in Table 1.

Independent prognostic factors for screening model construction

One-way Cox regression analysis of the training cohort showed that age, ECOG PS, smoking history, alcohol consumption history, complication, pathology, N stage, liver metastasis, bone metastasis, absolute neutrophil count, platelet count, absolute lymphocyte count, absolute monocyte count, fibrinogen, lactate dehydrogenase, D-dimer, neuron-specific enolase (NSE), squamous cell carcinoma-related antigen (SCC), Ki67, tumor-associated gene mutations, and treatment regimen were significantly associated with survival ($P < 0.05$). A multifactorial analysis of the above 21 variables was performed, and the best model was determined using stepwise backward regression with the lowest AIC value. Age, ECOG PS, bone

TABLE 2 Selection of variables independently associated with OS by univariate and multivariate Cox proportional hazards analysis in the training cohort.

Characteristics	Univariate			Multivariate		
	HR	CI95	P	HR	CI95	P
Age	1.02	1.01-1.04	0.001	1.03	1.01-1.04	0.001
Sex	1.29	0.98-1.70	0.075			
Weight	1.01	1.00-1.02	0.232			
Height	1.00	0.99-1.02	0.668			
ECOG PS	1.53	1.13-2.08	0.006	1.55	1.10-2.18	0.013
Smoke	1.38	1.06-1.80	0.017	0.94	0.64-1.40	0.740
Drink	1.33	1.01-1.74	0.042	1.24	0.93-1.67	0.142
Complication	1.53	1.17-2.00	0.002	1.18	0.88-1.56	0.266
History	0.53	0.27-1.04	0.064			
Pathology	1.49	1.06-2.09	0.022	0.74	0.50-1.09	0.129
AJCC T	1.05	0.94-1.17	0.418			
AJCC N	1.14	1.00-1.30	0.045	1.06	0.92-1.22	0.426
Chest radiation	0.88	0.60-1.30	0.526			
Liver metastasis	1.70	1.20-2.41	0.003	1.19	0.82-1.73	0.349
Bone metastasis	1.33	1.02-1.73	0.032	1.55	1.17-2.06	0.002
Brain metastasis	0.88	0.66-1.17	0.386			
NEUT	1.53	1.15-2.05	0.004	0.94	0.67-1.32	0.727
PLT	1.65	1.23-2.22	0.001	1.58	1.17-2.14	0.003
LYMPH	1.49	1.01-2.21	0.044	1.57	1.05-2.36	0.028
MONO	1.52	1.17-1.99	0.002	1.00	0.74-1.37	0.977
FIB	1.62	1.24-2.12	0.001	1.19	0.89-1.61	0.244
LDH	1.47	1.12-1.93	0.005	1.30	0.98-1.73	0.066
D-dimer	1.86	1.41-2.47	0.001	1.39	1.03-1.88	0.030
CEA	0.93	0.71-1.21	0.580			
NSE	2.04	1.23-3.40	0.006	1.42	0.79-2.55	0.235
SCC	1.68	1.16-2.44	0.006	1.60	1.07-2.40	0.021
CA199	1.03	0.76-1.40	0.839			
CA125	0.97	0.74-1.26	0.791			
CEA	0.93	0.71-1.21	0.580			
Ki67(%)	1.01	1.01-1.02	0.001	1.01	1.01-1.02	0.001
Mutation	0.56	0.43-0.73	0.001	0.51	0.38-0.69	0.001
Treatment	0.75	0.66-0.86	0.001	0.72	0.63-0.83	0.001

R, hazard ratio; CI95, 95% confidence interval; AJCC Stages, the eighth edition American Joint Committee on Cancer (AJCC) TNM staging system.

metastases, platelet count, absolute lymphocyte count,D-dimer, squamous cell carcinoma-associated antigen (SCC), Ki67, driver genes, and treatment regimen were ultimately identified as independent prognostic factors for modeling the prognosis of advanced NSCLC. The results of OS-based Cox regression survival analysis are shown in Table 2, respectively.

Model creation and validation

The model for predicting late survival in NSCLC patients was determined by the ten variables screened above and visualized in a nomogram (Figure 2). By calculating the sum of the scores of the ten variables from the nomogram, we can estimate the OS rates of advanced NSCLC patients at 1, 2, and 3 years. The performance of the model was validated using the C-index, ROC curve over time and calibration curve. The C-index of the OS-based prediction model was 0.711 (95% CI, 0.677-0.743) and 0.696 (95% CI, 0.614-0.717) for the training group and the internal validation group, respectively.

Figure 3 shows the AUC values of the column-line plots of predicted 1-, 2-, and 3-year OS in the two cohorts Training cohort: 1-year OS 0.771 (95% CI, 0.713-0.830); 2-year OS 0.781 (95% CI, 0.732-0.831); 3-year OS 0.789 (95% CI, 0.733-0.844); internal validation cohort: 1-year OS.

0.787 (95% CI, 0.709-0.865); 2-year OS 0.765 (95% CI, 0.686 -0.843); 3-year OS 0.755 (95% CI, 0.656-0.854). The C-index and the AUC values indicated that the prognostic model had a better discriminative ability for survival in advanced NSCLC patients.

Figure 4 shows the calibration curves of the prognostic model between the actual OS rates and the predicted probabilities of the two cohorts at 1, 2, and 3 years, respectively, demonstrating that the survival rates generated by the nomogram are in good agreement with those observed in the actual population.

Figure 5 shows the clinical benefits of the constructed model at 1, 2, and 3 years in both cohorts, suggesting that the model can achieve good benefits in clinical applications.

Risk stratification based on nomogram

Risk scores were calculated for all patients by nomogram, and the median risk score of the training cohort (OS: 205.3) was used as the threshold for categorizing patients into high-risk (OS:

risk score ≥205.3) and low-risk groups (OS: risk score <205.3).The Kaplan-Meier survival analysis showed a significant difference in OS between different risk groups (Figure 6), suggesting that column line plotting can help us accurately stratify the risk of patients with advanced NSCLC.

Prognostic value of immunotherapy in advanced NSCLC

With the development of innovative drugs, the use of immunotherapy in NSCLC patients is gradually increasing. To investigate the prognostic value of immunotherapeutic agents in patients with advanced NSCLC, we performed a controlled analysis of chemotherapy regimens combined with immunotherapy versus chemotherapy regimens alone. In our study, the R language MatchIt package was used for propensity score matching analysis. A 1:1 greedy nearest neighbor matching with a PS score of 0.1 was used to derive pairs of patients receiving chemotherapy combined with immunotherapy and chemotherapy only. Matching variables which

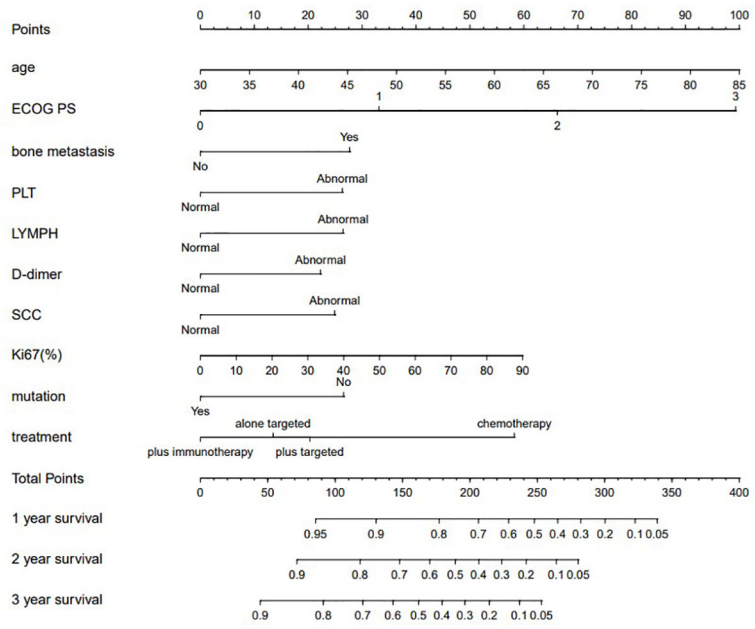

FIGURE 2
Nomograms for predicting 1, 2, and 3years OS of patients with advanced NSCLC.

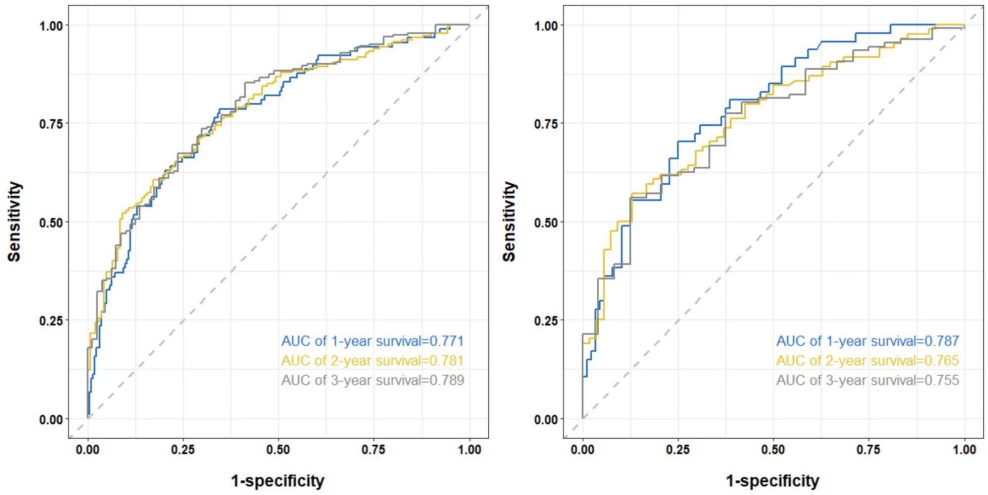

FIGURE 3
The time-dependent ROC curves of the nomogram predicting OS at (left) 1-year and 2-year and 3-year in the training cohort, and at (right) 1-year and 2-year and 3-year in the internal validation cohort.

include age, ECOG PS, bone metastasis, PLT, LYMPH, SCC, D-dimer, Ki67, and tumor driver gene mutations are nine variables. This strategy resulted in 68 matched pairs in each group, for a total of 136 patients included in the subgroup analysis. It was evident from the results that patients receiving chemotherapy combined with immunotherapy tended to have better OS in all subgroups, and all results were statistically significant (Figure 7), suggesting that the addition of immunotherapeutic agents to chemotherapy can provide a survival benefit for patients with advanced NSCLC.

Importance analysis of model variables

Figure 8 shows the visualization results of the ordering of the importance of the model variables, in which the treatment regimen accounts for the highest percentage, indicating that the treatment regimen is the most important among all the variables of the model, and that the treatment regimen is the most critical factor among the influencing factors of the survival prognosis of patients with advanced non-small cell lung cancer. Therefore, we analyzed the survival curves for all patients' treatment regimens (Figure 9). The results showed that targeted therapy alone, chemotherapy combined with targeted therapy and chemotherapy combined with immunotherapy all had better survival than chemotherapy alone.

Discussion

Advanced non-small cell lung cancer is one of the most common types of lung cancer, and thus its data are more readily available. We collected a large number of patient samples from

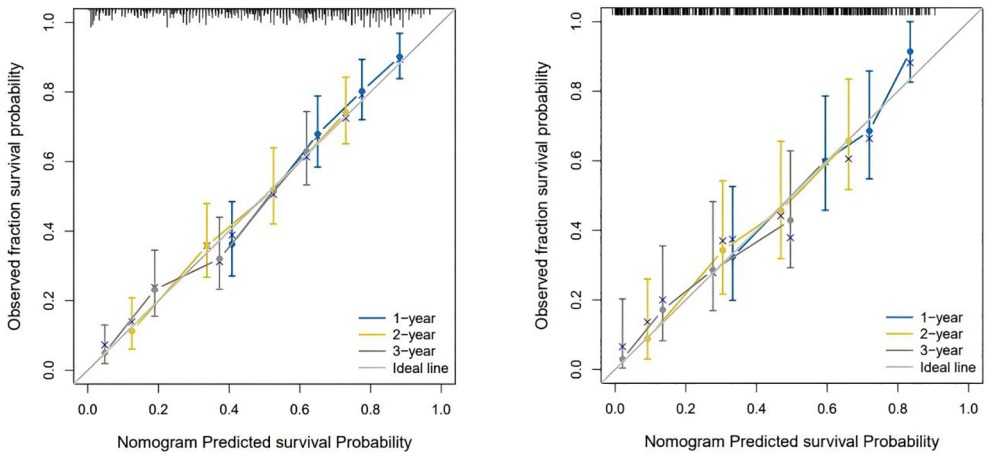

FIGURE 4
The calibration curves for predicting OS at (left) 1-year and 2-year and 3-year in the training cohort, and at (right) 1-year 2-year and 3- year in the internal validation cohort.

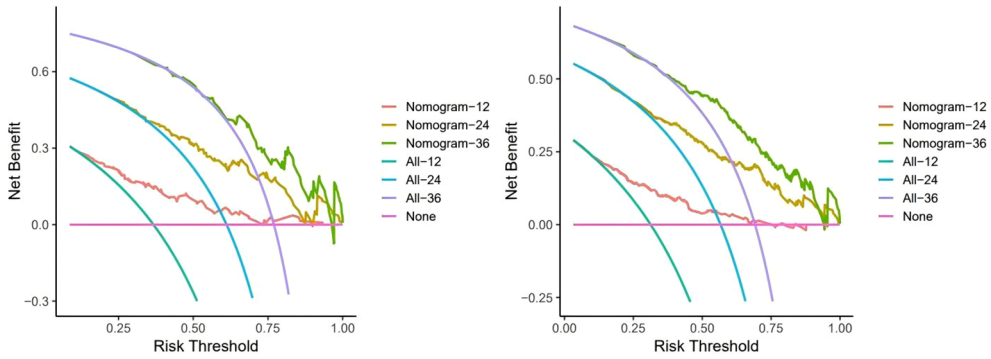

FIGURE 5
Decision curve analysis to assess clinical benefit. Here we use a monthly unit to record the benefits over three years. The training cohort (left) Internal validation cohort (right).

Shanxi Cancer Hospital, which facilitated our study of advanced non-small cell lung cancer.

In this work, we constructed a prognostic model based on basic information as well as clinical characteristics of patients with advanced non-small cell lung cancer. 462 patients from Shanxi Provincial Cancer Hospital were randomly assigned to the training cohort and the internal validation cohort, and were screened by Cox's one-way analysis for, age, ECOG PS, history of smoking, history of alcohol, complication, pathology, N-stage, liver metastasis, bone metastasis, absolute neutrophil value, platelet count, absolute lymphocyte value, absolute monocyte value, fibrinogen, lactate dehydrogenase, D-dimer, neuron-specific enolase (NSE), squamous cell carcinoma-associated antigen (SCC), Ki67, tumor-associated gene mutations, and treatment regimen were significantly associated with survival.

With the increase of age, the risk of death of patients increases (10). For middle-aged and elderly people, the function of human organs gradually decreases with age, and the invasion of non-small cell lung cancer accelerates this process, which seriously affects the survival and prognosis of patients.

Previous studies have shown that ECOG PS is an independent factor affecting prognosis (11, 12), which is consistent with the results of the present study, in which patients were subjected to tumor invasion resulting in a decreased physical activity status, compromised survival, and consequently a poor prognosis.

Bone metastasis is one of the prevalent metastases in advanced NSCLC patients, and it is also the most important factor leading to poor quality of life and low survival rate of lung cancer patients (13). Patients with bone metastases are often accompanied by severe bone pain, which seriously affects the quality of survival of patients.

In laboratory tests of patients with advanced NSCLC, platelets and lymphocytes were found to be independent influences on the survival prognosis of patients, and abnormalities in these two indices suggested a poor prognosis. Clemens Hinterleitner et al. found that platelets interact with lung cancer cells and transfer PD-L1 from tumor cells to platelets (14) suggesting that platelets are

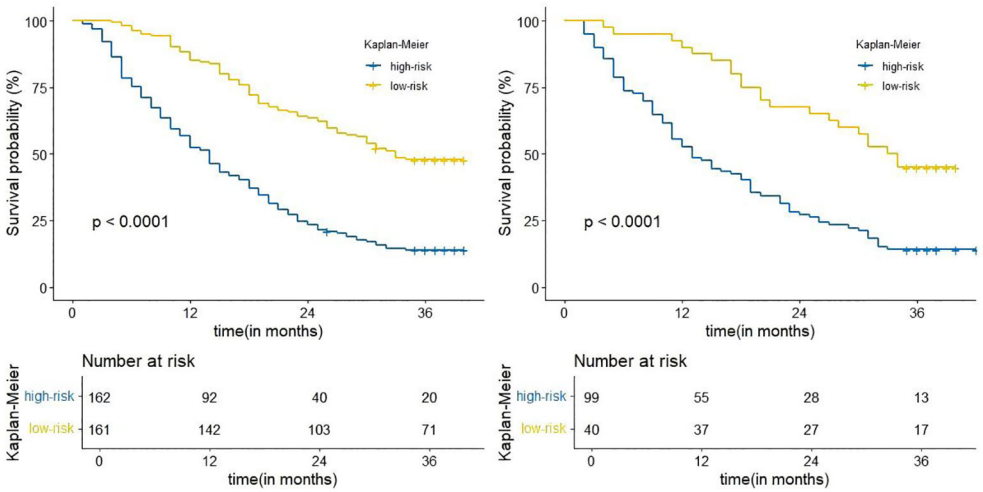

FIGURE 6
Kaplan-Meier curves for correlation with OS for the low and high-risk groups in the training cohort (left), internal validation cohort (right).

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Variable	alone chemotherapy	plus immunotherapy	HR(95%CI)	P value
Overall			**0.51(0.35~0.75)**	**0.001**
age				
<65	44 (64.7%)	43 (63.2%)	0.52(0.32~0.83)	0.006
>=65	24 (35.3%)	25 (36.8%)	0.50(0.26~0.95)	0.035
ECOG PS				
low	62 (91.2%)	62 (91.2%)	0.51(0.34~0.76)	0.001
high	6 (8.82%)	6 (8.82%)	0.54(0.15~1.89)	0.335
bone				
normal	35 (51.5%)	35 (51.5%)	0.37(0.21~0.64)	<0.001
abnormal	33 (48.5%)	33 (48.5%)	0.66(0.39~1.13)	0.129
PLT				
normal	55 (80.9%)	51 (75.0%)	0.53(0.24~1.16)	0.113
abnormal	13 (19.1%)	17 (25.0%)	0.50(0.32~0.78)	0.002
LYMPH				
normal	59 (86.8%)	62 (91.2%)	0.57(0.38~0.86)	0.007
abnormal	9 (13.2%)	6 (8.82%)	0.14(0.03~0.68)	0.015
Ddimer				
normal	33 (48.5%)	34 (50.0%)	0.59(0.34~1.04)	0.07
abnormal	35 (51.5%)	34 (50.0%)	0.43(0.25~0.72)	0.002
SCC				
normal	58 (85.3%)	59 (86.8%)	0.49(0.32~0.76)	0.001
abnormal	10 (14.7%)	9 (13.2%)	0.49(0.19~1.25)	0.136
Ki67				
<30	9 (13.2%)	9 (13.2%)	0.84(0.26~2.75)	0.769
>=30	59 (86.8%)	59 (86.8%)	0.46(0.31~0.69)	<0.001
mutation				
normal	43 (63.2%)	43 (63.2%)	0.41(0.20~0.82)	0.011
abnormal	25 (36.8%)	25 (36.8%)	0.58(0.36~0.92)	0.02

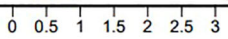

FIGURE 7
Subgroup analysis of chemotherapy and chemotherapycombined immunotherapy.

associated with tumor immune escape mechanisms thereby affecting patient prognosis. A study by Li Xiaohui et al. showed that platelets can promote the growth of lung adenocarcinoma (15). This further establishes that platelets are an independent influence on the prognosis of non-small cell lung cancer. In the study of Yoon Ya-Nam et al. neutrophil to lymphocyte ratio affects survival in patients with advanced non-small cell lung cancer (16). In this study although neutrophils to lymphocytes in the form of a ratio was not used as a variable, lymphocytes were still identified as an independent prognostic factor.

Previous studies have shown that D-dimer is associated with poor prognosis in non-small cell lung cancer (17–19), which is consistent with the findings of this study. Cancer cells usually regulate coagulation and fibrinolysis in cancer patients, and elevated plasma D-dimer suggests that patients may have a hypercoagulable state of the blood or thrombosis, which can cause damage to the organism leading to a poor prognosis.

Serum tumor markers (STMs) are circulating protein molecules produced by tumor cells or other cells in the body in response to cancer or certain benign diseases. Changes in their serum levels

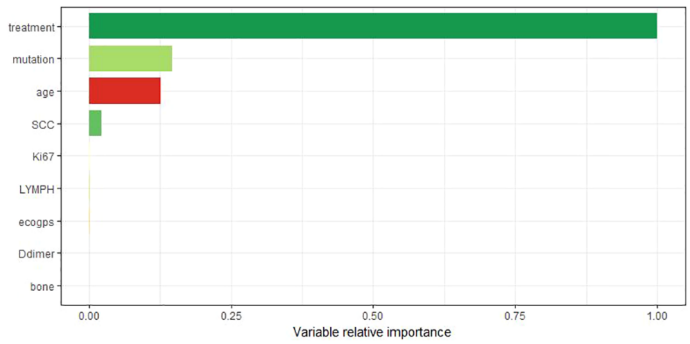

FIGURE 8
The importance ranking of variables takes the most important variable as the reference value.

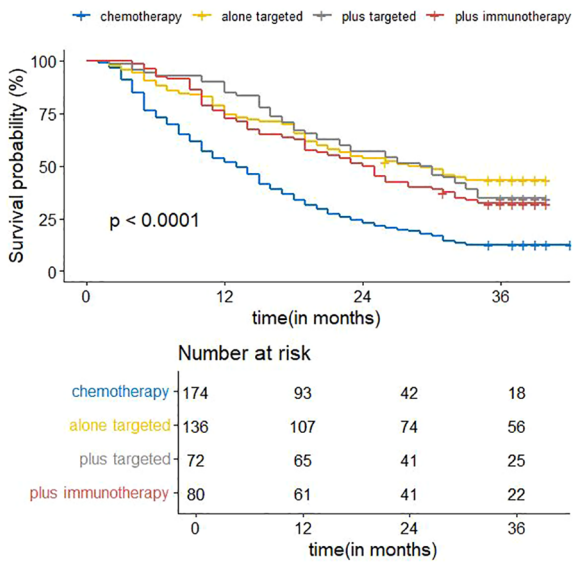

FIGURE 9
Kaplan-Meier curves for correlation with OS for the four treatment regimen.

have been shown to reflect tumor quality, making them valuable in predicting prognosis and assessing response to treatment during follow-up. Results have shown that CA 125 antigen (CA-125), carcinoembryonic antigen (CEA), cytokeratin 19 fragment (Cyfra 21.1) and squamous cell carcinoma antigen (SCC-Ag) are associated with NSCLC disease (20, 21). In this study squamous cell carcinoma antigen (SCC-Ag) was found to be a prognostic influencing factor in non-small cell lung cancer, when its value was elevated suggesting a poor prognosis for the patient.

Ki67 has significant clinical value in the treatment and prognosis of NSCLC (22, 23). Ki67 is an associated antigen of value-added cells, and its function is closely related to mitosis, which is indispensable in cell proliferation. The higher the proliferation index of Ki67, the higher the cell proliferation ability, the higher the degree of malignancy, and the worse the patient's prognosis.

Several studies have shown that mutations in tumor-associated genes affect the prognosis of patients with non-small cell lung cancer (24–28). In current therapeutic decisions, whether a tumor-associated gene is mutated or not is a key factor that can guide clinically available targeted therapies. We are rapidly discovering that more and more mutations occur in targetable pathways, and targeted therapies have dramatically altered treatment outcomes and disease prognosis (29).

Treatment regimen is significantly associated with survival prognosis in patients with advanced NSCLC (30). The importance of treatment regimen in the prognosis of advanced NSCLC can be seen in our variable significance analyses. The 2024 version of the Clinical Practice Guidelines in Oncology suggests that different treatment regimens should be chosen for patients with advanced or metastatic NSCLC, depending on their oncogenic drivers. For example, NSCLC with EGFR alterations is usually treated with targeted agents (gefitinib, ositinib, etc.) as the first line of therapy (31).

The emergence of immuno-oncology has revolutionized the treatment of metastatic non-small cell lung cancer (32). In recent years, immunotherapy has been increasingly used in the treatment of non-small cell lung cancer, and several clinical studies have shown that receiving immunotherapy can increase survival and effectively improve the prognosis of patients (33–35). Does immunotherapy still perform satisfactorily in the real world? Our subgroup analysis of patients receiving chemotherapy alone and chemotherapy combined with immunotherapy in the treatment regimen group showed that there was a significant difference in prognosis between patients receiving systemic chemotherapy and chemotherapy combined with immunotherapy in the advanced stages, with the group of patients with the addition of immunotherapy having a higher survival than the group receiving chemotherapy alone. Survival analysis plots of the treatment regimens also showed that immunotherapy improved survival, with a median survival time of 13 months for patients receiving chemotherapy alone and 24.5 months for patients receiving chemotherapy combined with immunotherapy. Immunotherapy combined with chemotherapy has a better therapeutic effect. Systemic chemotherapy, because of its lack of specificity, will damage normal body cells at the same time as it has a killing effect on the tumor, thus it is inefficient and produces serious adverse reactions, which may be the reason for the poor prognosis of the patients. When chemotherapy is combined with immunotherapy, the immune drug effectively improves the body's immune function, eliminates the escape mechanism of tumor cells, so that the tumor cells can be recognized by the body, which greatly improves the killing effect on the tumor cells, and effectively prolongs the survival of patients. It is recommended that immunotherapy be incorporated more into clinical regimens, which may benefit more patients with advanced non-small cell lung cancer. In addition, radiation therapy, as an adjuvant treatment, also occupies a certain position in patients with advanced non-small cell lung cancer, but the correlation with prognosis was not reflected in this study, and it is suggested that radiation therapy can be used as a palliative treatment to relieve localized pain, but symptomatic improvement may not be converted into OS benefit.

The data used in this study were collected from real-world clinical data, which can truly reflect the status of patients with advanced non-small cell lung cancer, and therefore have greater reference value. We hope that this model can provide a reference for clinical treatment, and that the construction of such models will help to discover new tumor-related prognostic factors. Due to regional limitations, we did not collect enough external data to serve as an external validation group, so this study lacks external validation of the model, and the extrapolation ability of the model is unknown. We hope that more internal and external cases can be collected subsequently to validate and optimize the model and better correct the model performance.

Conclusion

In summary, we successfully constructed and validated a prognostic model to predict the survival rate of patients with advanced NSCLC, which provides a more accurate basis for the treatment decision of such patients. Systemic chemotherapy dominates in advanced NSCLC patients, and chemotherapy combined with immunotherapy can improve the survival probability of advanced NSCLC patients, and it is suggested that immunotherapy should be incorporated into clinical treatment protocols more frequently.

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 Ethics Committee of Shanxi Hospital, Cancer Hospital, Chinese Academy of Medical Sciences (Shanxi Cancer Hospital). The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants\' legal guardians/next of kin because of the retrospective nature of the study.

Author contributions

KZ: Supervision, Writing – original draft. JUZ: Visualization, Writing – original draft. TX: Software, Writing – original draft. FaL: Validation, Writing – original draft. FeL: Data curation, Writing – original draft. JIZ: Formal analysis, Writing – original draft. YG: Writing – review & editing, Data curation, Methodology, Supervision. ZH: Supervision, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2025.1508721/full#supplementary-material

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