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REVIEW

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Parasite-enhanced immunotherapy: transforming the "cold" tumors to "hot" battlefields

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Abstract

Immunotherapy has emerged as a highly effective treatment for various tumors. However, the variable response rates associated with current immunotherapies often restrict their beneficial impact on a subset of patients. Therefore, more effective treatment approaches that can broaden the scope of therapeutic benefits to a larger patient population are urgently needed. Studies have shown that some parasites and their products, for example, *Plasmodium*, *Toxoplasma*, *Trypanosoma*, and *Echinococcus*, can effectively transform "cold" tumors into "hot" battlefields and reshape the tumor microenvironment, thereby stimulating innate and adaptive antitumor immune responses. These parasitic infections not only achieve the functional reversal of innate immune cells, such as neutrophils, macrophages, myeloid-derived suppressor cells, regulatory T cells, and dendritic cells, in tumors but also successfully activate $CD4^+$/$CD8^+$ T cells and even B cells to produce antibodies, ultimately resulting in an antitumor-specific immune response and antibody-dependent cellular cytotoxicity. Animal studies have confirmed these findings. This review discusses the abovementioned content and the challenges faced in the future clinical application of antitumor treatment strategies based on parasitic infections. With the potential of these parasites and their byproducts to function as anticancer agents, we anticipate that further investigations in this field could yield significant advancements in cancer treatment.

Keywords Immunotherapy, Parasite infection, Cancer, Immune response, Tumor progression

Overview

Malignant tumors are among the major threats to human health and causes of death. Although current cancer treatment has transitioned from a single surgical treatment to multiple comprehensive methods, such as surgery combined with chemotherapy, radiotherapy, or immunotherapy, the efficacy of these methods is unsatisfactory. In addition to high costs, a low chance of full recovery, and extensively demanding side effects, the poor quality of life of patients has perpetually been a dominant concern in cancer treatment. Therefore, it is necessary to develop new therapeutic strategies to improve the efficacy of cancer therapy.

Microbial-based cancer therapy has proven to be a promising cancer treatment strategy, by inducing

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tumor cell regression, either directly through the microbial destruction of tumor cells or indirectly through the activation of the host immune system during infection. This treatment strategy dates back to 1813 when Vautier [1] reported tumor regression in patients with gas gangrene. It attracted the attention of oncologists worldwide when Coley invented Coley's toxin in 1893 and applied it to the treatment of tumors such as sarcoma, lymphoma, melanoma, and myeloma [2]. To date, the therapeutic effects of many microorganisms and their attenuated strains on tumors, for example, *Clostridium*, *Salmonella*, *Bifidobacterium*, *Escherichia*, *Listeria*, *Shigella*, *Mycobacterium* (Bacillus Calmette-Guérin, BCG), and even bacteriophages, viruses, and protozoa, have been investigated. Notably, some of these microorganisms have proven to be valuable in tumor treatment to a certain extent. For example, BCG has been developed as a routine treatment for patients with high-risk bladder cancer [3]. Therefore, microbial-based cancer therapy is a promising adjuvant therapy for cancer.

Mechanistically, microorganisms may function as oncolytic "weapons" and induce an antitumor local or systemic immune response. For example, *Clostridium novyi* (*C. novyi*)-NT spores preferentially colonize the tumor necrosis (anaerobic) area and destroy adjacent cancer cells by releasing reactive oxygen species (ROS), proteases, etc. [2, 4]. Additionally, tumor antigens released by these ruptured cells undoubtedly increase the immunogenicity of tumor cells and initiate a targeted antitumor immune response [2, 4].

Furthermore, microbial-based adjuvant therapy for cancers may reshape the tumor microenvironment (TME) and achieve a transition from "cold" tumors to "hot" tumors through changes in cytokine secretion and immune cells infiltrations in the TME. "Cold" tumors, characterized by a lack of immune cell infiltration, low expression of PD-L1 and MHC-I, a low mutation burden, and a high presence of immunosuppressive cells, often exhibit resistance to conventional immunotherapy. In contrast, "hot" tumors, which are characterized by a strong immune response and a diverse mutational profile, tend to respond well to immunotherapeutic interventions. For example, *C. novyi*-NT infection may trigger an inflammatory response to produce cytokines such as IL-6, MIP-2, G-CSF, and TIMP-1, which attract neutrophils, monocytes, and lymphocytes into the tumor tissue, thereby resulting in tumor regression [4]. Another example is attenuated *Shigella flexneri* (*S. flexneri*), which may eliminate tumor-associated macrophages (TAM) in breast cancer and lead to tumor regression [5], indicating that a microbe with cancer therapy features likely has its own unique antitumor mechanism.

In recent years, the effects of some protozoa on tumors have drawn widespread attention. Studies have shown that some parasites and their products may exert antitumor effects by enhancing antitumor-immune responses, inhibiting tumor angiogenesis, or inducing tumor cell apoptosis, thereby triggering tumor regression [6, 7]. Here, we summarize the antitumor activities of the four main parasites and their products, *Plasmodium*, *Toxoplasma gondii* (*T. gondii*), *Trypanosoma cruzi* (*T. cruzi*), and *Echinococcus granulosus* (*E. granulosus*), providing new potential for developing parasitic protozoan-based adjuvant therapies for cancer.

Plasmodium-based cancer therapy

Plasmodium is a protozoan that parasitizes red blood cells and feeds on hemoglobin and can cause malaria in humans and animals [8]. Notably, *Plasmodium* infection in humans can produce periodic high fever in the acute phase. On the basis of this feature, Greentree [9] proposed in 1981 that *Plasmodium* infection might help treat tumors. Although several studies have shown that *Plasmodium* infection may promote the development of endemic Burkitt lymphoma [10, 11], some studies suggest that *Plasmodium* infection inversely associated with mortality in many cancers such as gastric cancer, breast cancer, and lung cancer [12, 13]. These findings suggest that some factors caused by *Plasmodium* infection may contribute to controlling carcinogenesis. Further studies have shown that *Plasmodium* may remodel the TME and activate antitumor immune responses [14]; additionally, it may inhibit tumor angiogenesis [15] and epithelial-mesenchymal transition (EMT) [16], ultimately triggering tumor regression (Fig. 1 and 2).

The characteristics of the TME in a solid tumor are vital factors in the success of anticancer therapy. Such microenvironment usually contains a variety of immune cells with suppressive phenotypes, such as TAM, myeloid-derived suppressor cells (MDSC), and regulatory T cells (Treg) [17]. Immune cells cooperate with tumor cells to preserve the undesirable TME, thereby intensifying tumor proliferation, metastasis, and EMT and increasing multidrug resistance. Therefore, targeting immunosuppressive cells will undoubtedly remodel the TME and improve antitumor efficacy.

Studies have shown that *Plasmodium* infection can remodel the TME [6, 7, 14]. Wang et al. [15] demonstrated that *Plasmodium* infection can decrease the proportions of M2-like TAM in a murine hepatocellular carcinoma (HCC) model. More importantly, *Plasmodium* infection can reversely shift the functional phenotype of M2-like TAM by inhibiting the IGF-1/MAPK and PI3K/AKT pathways, thereby suppressing HCC growth. Consistent with this finding, Adah [18] and Tao [19] reported

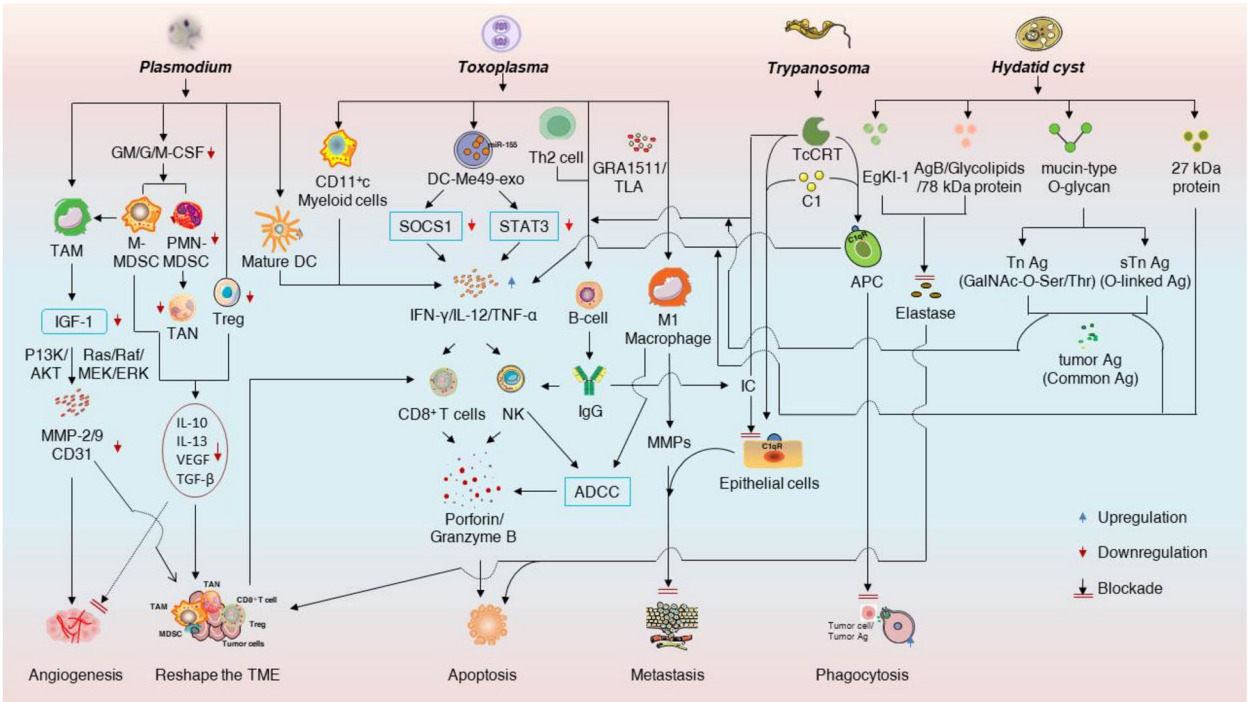

Fig. 1 Parasite infection triggers antitumor immune responses. *Plasmodium* infection hinders tumor angiogenesis by impeding the infiltration of tumor-associated macrophages (TAM), reshaping the TME, and suppressing the IGF-1/MAPK/PI3-K pathway, leading to reduced MMP-9 expression. This infection increases the expression of TNF-α and IFN-γ, activating natural killer (NK) cells and enhancing the $CD8^+$ T cell antitumor responses. This increased cytokine expression also prompts the maturation of dendritic cells (DC) and promotes the secretion of granzyme B and perforin, further augmenting the immune response against tumors. *Toxoplasma* activates a Th1 immune response upon infection, resulting in the secretion of cytokines to specifically target angiogenic factors such as VEGF and MMP while inhibiting the STAT-3 signaling pathway. These actions collectively hinder the formation of new blood vessels essential for tumor growth and survival. Additionally, this infection reduces TGF-β levels, further contributing to the suppression of angiogenesis. *T. gondii* infection also impairs tumor cell migration and invasion by reducing TNF-α and MMP-9 level. It promotes the polarization of macrophages toward an M1 activation state, known for potent antitumor effects. Moreover, the infection stimulates the production of IL-12, which is facilitated by the activation $CD8^+$ T cells or NK cells to secrete IFN-γ, whose signaling cascade enhances the immune response against tumor cells in vivo, contributing to tumor clearance. Antibodies against *T. cruzi* enhance the recognition of tumor cells through the use of host immune cells such as macrophages and NK cells. The upregulation of CD11b/c, His482, and MHCII expression could promote DC maturation, induce TNF and nitric oxide production by macrophages, and enhance Th1 polarization, thus increasing cytotoxic the ability of T cells to kill tumor cells. TcCRT may interact with endothelial cells (EC) in a C1- and cC1qR-dependent manner and inhibit EC proliferation, migration, and capillary morphogenesis, thereby inhibiting angiogenesis. In addition, TcCRT may initiate critical adaptive immune responses in the TME: on the one hand, it is recognized by cC1qR on APCs, which activates macrophages and enhances their phagocytosis; on the other hand, specific peptides in TcCRT may be cross-loaded onto MHC-I molecules after APC processing to activate $CD8^+$ T cells and their antitumor activity. *E. granulosus* upregulates the expression of several pronflammatory cytokines, including TNF-α and IFN-γ, through the secretion of mucin-type O-glycans, which induce a Th-1 response. This O-glycan also exerts antitumor effects by stimulating NK cell activation, inducing DC maturation, and upregulating IL-12 and IL-6 expression. Furthermore, EgKI-1 may play a role in remodeling the TME by increasing the number of $CD8^+$ T cells

that *Plasmodium* infection may inhibit the expansion and activation of MDSC and Treg in a murine Lewis lung cancer (LLC) model and glioma model, respectively. Mechanistically, *Plasmodium* infection can significantly reduce the expression of several crucial molecules, such as GM-CSF, G-CSF, and M-CSF, that determine the recruitment of MDSC to solid tumors; simultaneously, it may regulate the differentiation of recruited MDSC by modulating the level of multiple phosphorylated signal transducer and activator of the transcription (STAT) proteins. Additionally, this infection can reduce the proportion of Treg by suppressing the CCL-17/22-CCR4 pathway that modulates the accumulation of Treg in tumors. As a result, the level of immunosuppressive molecules, such as IL-10/13, VEGF, and TGF-β, are significantly reduced within solid tumors, which improves the TME and augments antitumor responses.

Plasmodium induces antitumor immune responses

Tumors often evade the attack of the immune system via multiple mechanisms. Disrupting these mechanisms will improve the efficacy of tumor immunotherapy. A practical strategy is to transfer a cold tumor to a hot tumor [14]. However, a prerequisite for this switch is the presence of

a suitable cytokine microenvironment, especially interferons (IFN). Coincidentally, proinflammatory cytokines, such as IL-12, TNF-α, and IFN-γ, are induced in response to *Plasmodium* invasion [6, 7, 14]. These findings provide a substantial basis for the "crosstalk" between *Plasmodium* infection and anticancer therapy.

Chen et al. [20] reported that *Plasmodium* infection elevates TNF-α and IFN-γ in bearing-LLC mice. Notably, IFN-γ not only activates natural killer (NK) cells but also increases $CD8^+$ T cell antitumor activity by encouraging the maturation of dendritic cells (DC), thereby inhibiting the growth and metastasis of LLC cells and increasing the survival rate of tumor-bearing mice. This strong specific antitumor-immune response mediated by $CD4^+$/$CD8^+$ T cells can be detected even in HCC-bearing mice infected with an attenuated *Plasmodium* strain [21]. Another study focused on murine breast cancer has shown that *Plasmodium* infection increases the percentage of effector and central memory T cells with antitumor activity and promotes the secretion of granzyme B and perforin by increasing the level of antigen-specific IFN-γ [22]. Together, these studies have shown that *Plasmodium* infection induces local and systemic tumor-specific immune responses through the "crosstalk" with tumors. Given these findings, *Plasmodium* immunotherapy has high potential as a prospective adjuvant therapy for cancer. Indeed, Tao et al. [19] demonstrated that *Plasmodium* immunotherapy combined with radiotherapy achieved the conversion of cold tumors to hot tumors, resulting in a synergistic antitumor effect that could cure approximately 70% of gliomas.

Plasmodium inhibits tumor angiogenesis and EMT

An important feature of solid tumors is that their growth requires new blood vessels to supply oxygen and nutrients [23]. Therefore, angiogenesis plays a central role in tumor proliferation, expansion, and metastasis and is an important therapeutic target. Studies have shown that TAM promote formidable tumor angiogenesis by producing proangiogenic factors and matrix metalloproteinases (MMP), such as VEGF-A, EGF, TGF-β, Tie2, angiopoietin, TNF-α, IL-1β, IL-8, CCL2, CXCL8, CXCL12, and MMP-2/9 [24, 25]. Wang et al. [15] reported that *Plasmodium* infection not only inhibits TAM infiltration in tumors but also reduces MMP-9 expression through negative regulation of the IGF-1/MAPK/PI3-K signaling pathway, thereby repressing tumor angiogenesis and HCC growth. Furthermore, Yang et al. [26] presented that *Plasmodium* infection decreases vascular endothelial growth factor receptor 2 (VEGFR2) expression by inducing the expression of an exosome containing microRNA-16/17/322/497, which specifically binds to the 3' UTR of *vegfr2*, resulting in a significant reduction in tumor angiogenesis and LLC growth in mice.

EMT is a process by which epithelial cells acquire mesenchymal characteristics [27]. It is the crucial step in the malignant transformation of tumors, endowing cancer cells with metastatic properties through enhanced mobility, invasion, and resistance to apoptotic stimuli [28]. Liang et al. [16] reported that *Plasmodium* infection significantly increases E-cadherin expression and reduces vimentin and Snail expression to prevent HCC recurrence and metastasis. Mechanistically, *Plasmodium* infection negatively modulates Akt and GSK-3β activation by inhibiting CCR10 expression, thus suppressing the accumulation of Snail, which is a significant inducer of EMT.

Taken together, *Plasmodium* infection restrains the proliferation and metastasis of malignant tumors, including lung cancer, liver cancer, breast cancer, and glioma, as its mechanism involves multiple stages and steps in the process of tumor progression.

Challenges of *Plasmodium*-based *cancer* treatments

Plasmodium as a cancer treatment is a double-edged sword. On the one hand, compelling laboratory evidence suggests that these parasites could inhibit the growth of specific tumors, including lung, liver, glioma, and breast cancers [14–16, 18–22]. Epidemiological studies have also demonstrated a suggestive association between a lower malaria incidence and higher incidences of these cancers [13]. Furthermore, limited clinical trials in China have suggested benefits for patients with advanced lung, liver, and prostate cancers. A patient with lung cancer exhibited the disappearance of metastatic lesions in the neck, a lack of blood vessels in lung tumor tissue, and significant infiltration of immune cells, including T cells (unpublished data).

On the other hand, the use of *Plasmodium* as a treatment modality for cancer patients faces challenges. These parasites are the causative agents of malaria, a severe infectious disease, which raises significant ethical and safety concerns. The potential for adverse reactions and complications, particularly in patients with compromised immune systems, necessitates meticulous research and stringent regulatory oversight to ensure patient safety and therapeutic efficacy.

To advance this treatment concept, thorough research, meticulously designed clinical trials, and appropriate ethical approval are essential. The development of attenuated *Plasmodium* strains and the training of specialized medical personnel are also critical. Furthermore, the willingness and compatibility of patients to undergo treatment with *Plasmodium* should be carefully considered,

as not all cancer patients may be comfortable with or suitable for this therapy.

In conclusion, while the potential of *Plasmodium* as an adjuvant for treating certain tumors is intriguing, the path to widespread application is complex and fraught with challenges. Extensive research, regulatory compliance, and patient-centered considerations are necessary before this therapy can be considered a viable option for cancer treatment.

Toxoplasma-based tumor biotherapy

Toxoplasma gondii (*T. gondii*) is a unicellular obligate intracellular protozoan parasite. Studies have shown that *Toxoplasma* is highly resistant to malignant tumor development. For example, *T. gondii* infection impedes the progression of spontaneous mammary tumors and leukemia [29]. Varga et al. [30] demonstrated that *T. gondii* infection or free cell extracts can reverse multidrug resistance in mouse lymphoma and human gastric cancer. Notably, a lysate antigen from *T. gondii* inhibited the growth of WEHI 164 fibrosarcoma in mice and tumors induced by methylcholanthrene in rats [31, 32]. Even formaldehyde-fixed *T. gondii* exhibited a favorable anti-LLC effect [33]. Deep sequencing analysis revealed that *T. gondii* infection significantly altered the cancer transcriptome, proteome, and cancer pathways [34]. Therefore, *T. gondii* may restrict tumor growth by inhibiting tumor angiogenesis, inducing apoptosis, regulating the cell cycle, and strengthening antitumor immunity (Fig. 1 and 2).

Toxoplasma inhibits tumor angiogenesis

Through a study conducted in 2001, Hunter et al. [35] reported that acute infection with *T. gondii* significantly inhibited angiogenesis in nonimmunogenic B16. F10 melanoma-bearing mice. This inhibitory effect was also observed in mice bearing LLC tumors [36]. The mechanism underlying this inhibition of angiogenesis by

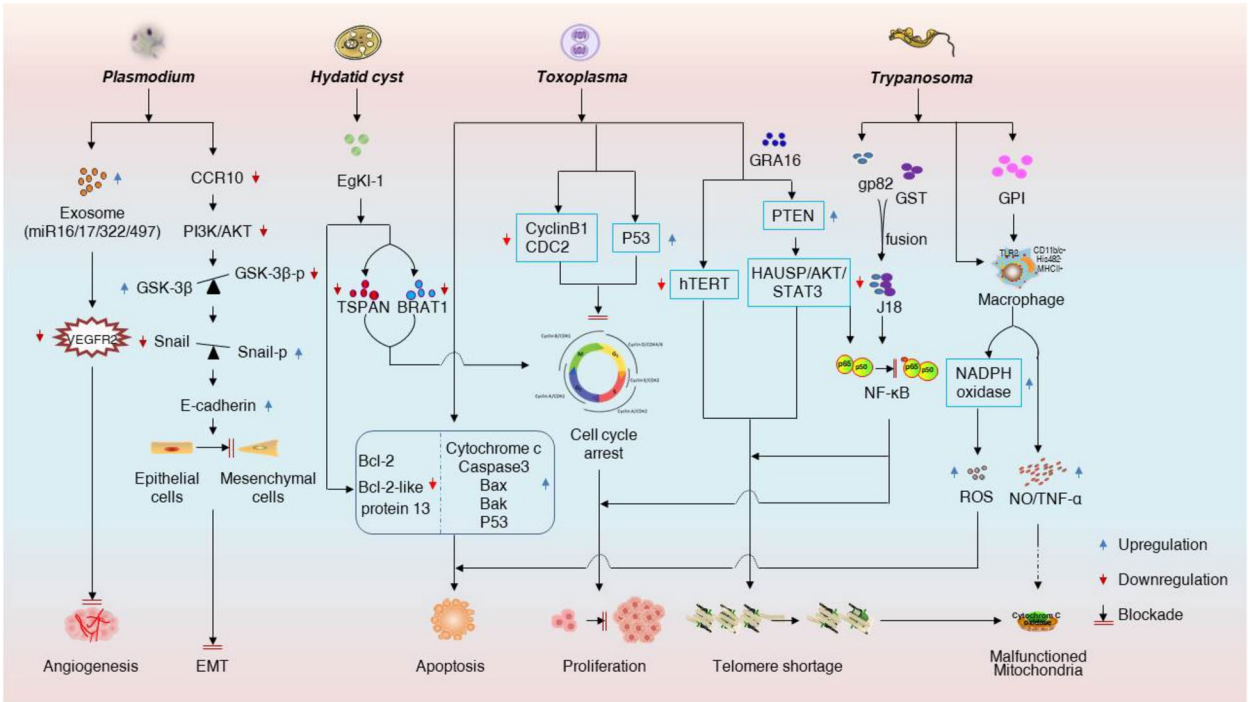

Fig. 2 Parasite infection directly counteracts tumor growth. *Plasmodium* infection induces the release of exosomes that specifically bind to VEGF, resulting in the decreased expression of vascular endothelial growth factor receptor 2 (VEGFR2) and the inhibition of tumor angiogenesis. Additionally, the infection resulted in the downregulation of CCR10 expression, which, in turn, triggered upregulation of E-cadherin expression by suppressing the AKT/PI3K pathway. This regulatory cascade ultimately curtailed the EMT in tumor cells. *E. granulosus* induces apoptosis in tumor cells by upregulating Bcl-2 expression through the secretion of the EgKI-1 molecule, which activates caspase 3. In addition, *E. granulosus* disrupts the cell cycle by downregulating the expression of activators of ATM-1 (BRAT1 and TSPAN). *Toxoplasma* infection potentiates apoptosis, a programmed cell death process, by downregulating Bcl-2 expression and increasing the expression of proapoptotic factors, including P53, Bax, Bak, Caspase3, and Cytochrome C. Furthermore, GRA16 from *T. gondii* has been observed to shorten telomeres in tumor cells by enhancing the PTEN/HAUSP/AKT/STAT3/NF-κB pathway and decreasing the expression of hTERT. *T. cruzi* potentially counteracts tumor growth by reducing J18 expression and blocking p65 phosphorylation through fusion of gp82 and GST. Furthermore, the infection elicits the activation of macrophages, which in turn increase their production of ROS and NO. This heightened secretion leads to the degradation of tumor cell mitochondria, causing tumor cell apoptosis

Toxoplasma infection involves a decreased proportion of significant molecules such as CD31, VEGF, and TGF-β. CD31 and VEGF play crucial roles in the formation of new blood vessels by facilitating cell–cell adhesion and binding to VEGFRs on the cell surface, thus promoting tumor angiogenesis [37, 38]. Pyo et al. [39] demonstrated that *Toxoplasma* lysate antigens (TLA) reduce CD31 expression and inhibit microvessel formation in a murine sarcoma-180 tumor model. Additionally, a significant reduction in VEGF levels was observed in Ehrlich ascites carcinoma-bearing mice infected with the gamma radiation-attenuated *T. gondii* ME49 strain [40]. *Toxoplasma* infection also leads to a decrease in TGF-β levels in tumor-bearing mice, contributing to the inhibition of tumor angiogenesis [40]. Furthermore, *Toxoplasma* infection induces a Th1 immune response, leading to the production of Th1-type cytokines such as IL-12 and IFN-γ. These cytokines prevent the expression of VEGF, integrins, and MMP; deactivate the STAT-3 signaling pathway, and ultimately result in the inhibition of tumor angiogenesis [36, 40].

Toxoplasma induces apoptosis and cell cycle arrest

Apoptosis, a form of programmed cell death regulated by genes, plays as an essential role in various physiological processes ranging from development to adaptive responses. The dysregulation of apoptotic processes is linked to numerous diseases, with excessive apoptosis resulting in cell shrinkage and insufficient apoptosis leading to uncontrolled cell proliferation, as observed in cancer [41, 42]. Therefore, targeting apoptosis represents a promising approach for cancer therapy.

In terms of mechanism, the balance among the Bcl-2 family members determines whether a cell will undergo apoptosis [43]. In general, the antiapoptotic proteins Bcl-2 and Bcl-xL inhibit apoptosis by binding to the proapoptotic proteins Bax and Bak. However, when cytoplasmic levels of free Bad increase in response to DNA damage, growth factor withdrawal, loss of contact with the extracellular matrix, or glucocorticoids, Bcl-2 and Bcl-xL bind to Bad to release Bax and Bak, promoting the sequential release of Cytochrome C and apoptosis [43, 44]. However, since this regulatory mechanism does not work well in cancer, the number of apoptotic cells is insufficient [42]. Thus, targeting antiapoptotic proteins and maintaining the balance between pro- and antiapoptotic family members are crucial for cancer therapy.

Wang and colleagues [45] reported that the tachyzoite of the *T. gondii* RH strain decreases Bcl-2 protein expression and increases Caspase-3 expression in H7402 cells, thus inducing their apoptosis. Furthermore, an attenuated *Toxoplasma* strain promoted the apoptosis of Ehrlich ascites carcinoma cells by decreasing Bcl-2 expression and increasing Bax, Bak, Cytochrome C, and Caspase 3 expression [40]. Other studies have established that excretory/secretory proteins (ESP) released by *T. gondii* increase p53 and reduce Bcl-2, triggering the apoptosis of multiple types of cancer cells including lung cancer A549 cells, breast cancer MCF-7 cells, prostate cancer DU145 cells, and esophageal cancer EC109 cells [46]. Notably, granule protein 16 (GRA16), a dense granule protein from *T. gondii*, induces HCT116 colorectal cancer cell apoptosis by directly decreasing telomerase reverse transcriptase (hTERT) expression and activity and indirectly shortening telomeres by activating the tumor suppressor PTEN and reducing HAUSP/AKT(S473)/STAT3/NF-kB expression [47].

Aberrant activity of the core cell cycle machinery is present in virtually all tumor types and is a typical driver of tumorigenesis [48]. An orderly cell cycle depends on the proper function of each cellular regulator. Following infection with *T. gondii* tachyzoites, the cell cycle in HCC H7402 cells is arrested with decreased cyclinB1 and cdc2 expression, which increases the proportion cells in the G0/G1 phase and decreases the ratio of cells in the S and G2/M phases [49]. Furthermore, the parasitic component, ESP, may induce cell cycle arrest and inhibit the proliferation of A549 cells by increasing the expression of p53, which plays a central role in triggering control mechanisms at both the G1/S and G2/M checkpoints [46, 49]. However, there is still a lack of in-depth investigations on the apoptosis and cell cycle arrest mediated by *Toxoplasma*, and the specific mechanism of action remains unclear.

Toxoplasma inhibits tumor metastasis

The MMP play a critical role in tumor metastasis because of their ability to degrade all extracellular matrix proteins. MMP-2 and MMP-9 are the most important mediators of tumor cell migration and invasion, which involves the degradation of ECM components [50]. Notably, after *Toxoplasma* inoculation, Ehrlich ascites carcinoma was shown to be less invasive due to low levels of MMP-2 and MMP-9 [40]. Further studies have shown that GRA15II released by *Toxoplasma* blocks the migration and invasion of Hepa1-6 tumor cells in a murine HCC transplant model by reducing MMP-2 and MMP-9 expression and driving macrophages toward classical activation [51]. Furthermore, studies from Pyo and colleagues [52] revealed that TLA decreases the level of TIMP-1, a metastatic marker, in CT26 tumor-bearing nude mice.

Toxoplasma enhances host antitumor immunity

As an obligate intracellular parasitic protozoan, *T. gondii* aggressively invades host cells, especially $CD11c^{+}$ myeloid cells such as macrophages and DC, which often

elicit immunosuppression in the TME [53, 54]. However, in tumor-bearing mice infected with *Toxoplasma* strains, myeloid cells with an immunosuppressive phenotype in the TME are transformed into antitumor immune cells with an immunostimulatory phenotype [54, 55]. A typical representative parasitic strain is CPS, a nonreplicating uracil auxotrophic *Toxoplasma* strain in which carbamoyl phosphate synthase II is deleted and there is no de novo pyrimidine biosynthesis pathway, which safely and significantly relieves immunosuppression in several types of tumors and successfully achieves the switch from "cold" tumors to "hot" tumors [51, 54–56]. First, CPS preferentially parasitizes ovarian and pancreatic cancer-resident $CD11c^+$ myeloid cells, resulting in increased expression of the costimulatory molecules CD80 and CD86, which are required for $CD8^+$ T cell activation, and a sequential significant improvement in the antigen-presenting ability of tumor cells [57, 58]. Aditionally, CPS increases Th1-type cytokine IL-12 production by triggering $CD8^+$ T or NK cell activation to produce IFN-γ, thereby leading to the regression or rejection of several established tumors, such as ID8-VegfA ovarian carcinoma [57], pancreatic cancer [58], and B16F10 melanoma [59]. Although $CD4^+$ T cells and NK cells are dispensable for the therapeutic benefit of pancreatic cancer and melanoma [58, 59], the activation of $CD4^+$ T cells and the production of tumor-specific IgG by the administration of the CPS strain may contribute to the development of effective long-term immunity in pancreatic tumor-bearing mice [60].

Notably, the components of *T. gondii* obtained in vitro also similarly enhance the immune response to clear cancer tumors in vivo, which may provide an effective solution to the safety problems associated with the direct use of *T. gondii* infection for immunotherapy. Payne and colleagues [61] reported that a subset of *T. gondii* proteins, termed soluble *T. gondii* antigens (STAg), which are composed of an immunodominant protein called profilin, elicited a marked therapeutic response in pancreatic cancer subcutaneous tumors with Kras and P53 mutations, resulting in a decrease in tumor volume, accompanied by an influx of $CD4^+$ and $CD8^+$ T cells into the tumor. Mechanistically, this treatment effect may depend on the secretion of IFN-γ and the activation of DC induced by STAg [61]. Furthermore, exosomes (DC-Me49-exo) derived from *Toxoplasma*-infected DC may regulate SOCS1 expression by delivering functional miR-155-5p, subsequently hindering macrophage polarization to the M2 phenotype in the murine CRC TME [62]. Interestingly, another study from the same research group revealed that DC-Me49-exo can suppress STAT3 signaling pathway to regulate the number of MDSC [63]. Together, these findings undoubtedly introduce new innovations for cancer immunotherapy.

Challenges of *Toxoplasma* -based cancer treatments

The antitumor properties of *T. gondii* have been the subject of extensive research, surpassing those of other antitumor parasites. Evidence suggests that an attenuated strain of *T. gondii* type I Δ GRA17 can potentiate the effects of immunotherapy, particularly when combined with PD-L1 inhibitors, leading to the regression of both primary and secondary tumors [64]. Despite the absence of clinical trials in the public domain, the therapeutic potential of this approach remains promising.

However, *T. gondii* is a pathogen that can cause toxoplasmosis, a condition that may be particularly severe for individuals with compromised immune systems or pregnant women. The prospect of using *T. gondii* in cancer patients as a treatment triggers concerns about the potential risk of toxoplasmosis and other adverse reactions, which could adversely affect patient health. To mitigate these risks and ensure the efficacy and specificity of this treatment modality, it is imperative to delve into the molecular mechanisms that govern the tumor-specific targeting of *T. gondii* and to gain a comprehensive understanding of the interaction of *T. gondii* with cancer cells. Additionally, strategies that enhance tumor-targeting ability of the parasites while reducing any unwanted side effects.

Furthermore, for *Toxoplasma*-based cancer biotherapies to advance toward clinical practice, adhering to regulatory standards, securing the required approvals, and addressing the ethical considerations associated with the application this strategy in a clinical setting are essential to ensure the safety and ethical application of this novel treatment option for the benefit of cancer patients.

Trypanosoma-based tumor biotherapy

Trypanosoma cruzi (*T. cruzi*), a single-celled protozoan parasite transmitted by *Triatominae* insects, causes Chagas disease (also known as American trypanosomiasis) in humans, leading to severe cardiac and stomach problems. However, studies have shown that *T. cruzi*-induced infection can be inversely correlated with cancer incidence [65, 66]. For example, after examining the pathological information of 894 patients with Chagas megacolon, Garcia and colleagues [66] reported no colonic neoplasia in patients with megacolon. Furthermore, as early as 1946, former Soviet scientists discovered that *T. cruzi* culture extracts had anticancer properties, showing marked therapeutic effects on cancer patients [67]. Additionally, studies have shown that *T. cruzi* infection or injection of *T. cruzi* lysate significantly inhibits the growth of xenograft

tumors in experimental mice [68–71]. Mechanistically, *Trypanosoma* may directly or indirectly enhance innate or adaptive immunity to kill tumor cells or induce tumor cell apoptosis by producing effector molecules (Fig. 1 and 2).

Anticancer activities of genetically differentiated *Trypanosoma* clones

Studies have shown that the anticancer activity of *T. cruzi* and its clonal lysates depends on the genetic differentiation of *T. cruzi* [68, 72–74]. Currently, *T. cruzi* is genetically classified into at least seven discrete typing units (DTU) [75]. DTU1 and DTU2 are well-analyzed genotypes for antitumor activity. Interestingly, the antitumor activity of the DTU1 clones was significantly greater than that of the DTU2 clones [72]. In addition, Batmonkh et al. [74] confirmed that the lysate of the DTU1 genetic group (clone P, G, Sp) had a direct anti-proliferative effect on Ehrlich adenocarcinoma cells, whereas the lysate of the DTU2 group clone Y7/2 had a pronounced delayed protective effect (70% tumor growth inhibition).

Specific antitumor immune responses induced by *T. cruzi* or its lysates

The chemical constituents of parasitic antigens typically include polypeptides, glycoproteins, lipoproteins, and polysaccharides. In some instances, there is a convergence of antigens between certain parasites and tumor cells, which can trigger immune cross-reactivity and augment the host's antitumor response. Studies have shown that vaccination with *T. cruzi* lysates elicits antitumor protection by enhancing both innate and adaptive immune responses [69]. For example, *T. cruzi* shares antigens with cells from Ehrlich adenocarcinomas [73, 76], acute lymphoblastic leukemia [77], and neuroblastoma [77]. Consequently, infection with *T. cruzi* or the administration of its lysate can trigger an antitumor immune response mediated by host-produced antibodies against Ehrlich adenocarcinoma, acute lymphoblastic leukemia, and neuroblastoma [73, 76–78]. Furthermore, antibodies generated against *T. cruzi* lysate can also enhance the recognition and targeting of diverse tumor cells, including rat and human colon and breast cancer cells, thereby facilitating tumor cell destruction through antibody-dependent cellular cytotoxicity (ADCC) [70, 79].

In addition, *T. cruzi* epimastigote lysates are capable of activating both $CD4^+$ and $CD8^+$ T cells and significantly inhibiting tumor growth [68, 70, 79]. Importantly, this T cell activation appears to be mediated by the production of Th1-type cytokines, such as interferon-gamma (IFN-γ), which enhances the activity of $CD8^+$ T cells, NK cells, and macrophages [69]. Alternatively, immunization with *Trypanosome* antigens can increase the number of CD11b/c(+) His48(−) MHC II(+) macrophages and dendritic cells, which in turn increase the activity of NADPH oxidase in these immune cells. This results in the production of reactive oxygen species (ROS) that can directly destroy tumor cells [70]. The increase in these antigen-presenting cells may effectively improve the tumor antigen presentation capacity, thereby enhancing the Th1 immune response and the ability of cytotoxic T cells to kill tumor cells.

Notably, *T. cruzi* lysates also activate and trigger Toll-like receptor (TLR) signaling in mice and humans [69]. Further studies have shown that mannose residues in lysates can activate mouse and human TLR4 as well as human TLR2, which may help promote the maturation of APCs such as dendritic cells [69]. In addition, studies have reported that the glycosylphosphatidylinositol (GPI) of *T. cruzi* is a ligand of TLR2 [80], which may induce macrophages to produce TNF and nitric oxide and strengthen Th1 polarized immune responses [81, 82]. In this context, *T. cruzi* lysate-induced immunity and tumor protection are not associated with IFN-γ production.

Antitumor effects of *T. cruzi*-derived calreticulin

T. cruzi calreticulin (TcCRT), an endoplasmic reticulum (ER) resident chaperone protein with a molecular weight of 45 kDa [83, 84], plays a central role in the interaction between *T. cruzi* and the host [79, 84]. As a complement inhibitor, TcCRT inhibits the activation of the complement system by interacting with complement proteins such as complement factor 1 (C1), mannose-binding lectin (MBL), and ficolins, thus leading to an increase in host infectivity [84–86]; it also acts as a vital virulence factor to promote persistent infection of the host by *T. cruzi* [84–86]. However, although TcCRT plays a substantial role in the invasion and dissemination of *T. cruzi*, it also has unique antitumor potential [84, 87, 88].

Studies have shown that TcCRT can inhibit the growth of various tumors, including colon [89] and breast [89–93] cancer and melanoma in mice in vitro and in vivo [94]. Mechanistically, TcCRT may interact with endothelial cells (EC) in a C1 and cC1qR-dependent manner, suppressing EC proliferation, migration, and capillary morphogenesis and effectively combating angiogenesis [79, 90, 93, 94]. Furthermore, TcCRT may also initiate crucial adaptive immune responses in the TME: on the one hand, TcCRT translocated into the tumor recruits complement C1 and is recognized by cC1qR on APCs, activating macrophages and enhancing their phagocytosis [94]. On the other hand, the specific peptides in TcCRT may be cross-loaded to the MHC-I molecule after APC processing to activate $CD8^+$ T cells and increase their antitumor activity [92]. In addition, on the base of the immunogenicity of this protein, TcCRT may also

induce humoral immunity against TcCRT [69, 94, 95]. These antibodies may interfere with antiangiogenesis by disrupting the interaction of parasite molecules with their receptors on endothelial cells [69, 94, 95]. Another possibility is that the immune complexes formed by TcCRT and anti-TcCRT antibodies are taken up by APC (B cells, macrophages, and dendritic cells), promoting the antitumor humoral immune response [94]. Structurally, these functions of TcCRT are dependent of its N-terminal domain, especially the polypeptide segment containing residues 131–159, which is a strong dipole that can interact with charged proteins (e.g., collagen tails and scavenger receptors) [96]. Therefore, TcCRT may have high development potential as an antiangiogenic and antitumor drug.

Prospects for the development of *T. cruzi* as an anticancer agent

Although studies have demonstrated that *T. cruzi* has beneficial anticancer activity, as a pathogen with a wide range of pathogenicity, its potential danger cannot be ignored. Therefore, the rational development of anticancer preparations for *T. cruzi* is imperative. J18 is a recombinant protein developed on the base of the *T. cruzi* surface glycoprotein gp82 fused to glutathione-S-transferase (GST) [97]. Atayde and colleagues [96] reported that J18 can destroy the actin cytoskeleton of the melanoma cell line Tm5 and induce apoptosis. By preventing NF-kappaB from entering the nucleus, J18 hinders tumor growth, ultimately prolonging the survival of mice with melanoma. In addition to targeting the development of active ingredients of *T. cruzi*, producing attenuated *T. cruzi* strains as cancer antigen delivery vehicles is also an effective strategy [98, 99]. Junqueira et al. [99] developed an anticancer strain expressing the cancer-testis antigen (NY-ESO-1) using the attenuated *T. cruzi* CL-14 clone. The Immunization of tumor-bearing mice with this strain can kill tumor cells and hinder tumor development by inducing a strong NY-ESO-1 antigen-specific immune response [99], indicating the potential of using *T. cruzi* to develop tumor vaccines. Ultimately, developing effective *T. cruzi* anticancer active ingredients or attenuated *T. cruzi* strains may be a successful strategy for the large-scale application of *T. cruzi* in tumor therapy.

Challenges of *Trypanosoma*-based tumor biotherapy

Trypanosoma parasites, which are responsible for diseases such as African sleeping sickness and Chagas disease, provoke immune system activation and inflammation in hosts. The concept of using these parasites as adjuvants in cancer therapy for patients raises concerns about triggering immune and inflammatory responses, which could result in adverse reactions and complications. Additionally, trypanosomes have developed intricate mechanisms to circumvent the host immune system, allowing them to establish persistent chronic infections and thrive within the host. This ability to evade immune surveillance poses a significant challenge for cancer biotherapeutics, potentially hindering the ability of *Trypanosoma* to specifically target and eradicate cancer cells without being countered by the host immune response.

To ensure the safety and efficacy of *Trypanosoma*-based cancer biotherapy, a thorough understanding of the interactions between parasites and cancer cells and potential off-target effects on healthy tissues is essential. The development of strategies to increase the specificity of *Trypanosoma* in targeting cancer cells while minimizing collateral damage to normal tissues is critical for the success of this therapeutic approach.

Moreover, ensuring patient safety, obtaining regulatory approval, and adhering to ethical guidelines are vital aspects that require meticulous consideration when exploring *Trypanosoma*-based tumor biotherapy as a potential treatment option for cancer.

Echinococcus-based tumor biotherapy

Echinococcus granulosus (*E. granulosus*) is a worm that causes echinococcosis, an endemic infectious disease that affects individual health and socioeconomic development. However, several studies have suggested that *E. granulosus* has anticancer effects and that molecules derived from *Echinococcus* induce specific anticancer immune responses in the host [100, 101]. For example, hydatid cyst protoscolices inhibit the proliferation of WEHI-164 fibrosarcoma cells and baby hamster kidney (BHK) fibroblasts in vitro and increase the lysis of fibrosarcoma cells [102]. Similarly, injection of hydatid fluid into the peritoneum or tumor margin reduced melanoma tumor size in tumor-bearing mice [103]. Furthermore, immunization with antigens derived from hydatid cysts in tumor-bearing mice can effectively eliminate CT26 colon cancer [100], breast cancer [104, 105], and melanoma [106]. Indeed, host infection by *Echinococcus* is a complex process, and its anticancer effect may involve multiple mechanisms, including the inhibition of neutrophil elastase and neutrophil chemotaxis, the induction of the antitumor immune response, and tumor cell apoptosis (Fig. 1 and 2).

Direct antitumor effects induced by *E. granulosus*-derived molecules

The anticancer effect of *E. granulosus* involves an intricate process. Although the specific molecules involved in this process are still highly controversial, hydatid molecules, especially protoscolices excretion/secretion (ES)

molecules, have demonstrated high anticancer potential [107]. Among them, the typical representative molecule is EgKI-1, a Kunitz-type protease inhibitor highly expressed in the oncosphere of *E. granulosus* that can effectively inhibit chymotrypsin and neutrophil elastase [108]. Recent studies have shown that EgKI-1 not only restricts the proliferation and migration of multiple human cancers, such as breast cancer, melanoma, and cervical cancer, in a dose-dependent manner in vitro, but also significantly inhibits the growth of triple-negative breast cancer and melanoma in vivo [109, 110]. Mechanistically, EgKI-1 may activate caspase-3 by upregulating B-cell lymphoma 2 (BCL-2)-like protein 13 expression, thereby inducing tumor cell apoptosis; on the other hand, it may also disrupt the cell cycle by downregulating the expression of tetraspanin (TSPAN, H7BXY6) and BRCA1-related ATM activator-1 (BRAT1), which are crucial for controlling tumor initiation, growth, metastasis and DNA repair [109]. Furthermore, owing to the role of EgKI-1 as an elastase inhibitor, another possible mechanism is that EgKI-1 may reduce cancer cell migration by effectively blocking the infiltration and function of tumor-associated neutrophils (TAN) [109], which play pivotal roles in the TME and cancer metastasis [111, 112]. Additionally, EgKI-1 treatment may favorably reshape the TME by increasing $CD8^+$ T cell populations in the TDLN of tumor-bearing mice, thereby attacking melanoma cells [110]. Undoubtedly, the specific antitumor mechanism of EgKI-1 still requires further research. As an anticancer agent with significant potential for development, the potential of EgKI-1 to combat the proliferation and metastasis of malignant cells is worth examing.

In addition, other hydatid molecules, including antigen B (AgB), glycolipids, glycoproteins, and 78 kDa components, have also exhibited some anticancer potential, such as the ability to induce apoptosis in breast cancer cells [104, 105]. Intriguingly, AgB is also a potent neutrophil elastase inhibitor highly expressed in hydatid cysts and may mediate anticancer effects in chronic hydatid infection [107]; however, its possible role requires further exploration. Overall, the available evidence suggests that the anticancer effect of *E. granulosus* depends, to some extent, on the functions of hydatid cyst-derived molecules, especially ES molecules [107].

Antitumor immune response induced by *E. granulosus*

Emerging evidence indicates that *E. granulosus* and various cancers share structurally similar or common antigens [100, 106, 113–115]. As early as 1979, Yong et al. [115] reported that the hydatid fluid and serum of lung cancer patients could form a strong precipitin band, indicating that there may be common antigens between *E. granulosus* and lung cancer cells. Studies from other groups have consistently confirmed that there may be a wide range of antigenic similarities between *E. granulosus* and various cancers such as melanoma and breast and colon cancer. Mucin-type O-glycans play a major role in cancer metastasis and immune evasion and are significant tumor-associated antigens [116]. However, *E. granulosus* abundantly expresses two carcinoma-associated mucin-type O-glycans, Tn antigen (GalNAc-O-Ser/Thr) and sialyl Tn (sTn) antigen (a related O-linked antigen), which can be detected in larvae or adult worm extracts, and even in the serum of patients infected with parasites [117, 118]. In addition, a glycan antigen from the hydatid cyst wall with a molecular weight of approximately 53 kDa was detected in both the serum of patients with hydatid disease and the serum of healthy volunteers [118]. Furthermore, it has been determined that *E. granulosus* and human breast cancer share a nonglycosylated 27 kDa molecule [114]. Similarly, heat shock protein (HSP) 70 of *E. granulosus* shares 60% homology with moralin in CT26 colon cancer cells [100]. These findings suggest that there may be potential for developing effective strategies for the diagnosis and treatment of cancers.

Since specific tumor antigen recognition is crucial for initiating antitumor immune responses, those antigens excreted or secreted by *E. granulosa*, which are structurally identical or similar to tumor antigens, can also induce specific antitumor responses. For example, studies have revealed that antigens from hydatid cysts or antisera raised against hydatid cysts can react with sera from breast cancer patients or ES products of cancer cells in vitro [114, 119–121], suggesting that *E. granulosus* may have the ability to trigger antitumor immunity via the antiparasitic adaptive immunity induced by common antigens. Similarly, this immune cross-reactivity is also present in colon cancer: vaccination with *E. granulosus* effectively induces antitumor immunity and thereby prevented CT26 colon cancer growth in a mouse model [100, 122]. These findings indicate that the development of highly immunogenic antitumor drugs may be a promising strategy for antitumor treatment.

However, the antitumor effects induced by *E. granulosus* are not limited to humoral immunity alone; the cellular immunity activated by this parasite also has significant potential in combating tumors [123]. Although the development and growth of cysts typically lead to a shift from a Th-1 immune response to a Th-2 response, which may not favor *E. granulous*-mediated antitumor effects, studies have shown that the antitumor potential is more likely associated with the Th-1 response induced by *E. granulosus* [107, 124]. For example, hydatid cyst wall (HCW) antigens, especially the 27 kDa protein band, increase the amount of IL-2, TNF-α, and IFN-γ, which inhibits mouse mammary tumor growth and

metastasis, ultimately increasing the survival rate [125]. Furthermore, immunization of tumor-bearing mice with the Tn-like peptide of *E. granulosus* produced high levels of IFN-γ [126]. Similarly, immunization of melanoma-bearing mice with antigens from hydatid cysts induced IFN-γ and inhibited tumor growth [127]. In addition, melanoma growth inhibition mediated by adoptively transferred splenocytes from hydatid cysts, hydatid fluid, or protoscoleces-immunized mice [124, 128] appears to confirm the antitumor effects of *E. granulosus* induced through Th-1 responses.

Notably, mucin peptides originating from *E. granulosus* have been shown to stimulate increasing numbers of activated NK cells within the spleens of immunized mice, an effect that is positively associated with the ability of splenocytes to destroy tumor cells [126]. Mechanistically, these peptides may induce DC maturation by upregulating IL-12p40/p70 and IL-6 expression. Consequently, this heightened maturation activates NK cells, implying a potential antitumor effect resulting from the activation of innate immunity by *E. granulosus*. However, further evidence is necessary to establish this finding concretely.

Challenges of *Echinococcus*-based tumor biotherapy

Although humans are incidental hosts for this tapeworm, infection with *E. granulosus* can result in hydatid cysts within the body, leading to cystic echinococcosis. The treatment for this condition is often protracted and expensive, potentially involving major surgery and extended pharmaceutical interventions. Consequently, the use of *E. granulosus* as a therapeutic agent in cancer patients is associated with the risk of unforeseen outcomes, including adverse reactions and additional complications. Despite animal evidence indicating that *E. granulosus* may inhibit certain cancers, the path to clinical application is arduous. The development of attenuated strains that consider the biological attributes of the tapeworm and the immunological responses of host is needed. A comprehensive understanding of the interaction between *E. granulosus* and cancer cells and the potential impact of *E. granulosus* on healthy tissues is also essential. Moreover, ethical approval, stringent medical supervision, and the establishment of safety and efficacy are crucial for determining whether this therapeutic approach can ultimately benefit cancer patients. Another pivotal task is to create safe and effective antitumor products derived from *E. granulosus*.

Conclusion and future perspectives

In summary, infection with parasites or the injection of their products can trigger or reestablish the immune response against tumors in vivo (summarized in Table 1). This activation can lead to several beneficial effects, including the reversal of functions of immunosuppressive cells, such as TAM, TAN, MDSC, and Treg; it can also activate DC, reduce their secretion of inhibitory cytokines, and increase the production of proinflammatory factors such as TNF-α, IL-12, and IFN-γ. These changes can transform "cold" tumors, which have a minimal immune response, into "hot" tumors, which are actively engaged by the immune system. Additionally, activating $CD8^+$ T cells with Th1-type cytokines can enhance antitumor-specific immune responses, significantly inhibiting tumor growth and spread. Furthermore, B cells activated by certain parasites can produce antibodies that enable NK cells to carry out ADCC, leading to tumor cell apoptosis.

Although parasites may employ various mechanisms to exert their antitumor effects and the specific mechanisms by which they elicit these immune responses are not fully understood, this does not diminish their potential as adjuvants in cancer immunotherapy. For example, the combination of *T. gondii* ΔGRA17 tachyzoite therapy with anti-PD-L1 treatment has been shown to significantly prolong the survival of mice and inhibit the growth of tumors in preclinical models of melanoma, Lewis lung cancer, and colon adenocarcinoma [64]. This discovery offers a potential therapeutic strategy for treating "cold" tumors and holds promise for the future of parasitic protozoan-based immunotherapy.

Furthermore, many parasites and some tumors share common antigens, which have the potential to generate immune responses and exhibit antitumor activity. These specific antigens derived from parasites not only possess T cell epitopes that can trigger immune responses specific to tumors but also effectively circumvent central thymic tolerance mechanisms, giving them an edge over some tumor-associated antigens (TAA). Thus, there is potential to harness parasite antigens as targets or adjuvants for mRNA-based tumor vaccines. Undoubtedly, this innovative approach will enhance the efficacy of tumor vaccines.

Notably, the aforementioned parasites are pathogens that can cause parasitic diseases in humans. The treatment of these diseases can be complex and costly, sometimes necessitating surgery and long-term medication. Consequently, the introduction of parasites into cancer patients as a tumor therapy strategy must be performed with great care to avoid unpredictable consequences, including adverse reactions and patient complications. Such outcomes could not only harm patient health but also lead to concerns and skepticism regarding this therapeutic strategy. Therefore, caution is imperative. Researchers must thoroughly investigate the molecular mechanisms by which parasites specifically target tumor tissues and interact with cancer cells and develop

Table 1 Summary of parasitic protozoan-based adjuvant therapy for cancer

Parasites with antitumor properties	Parasites-derived components	Attenuated strains	Mechanisms	Benefits	Limitations	Clinical trail	References
Plasmodium (Mouse: *P. yoelli*)	metabolites (such as HZ), lysate Ag		① reshape TME by regulating TAM, TAN, MDSC, DC; ② reactivate NK, CD8 + T cells; ③ secret exosomes to inhibit tumor angiogenesis; ④ inhibit EMT by downregulating CCR10/PI3K/AKT pathway	①against lung, liver, and breast cancer, as well as glioma; ② sensitize radiotherapy for solid Tumors	may cause fever, anemia, and severe malaria in patients	Small-scale clinical trial in China	[13–16, 18–22, 26]
T. gondii	GRA1511/TLA, GRA16	ME49/RH/CPS/ΔGRA17	① polarize Mϕ to M1-type TAM ② inhibit angiogenesis by suppressing the expression of MMP, TGF-β, CD31, etc.; ③ shorten telomere by upregulating PTEN expression and reducing HAUSP/AKT/STAT3/NF-κB activities; ④ arrest cell cycle by upregulating P53 expression and downregulating CyclinB1 and CDC2 expression; ⑤ reactivate reactivate NK, CD8 + T cells by increasing IFN-γ and IL-12 expression; ⑥ carry out ADCC cells by activating B cells to produce Ab; ⑦ reshape TME by regulating the infiltration of TAM and MDSC through exosome	① against breast, colon, lung, prostate, esophageal, pancreatic and gastric cancer, as well as melanoma, fibrosarcoma, leukemia, lymphoma, and Ehrlich ascites carcinoma; ② synergizes with anti-PD-L1 therapy to inhibit both targeted and distal tumors	may cause toxoplasmosis in patients, especially immunocompromised persons and pregnant women		[29–36, 39, 40, 45–47, 51, 52, 54–65]

Table 1 (continued)

Parasites with antitumor properties	Parasites-derived components	Attenuated strains	Mechanisms	Benefits	Limitations	Clinical trail	References
T. cruzi	gp82, TcCRT, Lysate Ag, Glycosylphosphatidylinositol	DTU1 clone: P, G, Sp; DTU2 clone:Y7/2	① inhibit tumor cell proliferation by suppressing NF-κB pathway; ② inhibit angiogenesis by epithelial cell migration and capillary morphogenesis ③ carry out ADCC of NK cells by activating B cells to produce Ab; ④ reactivate CD8 + T cells by enhancing phagocytosis, antigen process, DC maturation, and Th 1-type cytokine expression ⑤ destroy tumor cells by producing ROS; ⑥ malfunction mitochondria by producing NO and TNF-α	against colon and breast cancer, as well as melanoma, neuroblastoma, and Ehrlich adenocarcinoma, leukemia	may cause diseases such as African trypanosomiasis (sleeping sickness) and Chagas disease		[66–75, 77, 78, 80, 82, 84, 86, 88–90, 92–97, 99]

Table 1 (continued)

Parasites with antitumor properties	Parasites-derived components	Attenuated strains	Mechanisms	Benefits	Limitations	Clinical trail	References
E. granulosus	EgKI-1, AgB, Glycolipids, Glycoproteins, mucin-type O-glycan, 27 kDa protein, 78 kDa protein		① inhibit tumor cell proliferation by arresting cell cycle through the down-regulation of TSPAN and BRAT1; ② inducing apoptosis by increasing BCL-2-like protein 13 and caspase-3 expression; ③ reshape TME by regulating the functions of TAN and activating CD8 + T cells ④ reactivate CD8 + T cells by enhancing antigen process ability of APC and increasing INF-γ/IL-2/IL-6/IL-12/TNF-α expressions ⑤carry out ADCC of NK cells and macrophages by activating B cells to produce Ab;	against lung, breast, colon, and cervical cancer, as well as melanoma, fibrosarcoma	may cause cystic echinococcosis		[100–107, 109, 110, 113–128]

strategies to prepare attenuated strains that enhance tumor targeting while minimizing off-target effects. Furthermore, strict adherence to regulatory guidelines, securing the necessary approvals, and addressing ethical concerns related to the use of parasites in clinical settings are crucial steps for parasitic protozoan-based cancer biotherapy to progress toward clinical benefits for cancer patients.

Authors' contributions
BW conceived the idea for this manuscript. BW and YX wrote the manuscript. BW and YFW revised the manuscript. JYW, YPP, and YLW provided insightful suggestions.

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No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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