Infectious Agents and Cancer

REVIEW

Open Access

From pathogen to cure: exploring the antitumor potential of *Toxoplasma gondii*

Parisa Alipanahi[1], Arezou Khosrojerdi[2], Abdol Satar Pagheh[2], Kareem Hatam-Nahavandi[3] and Ehsan Ahmadpour[1,4,5*]

Abstract

Toxoplasma gondii (*T. gondii*), an intracellular protozoan parasite, has attracted significant attention in recent years for its dual role in both promoting and inhibiting cancer. Although traditionally recognized as a potential risk factor for tumor development, particularly in immunocompromised individuals, emerging research suggests that *T. gondii* may possess anti-cancer properties. This paradox is rooted in the parasite's ability to modulate the host's immune system, triggering antitumor immune responses through the activation of immune cells and the secretion of cytokines such as TNF-α and IFN-γ. *T. gondii* has demonstrated efficacy in reversing tumor-associated immunosuppression, inhibiting angiogenesis, and promoting tumor regression in preclinical models. However, its potential as an immunotherapeutic agent is tempered by the risks associated with administering live parasites, including infection and immune system complications. This article reviews the current understanding of *T. gondii* impact on cancer and its potential role in cancer therapy. Despite promising preclinical results, challenges remain, including the need for safer therapeutic approaches. Future research should focus on genetically modified or attenuated strains of *T. gondii* that retain their antitumor capabilities while minimizing risks. Understanding the underlying mechanisms by which *T. gondii* modulates the tumor microenvironment will be crucial for translating these findings into clinical applications, potentially offering new avenues for cancer treatment.

Clinical trial number

Not applicable.

Keywords *Toxoplasma gondii*, Antitumor immunity, Immunotherapy, Cytokine modulation, Tumor microenvironment

*Correspondence:
Ehsan Ahmadpour
ehsanahmadpour@gmail.com; ahmadpoure@tbzmed.ac.ir
[1]Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran
[3]Department of Parasitology and Mycology, School of Medicine, Iranshahr University of Medical Sciences, Sistan and Baluchestan, Iranshahr, Iran
[4]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[5]Department of Parasitology and Mycology, Tabriz University of Medical Sciences, PO Box: 14155-6446, Tabriz, Iran

Introduction

Cancer is a disease characterized by the uncontrolled growth of abnormal cells, which invade nearby tissues and spread to other parts of the body [1]. Current research identifies various mechanisms, including cell cycle mutations [2], disruptions in apoptotic pathways [3], aberrant signal transduction [4, 5], angiogenesis [6, 7], and invasion and metastasis [8], as contributing significantly to cancer progression. Cancer's multifactorial nature is further influenced by genetic factors, lifestyle, and the tumor microenvironment [1, 9, 10]. Consequently, the disease remains a global health challenge, with approximately 20 million new cases and 10 million deaths reported annually despite ongoing advances in research and treatment [11].

Traditional cancer treatments, such as surgery, radiation therapy, and chemotherapy, are still widely used. Still, their efficacy is often limited by factors such as patient tolerance, tumor stage, and the potential for severe side effects. Radiation therapy can damage healthy cells and even induce secondary cancers, while chemotherapy is known to harm healthy organs alongside cancerous tissues [12, 13]. As a result, there is a growing need for innovative cancer therapies that minimize these drawbacks.

Immunotherapy has emerged as a promising alternative to conventional treatments, focusing on boosting the immune system's ability to target and eliminate cancer cells [14]. Recent advancements, such as immune checkpoint inhibitors that enhance immune responses to tumors [15], and chimeric antigen receptor T-cell therapy, which allows T cells to specifically recognize and attack cancer cells [16], have already begun to revolutionize clinical cancer treatment. Intriguingly, certain parasites, particularly intracellular protozoan parasites, have demonstrated potential to stimulate immune responses against tumors. These parasites can counteract tumor evasion and promote sustained immune activity through mechanisms such as inducing cancer cell apoptosis, enhancing host immune responses, and inhibiting angiogenesis and metastasis [17, 18]. Persistent protozoan infections continuously release antigens, resulting in long-term, targeted immune responses [19]. Furthermore, parasite-derived components, including lysates and functional proteins, have been shown to suppress tumor progression by inducing the release of antitumor cytokines and other non-immunological pathways [20].

Among these parasites, apicomplexans- such as *Plasmodium*, *Toxoplasma*, *Cryptosporidium*, *Babesia*, and *Theileria* have garnered significant attention for their potential anticancer effects [21]. Studies suggest that infections with apicomplexans, including *Eimeria*, *Plasmodium*, and *Toxoplasma gondii* (*T. gondii*), may exhibit antitumor properties and offer therapeutic benefits for cancer treatment [22]. Evidence of an inverse relationship between parasite infection rates and cancer mortality further supports the notion that parasites may reduce the likelihood of cancer-related deaths [23]. This competitive interaction between parasites and tumors highlights the potential of parasite-based therapies in oncology [24].

Unlike bacteria and viruses, protozoa present unique advantages in cancer treatment due to their eukaryotic nature, enabling them to mimic human cells more closely. This allows protozoa to express human proteins and tumor antigens more accurately, thereby triggering specific antitumor immune responses. However, ethical and legal concerns about the pathogenicity of protozoa remain significant barriers to their clinical application [25]. Nevertheless, a successful clinical trial almost a decade ago demonstrated that weakened strains of non-replicating sporozoites could be safely tolerated in humans, suggesting the potential viability of protozoa as a novel cancer treatment strategy [25]. This review explores the existing body of knowledge on the relationship between *T. gondii* infections and cancer, focusing on the parasite's potential to influence cancer development, progression, and treatment response.

Toxoplasma gondii

T. gondii is an obligate intracellular protozoan parasite that infects a wide variety of mammals and birds, with about 33% of the global population being carriers [26, 27]. It is primarily transmitted through food or water contaminated with the parasite or passed vertically from mother to child during pregnancy [28]. Traditionally, the parasite is classified into three main strains (Types I, II, and III) based on their virulence profiles in mice [29]. Type I is the most virulent strain, with an LD_{50} (lethal dose for 50% of the population) of approximately one tachyzoite, meaning that a single organism can kill a mouse. Mice infected with this strain typically die within 8 to 10 days due to its rapid replication and spread. Chronic infections are rare because the formation of bradyzoite cysts poses a high risk of host mortality. This strain causes severe immunosuppression and is often used in research to study acute infections and virulence mechanisms [30]. Strain II of this parasite exhibits moderate virulence, with an LD_{50} of approximately 1,000 tachyzoites. Acute infections with this strain are generally less severe than those caused by type I, and mice often survive the acute phase of the illness. Additionally, infection with Strain II facilitates the efficient formation of tissue cysts, making it a common choice for studies of chronic toxoplasmosis. This strain also triggers a strong immune response in the host, making it valuable for investigating host-pathogen interactions [30]. Type III strain is classified as non-virulent or of low virulence, with an LD_{50} greater than 1×10^6. It typically exhibits low pathogenicity but is capable of

forming effective cysts, much like type II strains. This strain induces a weaker immune response compared to type II and is commonly used in studies focused on chronic infection and parasite persistence [30].

In addition, atypical strains of *T. gondii* are non-clonal and result from sexual recombination in wild hosts, primarily found in tropical regions. Often referred to as haplogroups IV–XII, these strains differ from the classical Types I, II, and III that are common in Europe and North America. The virulence of atypical strains can vary significantly. Some, like strain GUY-DOS from French Guiana, are hypervirulent, while others may be avirulent. This variability is associated with unique alleles of virulence factors [31]. Atypical strains can lead to more severe disease, notably in immunocompetent individuals and congenital infections, causing issues like disseminated toxoplasmosis and neurological complications [31].

While *T. gondii* infections are often asymptomatic in healthy individuals, they can cause severe complications in pregnant women and immunocompromised individuals, including birth defects and toxoplasmosis, which can affect the brain, muscles, and eyes [32–34]. Research has shown that *T. gondii* can induce a strong Th1 immune response and inhibit tumor growth by promoting vascular necrosis, hypoxia, and inhibiting tumor angiogenesis [35, 36]. The parasite plays a dual role in apoptosis, influencing both pro-apoptotic and anti-apoptotic pathways in cells [37].

On the one hand, *T. gondii* exerts antitumor effects by promoting apoptosis and selectively targeting malignant cells for destruction, leading to suppressed tumor growth. This apoptosis-promoting effect is believed to involve modulation of mitochondrial pathways, activation of caspases, and suppression of prosurvival signaling in tumor cells. Such targeted cytotoxicity results in reduced tumor burden and is part of the reason for investigating attenuated strains of *T. gondii* as oncolytic or immunotherapeutic agents. In addition, the parasite can remodel the tumor microenvironment, reduce immunosuppression, and enhance antitumor immunity [37, 38].

In contrast, *T. gondii* blocks apoptosis in host immune cells, allowing the parasite to persist in the body longer while stimulating immune responses that fight tumors. In immune cells, particularly dendritic cells and macrophages, *T. gondii* often inhibits apoptosis and increases their survival. This allows the parasite to persist in host tissues, evade immune clearance, and continuously stimulate proinflammatory responses. These immune activations, including increased production of IL-12, IFN-γ, and activation of CD8 + T cells and NK cells, could indirectly enhance antitumor immune surveillance and further contribute to its tumor suppressive properties. This contradictory behavior makes it a compelling candidate for engineered immunotherapies, but also emphasizes the need to carefully control its pathogenic potential [37, 38].

Studies have shown that *T. gondii* directly inhibits cytochrome c-mediated caspase-3 activation, a key mechanism that prevents apoptosis in host cells [39]. This was the first evidence of *T. gondii* interfering with the caspase pathway independent of cytochrome c release from mitochondria; however, the functional significance of this in living cells remains unclear [40]. Additionally, the ability of *T. gondii* to block apoptosis has been linked to its reduction of poly (ADP-ribose) polymerase (PARP) interactions, without changes in mRNA levels [40]. PARP is a crucial pro-apoptotic factor, and when excessively activated, it can deplete NAD + and ATP levels, leading to mitochondrial outer membrane permeabilization (MOMP) and caspase-independent cell death [41]. By preventing this overactivation, *T. gondii* helps preserve the survival of certain host cells [42]. This dual action of promoting apoptosis in breast cancer cells while inhibiting it in immune cells enhances the potential of *T. gondii* as an immunotherapeutic agent, allowing for tumor suppression while maintaining immune function. A study involving cancer patients and healthy individuals found that asymptomatic *T. gondii* infection might enhance immunity against cancer, with lower anti-*T. gondii* antibody titers correlating with better prognoses [43].This suggests a possible link between *T. gondii* infection and improved cancer outcomes.

The effect of *T. gondii* on the immune system

Cellular immunity

Tumor-infiltrating dendritic cells (DCs)

Tumor-infiltrating DCs play a pivotal role in linking the innate and adaptive immune systems, as they process antigens from the tumor environment and present them to CD8 + T cells via major histocompatibility complex (MHC) class I, initiating a targeted immune response. These DCs guide the functional programming of T cells, determining their capacity to protect the host from cancer spread [44, 45]. When immature DCs encounter inflammatory signals, they mature and acquire enhanced antigen-presenting capabilities, critical for initiating immune responses in tumor-draining lymph nodes [46]. However, in certain cancers like ovarian cancer, tumor-associated DCs often exhibit an immature and immunosuppressive phenotype, which can promote tumor growth and angiogenesis [47]. These immature DCs, such as CD11c + DCs, can also express immunosuppressive molecules like PD-L1, which impairs T cell activation [48].

Interestingly, *T. gondii* infection has been shown to reprogram tumor-associated DCs and macrophages into an immunostimulatory phenotype, enabling them to trigger a strong Th1-mediated immune response. This

infection, through TLR11 signaling, induces the production of cytokines such as interleukin-12 (IL-12), which is essential for activating CD8 + T cells that can destroy cancer cells [49]. *T. gondii* infection or its components, such as novel recombinant *Toxoplasma* uracil auxotrophs (NRTUAs), can overcome the immunosuppressive state of myeloid cells within the tumor microenvironment, converting tumor-associated DCs and macrophages into effective antigen-presenting cells. This reprogramming enhances CD8 + T cell infiltration, leading to a robust anti-tumor immune response, ultimately shrinking tumor tissue [50, 51].

Tumor-associated macrophages (TAMs)

TAMs are the most abundant immunosuppressive cells within the tumor microenvironment. TAMs are generally classified into two phenotypes: M1 macrophages (classically activated) and M2 macrophages (alternatively activated) [52]. M1 macrophages are activated by Toll-like receptor-4 (TLR4), lipopolysaccharide (LPS), granulocyte-macrophage colony-stimulating factor (GM-CSF), or Th1 cytokines like interferon-gamma (IFN-γ), and are known for producing proinflammatory cytokines (interluekinIL-1β, IL-12, IL-23, TNF-α), reactive oxygen species (ROS), and nitrogen intermediates. M1 macrophages also express high levels of MHC class II receptors and co-stimulatory molecules such as CD86, making them crucial for initiating inflammatory responses and promoting antitumor immunity [53–55].

Conversely, M2 macrophages are activated by Th2 cytokines such as IL-4, IL-10, and IL-13, and express surface molecules like CD163 and CD206. These macrophages are involved in wound healing, tissue repair, and immunosuppression, and are characterized by high levels of inhibitory cytokines (IL-10, TGF-β) and Arginase-1 (Arg-1) [56, 57]. M2 macrophages typically contribute to tumor growth by mediating angiogenesis and immunosuppression. TAMs, in most tumors, predominantly exhibit an M2 phenotype, which fosters tumor progression [58, 59].

T. gondii tachyzoites exert antitumor effects in macrophages by upregulating the expression of key pro-inflammatory cytokines, including TNF-α, IL-12, and IFN-γ, and by stimulating immune cells to mount a robust immune response [60–64]. In immunocompromised mice, *T. gondii* infection of macrophages can activate macrophages to eliminate cancer cells, including those frequently investigated in solid tumors, like melanoma or ovarian cancer [51, 65]. Additionally, exosomes derived from *T. gondii*-infected DCs (Me49-DC-Exo) have demonstrated antitumor effects by inhibiting the polarization of macrophages into the tumor-promoting M2 phenotype. The miRNAs within these exosomes, particularly miR-155-5p, promote the M1 macrophage phenotype while downregulating M2-associated genes by targeting the suppressor of cytokine signaling 1 (SOCS1) and signal transducer and activator of transcription 3 (STAT3) pathways, thus enhancing the immune system's ability to fight cancer [66].

Innate lymphoid cells (ILC1) and natural killer (NK) cells

Group 1 innate lymphoid cells (ILC1) and natural killer (NK) cells are pivotal components of the innate immune system, playing crucial roles in early defense against infections and tumors. While NK cells are known for their direct cytotoxic activity, targeting and destroying tumor cells, especially those lacking major histocompatibility complex (MHC) class I molecules-ILC1 cells mainly contribute by producing pro-inflammatory cytokines like interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) [67, 68]. These cytokines enhance immune responses, particularly in tissues such as the liver and mucosal surfaces where ILC1s are predominantly found. Together, NK cells and ILC1s are instrumental in controlling intracellular pathogens, including viruses and protozoa, and play key roles in tumor surveillance and suppression [69].

Activated NK cells are tightly regulated by a balance of activating, costimulatory, and inhibitory receptors. Their ability to eliminate tumor cells makes them especially effective against blood cancers and metastatic tumors. Additionally, NK cells indirectly enhance immune responses by promoting T cell and antibody activity. Cytokines secreted by NK cells, such as IL-12, are vital in controlling tumor growth. IL-12 drives Th1 differentiation and stimulates IFN-γ production, which enhances the immune system's ability to target tumors, creating a synergistic effect with other immune cell [70–72].

In response to *T. gondii* infection, both NK cells and ILC1s are activated. During acute infection, *T. gondii* stimulates dendritic cells to release IL-12, which prompts NK cells to secrete IFN-γ and ILC1s to produce both TNF-α and IFN-γ. This cytokine production is crucial in limiting parasite replication and also plays a role in inhibiting tumor growth. Moreover, *T. gondii* infection can cause long-lasting changes in NK cells, converting them into Eomes-dependent ILC1-like cells, which may enhance tumor defense [73–75].

Despite this, the molecular mechanisms by which *T. gondii* stimulates NK cell activity remain poorly understood. Experiments have shown that infection with the Type II Prugniaud (Pru) strain of *T. gondii*, following anti-NK1.1 antibody administration, increases parasite load and mortality compared to control-treated mice [76]. Anti-NK1.1 targets both NK cells and ILC1s, underscoring their vital roles in the immune response to *T. gondii* [77, 78].

T cells

CD4 + and CD8 + T cells play crucial roles in the antitumor immune response. CD8 + cytotoxic T lymphocytes are primarily responsible for directly targeting and killing tumor cells by recognizing specific antigens presented by MHC class I molecules. Their activation leads to the secretion of effector molecules, such as perforin and granzymes, which induce apoptosis in malignant cells [79, 80]. Meanwhile, CD4 + T helper cells enhance the immune response by providing necessary signals and cytokines that support CD8 + T cell activation, proliferation, and differentiation into memory T cells. CD4 + T cells also aid in the activation of antigen-presenting cells, promoting a robust immune environment that enhances the overall antitumor activity. Together, these T cell subsets collaborate to mount a coordinated attack against tumors, improving the body's ability to recognize and eliminate cancerous cells [81].

In mice vaccinated with attenuated *T. gondii* to prevent pancreatic cancer recurrence, a robust cell-mediated immune response involving both CD4 + and CD8 + T cells was sustained for over 200 days [82]. Furthermore, the autoclaved *Toxoplasma* vaccine (ATV) enhances tumor-infiltrating CD8 + T cell populations, resulting in a higher CD8 + T/Treg ratio and significant tumor growth inhibition [83]. In addition, the effectiveness of the antitumor response is often gauged by the ratio of CD8 + T cells to CD4 + T cells, with higher proportions indicating stronger antitumor immunity [84]. Notably, research has shown a significant increase in CD8 + T cell numbers and the CD8+/CD4 + ratio in mice treated with ME49Δgra5 (attenuated live vaccine against *T. gondii* infection) [67].

Humoral immunity

During a prolonged and efficient anti-tumor immune response, which is crucial for tumor treatment, tumor-associated antigens (TAA) that are present at low levels in normal cells are recognized, leading to the activation of humoral immunity [85]. Protozoan infections, such as those caused by *T. gondii*, can further induce humoral antitumor immunity, promoting the release of antibodies that may influence tumor biology by blocking specific receptors on the surface of tumor cells [86]. In a study involving *T. gondii* ME49 strain infection, Lewis lung carcinoma-bearing mice showed significantly elevated levels of IgG1 and IgG2a antibodies, suggesting an enhanced humoral response [60] (See Fig. 1).

Reduction of parasite pathogenicity: essential approaches

The virulence of *T. gondii* strains poses challenges for cancer treatment, particularly for immunocompromised individuals like cancer patients, who are more vulnerable to infections [87]. The parasite's invasive nature triggers the antitumor response, but balancing its therapeutic benefits without compromising the immune system is difficult. Ongoing research explores ways to reduce the parasite's virulence while preserving its therapeutic properties [88]. The idea of using live, non-pathogenic protozoan parasites as therapeutic tools to combat cancer has gained significant global attention [19].

Various strategies have been explored to attenuate the parasite's virulence. For instance, in 1985, formalin-fixed *T. gondii* tachyzoites were injected intralesionally into mice with Lewis lung carcinoma, and two out of six mice completely rejected the tumor [89]. Gamma-irradiated *T. gondii* has also been used to attenuate the parasite's virulence, preventing tumor development and suppressing pre-existing tumor growth [90]. Genetically engineered strains such as ΔCPS, ΔOMPDCΔUP, ΔGRA17, and Δldh1-Δldh2 have shown the potential to halt the growth of deadly tumors in preclinical models [61, 64, 91]. Recently, live, attenuated *T. gondii* parasites have been injected intratumorally as biological antitumor agents, successfully slowing tumor growth in preclinical models [19, 91].

In the following section, select components and genetically attenuated strains of *T. gondii* that have been rendered nonpathogenic through genetic modification or other laboratory techniques, yet retain the ability to stimulate antitumor immune responses, will be reviewed.

Attenuated *T. gondii*

ME49Δgra5

ME49Δgra5 refers to a genetically modified strain of *T. gondii* based on the ME49 strain, where the gene encoding GRA5 (a dense granule protein) has been deleted or knocked out. GRA5 maintains the structure and integrity of the parasitophorous vacuole membrane (PVM), which is essential for the parasite's survival and manipulation of host cell functions. ME49Δgra5, as an attenuated live vaccine, has been reported to inhibit tumor growth and metastasis [92]. This strain induces significant production of pro-inflammatory cytokines, such as TNF-α, IL-12, and IFN-γ, which are crucial in suppressing tumor growth [92]. In a 4T1 mouse breast tumor model, researchers investigated the antitumor effects of ME49Δgra5. On days 9, 11, and 13 after the injection of 4T1 tumor cells, mice received intratumoral inoculations with ME49Δgra5 tachyzoites at a dose of 10^5 tachyzoites per inoculation [67]. Compared to the control group treated with PBS, the ME49Δgra5-treated mice exhibited significant tumor growth inhibition, with reductions in tumor size and weight. By day 27, tumor volume in the control group was six times larger than in the ME49Δgra5-inoculated group [67]. Furthermore, ME49Δgra5 treatment dramatically improved the survival rate of 4T1 tumor-bearing mice [67]. These findings suggest that ME49Δgra5 may serve as an effective

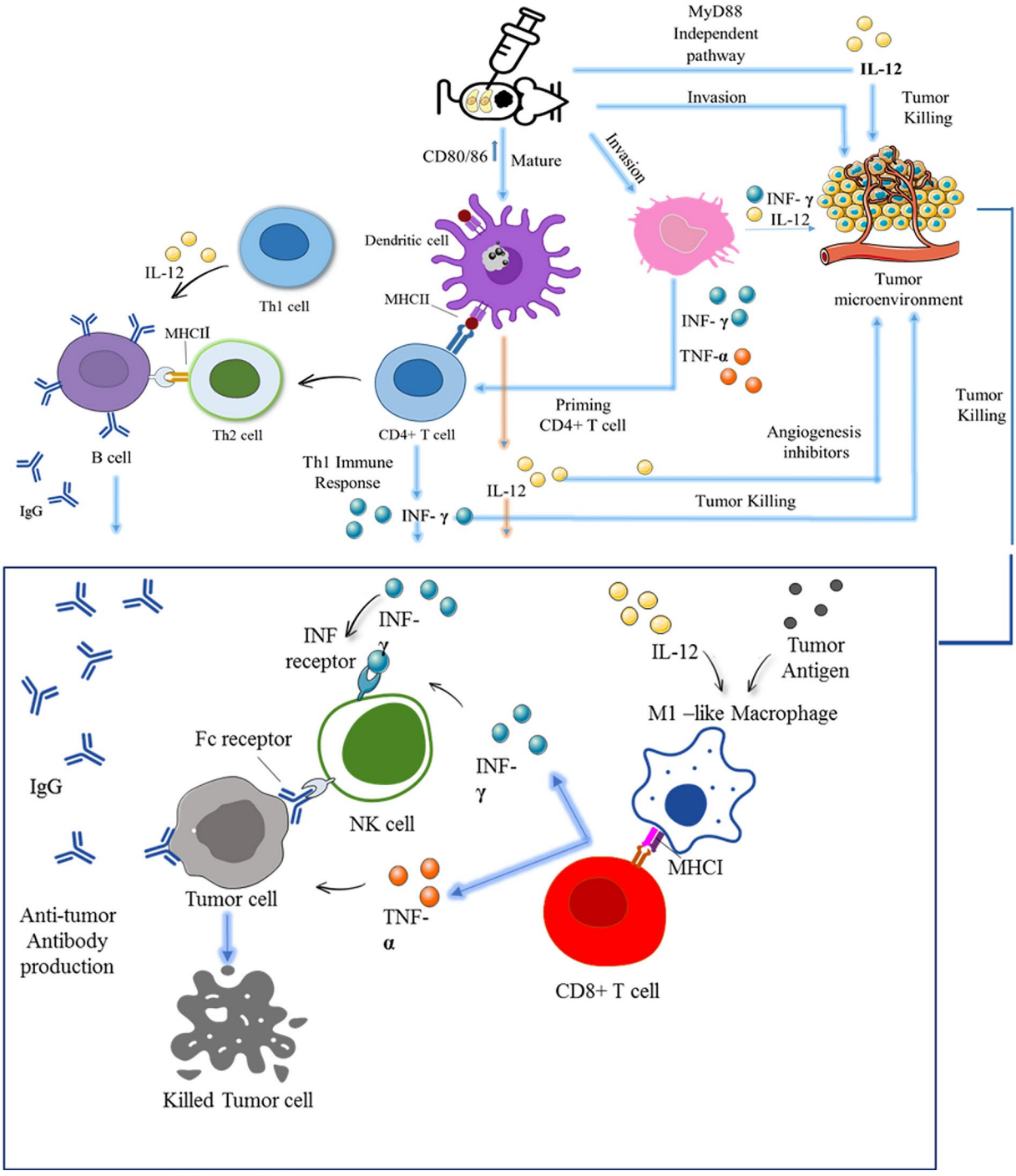

Fig. 1 Immunomodulatory Effects of *Toxoplasma gondii* in Enhancing Antitumor Responses. *T. gondii* infection significantly increases the expression of inflammatory markers, such as CD80 and CD86, in dendritic cells (DCs), which are key antigen-presenting cells in the immune system. This upregulation is critical for the maturation of DCs, enabling them to effectively present antigens in conjunction with MHC class II molecules and activate T cells. Mature DCs stimulate the differentiation of CD4 + T cells into the Th1 subset, which secretes interleukin-12 (IL-12) and interferon-gamma (IFN-γ). The IL-12/IFN-γ axis is pivotal in the antitumor response, as IFN-γ induces apoptosis in cancer cells and enhances the cytotoxic activity of CD8 + T cells and natural killer (NK) cells. In addition to cellular immunity, *T. gondii* also promotes a humoral response by stimulating some CD4 + T cells to activate B cells, leading to the production of specific IgG antibodies against tumor antigens. This antibody-mediated response further supports the immune system in combating cancer cells. Moreover, *T. gondii* infection recruits CD4 + and CD8 + T cells and macrophages into the tumor microenvironment (TME). These immune cells secrete IL-12 and IFN-γ, thereby amplifying the antitumor immune response and inhibiting tumor growth

immunotherapeutic agent against 4T1 tumors by stimulating the immune system to attack tumor cells in the breast. Additionally, as a potential vaccine, it could protect *T. gondii* infection. ME49Δgra5's capacity to induce strong immune responses, while maintaining safety, is key to its potential success in these roles. However, further research is necessary to fully understand its mechanisms and optimize its use for both cancer treatment in breast and protection against *T. gondii* [67].

Non-replicating uracil auxotrophic strains (NRTUAs)

NRTUAs are mutants of *T. gondii* that cannot replicate due to deletions in key genes required for uracil biosynthesis. Specifically, these strains lack either the carbamoyl phosphate synthetase II (cps) gene or the orotidine 5'-monophosphate decarboxylase (OMPDC) gene [93]. Both genes are essential for synthesizing uracil nucleotides (UTP), which are necessary for parasite replication. Without these enzymes, the parasites become auxotrophic for uracil and lose their ability to reproduce in the host without external uracil supplementation. NRTUAs, also referred to as "immunotherapeutically attenuated vaccine strains," hold promise as safe alternatives for cancer treatment, particularly due to their ability to stimulate antitumor immune responses. Their effectiveness against a variety of tumor types, including melanoma, breast, ovarian, lung, and pancreatic cancers, is currently under investigation [51].

These non-replicating *Toxoplasma* strains can infect host cells in vitro when supplemented with uracil, but they cannot proliferate in vivo due to the absence of uracil in the host environment. This makes them non-pathogenic and unable to cause disease, which has led to their investigation as potential vaccine candidates due to their safety profile [93, 94]. In mouse models, a cps II knockout strain (Δcps) is cleared within approximately five days, but despite its rapid clearance, it significantly slows tumor growth and even causes pre-existing tumors to shrink [95].

The immune response, particularly involving CD8 + T cells and NK cells plays a critical role in tumor suppression by NRTUAs. Following vaccination with NRTUAs, a strong Th1 immune response is triggered, marked by the rapid proliferation of CD19 + B cells, CD3+, and CD8 + T cells at the injection site [94, 96]. Additionally, a Th2 humoral immune response against cancer cells is stimulated, and in pancreatic cancer mouse models, tumor-specific IgG production has been observed [19, 82]. Key cytokines such as IL-12 and IFN-γ are also produced, further enhancing the antitumor activities of T cells and NK cells [97, 98].

The cps vaccine model has proven useful for investigating how IL-12 signaling influences the development and differentiation of protective CD8 + T cell responses [99, 100]. The cps model has been shown to effectively produce robust CD8 + T cell immunity against *Toxoplasma* and potentially other infections and tumors [93, 94]. For example, heterologous expression of the model antigen ovalbumin (OVA) in cps-modified *T. gondii* leads to high levels of OVA-specific CD8 + T cell responses. These antigen-specific CD8 + T cells express perforin and cytolytic granzyme B and can target and kill OVA-expressing cells both in vitro and in vivo [101].

The type I auxotrophic mutant strain RH-Δcps has been shown to significantly increase CD8 + and CD4 + T cell infiltration into tumors, leading to large amounts of IFN-γ production, which plays a key role in the antitumor response [102]. RH-Δcps specifically targets CD11c + cells in the tumor microenvironment, enhancing the expression of costimulatory molecules CD80 and CD86, which improves antigen presentation and immune response [82]. Similarly, the RH-Δompdc mutant strain, which lacks the OMPDC gene, has demonstrated potential as an immunotherapeutic agent for breast cancer. This strain was developed using CRISPR/Cas9 technology and has been shown to reduce tumor size and metastasis in a 4T1 breast cancer mouse model [103]. RH-Δompdc stimulates the production of pro-inflammatory cytokines, such as IL-12 and IFN-γ, and boosts immune responses, inhibiting tumor angiogenesis and activating APCs and infiltrating T cells [103].

The RHΔompdcΔuprt mutant, generated by CRISPR/Cas9 deletion of both the ompdc and uprt genes, has also shown promise as a live attenuated vaccine candidate. This strain is unable to synthesize uracil or UMP, significantly reducing its virulence while maintaining strong immunogenicity [104, 105]. The RHΔompdcΔuprt mutant has been shown to elicit robust immune responses in both mice and cats, providing protection against toxoplasmosis and reducing oocyst excretion in vaccinated animals [106]. The safety and immunogenicity of these uracil auxotrophic strains make them valuable candidates for the development of novel cancer immunotherapies and vaccines against *T. gondii*. Further research will continue to explore their potential for enhancing antitumor immunity and preventing both parasitic infection and tumor progression through targeted immune modulation.

T. gondii components

T. gondii profilin protein

T. gondii profilin (Tgprofilin) is a class of actin-binding proteins originally identified from soluble *T. gondii* antigens (STAg), known for stimulating immune responses and reducing parasitic, viral, and bacterial loads [107]. Profilin-like protein (TgPLP), with a predicted molecular weight of 17.5 kDa, shares significant homology with Tgprofilin [108]. Since some studies do not differentiate

between Tgprofilin and TgPLP, we will consider TgPLP a subset of Tgprofilin. Tgprofilin is essential for *T. gondii*g liding motility and host cell invasion. It also acts as a Toll-like receptor 11 (TLR11) agonist, promoting IL-12 production via the MyD88 pathway [109]. TgPLP contributes to antitumor immune responses in pancreatic tumor models by enhancing antigen-presenting cell markers in bone marrow-derived macrophages (BMMs), boosting IL-12 production, and facilitating pancreatic tumor cell phagocytosis during autologous whole tumor cell vaccination [110].

By increasing IL-12 and IFN-γ levels, TgPLP enhances immune responses, including the expression of MHC class I and II, and costimulatory molecules B7.1 and B7.2 on macrophages. In mice vaccinated with TgPLP alongside autologous whole tumor vaccine (AWV), higher levels of total IgG and IgG2a were detected, along with a greater number of immune cells, including CD4 + and CD8 + T cells, NK cells, and macrophages, compared to mice vaccinated with AWV alone [110].

In summary, *T. gondii* profilin-like protein (TgPLP) functions as a TLR-based vaccine adjuvant that enhances antitumor immunity against pancreatic cancer by activating macrophages, increasing IL-12 production, and promoting tumor cell clearance through the MyD88 pathway. However, IFN-γ is also essential in preventing pancreatic cancer in this immune response [110].

T. gondii lysed antigen(TLA)

Studies have demonstrated that TLA exhibits potent antitumor activity in mice and rats [111]. Intramuscular injection of TLA has been shown to inhibit the growth of tumor cells induced by the chemical 20-methylcholanthrene [112]. Additionally, TLA treatment significantly increased the cytotoxic activity of spleen cells, enabling them to effectively kill feline FL74 lymphoma cells [113]. In another study, TLA vaccination in mice led to a reduction in both the size and weight of sarcoma 180 tumors and inhibited the expression of the angiogenic marker CD31, which is associated with tumor blood vessel formation [114].

A subsequent experiment compared the antitumor effects of TLA in athymic nude mice (which lack T cells) and non-athymic mice, using CT26 tumors. TLA injections into the tumors of both groups reduced tumor growth and lowered levels of the metastasis marker TIMP-1. Notably, TLA treatment activated the innate immune system in these mice, as evidenced by increased MyD88 signaling in bone marrow-derived macrophages and elevated IL-12 expression in the serum of athymic mice. This suggests that TLA's antitumor effects are primarily driven by innate immune activation, particularly via IL-12, a cytokine with potent antitumor properties [115].

Further studies have shown that *T. gondii* proteins, such as those in TLA, can strongly stimulate DCs, leading to significantly higher levels of IL-12p70 production compared to DNA-based immunogens. These findings suggest that TLA can inhibit tumor growth by selectively promoting early IL-12 expression, which is critical for initiating effective antitumor immune responses [116].

T. gondii secreted proteins

Tachyzoite excretory/secretory proteins (ESPs) are released by *T. gondii* into the tachyzoite culture medium [117]. These proteins include proteases and elements that specifically bind to serum antibodies in individuals previously infected with the parasite [39]. Similar to TLA (lysate antigen) components, ESPs are strong candidates for vaccines with potential antitumor effects [118]. ESPs have been shown to induce apoptosis in various cancer cell lines, including MCF-7 breast cancer cells, K562 erythroleukemic cells, and DU145 prostate cancer cells [119].

In the study, cancer cells were treated with various concentrations of *T. gondii* tachyzoites during their mid-exponential growth phase. The results demonstrated a dose-dependent inhibitory effect on tumor cell proliferation. The treatment downregulated the anti-apoptotic Bcl-2 gene and upregulated the tumor-suppressor protein p53 in A549 lung tumor cells. Additionally, *T. gondii* ESPs have been shown to inhibit the growth of B16F10 melanoma, Lewis lung carcinoma, and prostate cancer cells. Research involving subcutaneous injection of Lewis lung cancer and B16F10 melanoma cells into mice revealed that ESP administration significantly reduced the population of CD4 + CD25 + Foxp3 + T regulatory (Treg) cells while increasing NK cell levels in the spleen, contributing to enhanced antitumor immunity [120].

Interestingly, *T. gondii* releases certain proteins that adhere to the parasitophorous vacuole membrane (PVM), supporting parasite survival. *T. gondii* secretes dense granule proteins such as GRA2 and GRA12, which are crucial for sustaining long-term infections and modulating the host immune response, both key factors in the parasite's antitumor effects. GRA2 plays a key role in forming the intravacuolar network (IVN), which aids nutrient exchange, parasite replication, and immune modulation [121, 122]. GRA12 also contributes to the IVN's structure but is less understood [62, 123, 124]. Together, they facilitate the export of GRA proteins from the PVM into the host cell, triggering immune responses,

including T-cell activation and pro-inflammatory cytokine production, essential for antitumor activity. They also reduce immunosuppressive cells, like Tregs, in the tumor microenvironment, making cancer cells more vulnerable to immune attack. GRA2 and GRA12 interactions with host cell signaling disrupt tumor growth pathways in ovarian carcinoma, enhance antigen presentation, and recruit immune cells like NK cells and macrophages, further promoting tumor cell elimination. Deletion of GRA2 reduces heterophagy and weakens the antitumor response, while GRA12 localizes to IVN structures and associates with ROP5/ROP18 complexes [125]. Removing either protein significantly weakens the antitumor immune response.

The dense granule protein GRA8 is a key regulator of mitochondrial metabolism with potential anticancer effects in colorectal cancer [126]. It interacts with proteins such as ATP5A1 and SIRT3 to enhance ATP production, which can disrupt the energy balance in cancer cells in colorectal, often reliant on glycolysis (the "Warburg effect") [127, 128]. By boosting mitochondrial ATP synthesis, GRA8 creates energy stress in cancer cells in colorectal, making them more susceptible to apoptosis [129]. This modulation of mitochondrial activity can trigger cell death pathways, targeting cancer cells while sparing healthy ones. Ultimately, GRA8's ability to reprogram mitochondrial function may inhibit cancer cell in colorectal proliferation and survival [126].

GRA16, another dense granule protein of *T. gondii*, is critical in influencing host cellular processes, particularly in lung carcinoma. GRA16 translocates into the host cell nucleus, where it interacts with ubiquitin-specific protease 7 and protein phosphatase 2 A (PP2A), key regulators of the cell cycle and the p53 tumor suppressor pathway [130]. By affecting host gene transcription, GRA16 helps restore p53 function and regulate cell cycle progression. Furthermore, GRA16 enhances the efficacy of chemotherapy drugs like irinotecan and inhibits NF-κB activity in H1299 lung cancer cells [131]. NF-κB is known to regulate immune surveillance, apoptosis, and inflammatory cytokine release, as well as cell proliferation via interactions with transcription factors [132, 133]. Inhibiting NF-κB activation in non-small cell lung cancer cells is a crucial mechanism through which *T. gondii* proteins, like GRA16, exert antitumor effects and modulate immune responses in lung carcinoma [131].

Researchers have discovered that GRA24, also known as TgBRADIN, plays a critical role in triggering antitumor responses in ovarian carcinoma [62, 134]. GRA24 can cross the PVM and reach the host cell nucleus, where it maintains the activation of the host's p38α MAPK pathway. This is essential for immune response modulation in both type I and type II *T. gondii* strains [135]. Notably, GRA24 increases IL-12 production in type II strains but not in type I strains, suggesting strain-dependent antitumor effects mediated through this pathway. However, the study showed that both type I and type II uracil auxotrophs induced equally effective antitumor responses in Ovarian carcinoma, implying that p38α MAPK signaling may be a crucial factor in these effects [62].

Research into *T. gondii* Rhoptry proteins, specifically ROP35 and ROP38, has shown that these proteins play important roles in controlling the antitumor response in ovarian carcinoma [62]. ROP38 is involved in regulating several key processes in host cells, such as cell growth (proliferation), programmed cell death (apoptosis), and signaling pathways like MAPK, which are important for cell survival and stress response. ROP35, on the other hand, helps the parasite establish long-term (latent) infections, but this effect is not linked to the parasite's virulence (ability to cause disease). Both ROP35 and ROP38 work independently of the well-known ROP5/ROP17/ROP18 protein complexes, which means they might use different, yet unknown, methods to trigger their antitumor effects [136, 137]. This independent action of ROP35 and ROP38 suggests there are unique mechanisms behind their ability to fight tumors, which requires further research to understand fully (Table 1).

Combining *T. gondii* with modern immunotherapies

The field of immunotherapy, particularly immune checkpoint-based therapies, has gained increasing attention in clinical trials for solid tumors. Research on advanced metastatic cancers such as bladder, kidney, and melanoma has shown that these therapies can yield long-lasting effects [140, 141]. However, the effectiveness of immunotherapy in certain tumors is limited due to factors such as poor MHC presentation, inadequate T-cell infiltration, low or absent neoantigen loads, and resistance to immune checkpoint blockade [142–146]. To overcome these challenges, novel strategies are needed to provoke a strong and sustained antitumor immune response, which could significantly slow tumor progression and improve patient survival.

Recent studies have explored the potential of combining an attenuated strain of *T. gondii*, ΔGRA17, with anti-PD-L1 therapy to enhance tumor immunogenicity and overcome immune checkpoint resistance. In mouse models of LLC lung carcinoma, MC38 colon carcinoma, and B16-F10 melanoma, intratumoral injection of ΔGRA17 tachyzoites modified the tumor immune environment,

Table 1 *Toxoplasma gondii*-derived peptides with antitumor effects

Toxoplasma strain	protein from Toxoplasma	Tumor type	Tumor cell line	Mice	Methods of tumor cell inoculation	Immune effectors and signals	References
T. gondii excretory/ secretory proteins (ESP)/RH strain (Type I)	ESP	Melanoma Lung cancer	B16F10 Lewis	C57BL/6	Subcutaneous inoculation (s.c.)	CD4 + CD25 + FOXP3 + T cells (Treg), NK cells	[120]
T. gondii profiling (Tg-profilin) and profilin-like protein (TgPLP)/ N28E2 and RH88 (Type II and Type I)	STAg and profilin	Pancreatic tumor	allografting pancreatic tumors from KPC mice	mice lacking Batf3 and IFN-g in C57BL/6J.	Subcutaneous inoculation (s.c.)	FOXP3+, CD4+, or CD8 + T cells.	[138]
T. gondii rhoptry proteins (ROPs) and dense granule antigens (GRAs)/ RH strain (Type I)	ROP5, ROP17, ROP18, ROP35 or ROP38; GRA2 or GRA12, and GRA24	Ovarian carcinoma	ID8	mice lacking MyD88, Batf3, IFN-g, CD8, MHCII, IL-12p40, IL-12p35 in C57BL/6	intraperitoneal inoculation (i.p.)	CD4+, CD8+, IFN-g, and IL-12 T cells.	[62]
PRU strain (Type II)	GRA15	Hepatic carcinoma	Hepa1-6	C57BL/6	Subcutaneous inoculation (s.c.)	IL-12, IL-6, and TNF-a.	[139]
RH strain (Type I)	GRA16	Non-small-cell lung carcinoma	H1299	BALB/c	Subcutaneous inoculation (s.c.)	NF-kB	[131]
RH-Dcps (a virulent *T. gondii* strain)	---	Ovarian Cancer	ID8 cells	C57BL/6: mice lacking IL-12p40, IL-12p35, IL-17a, MyD88 /mice with Foxp3GFP and OT-1	intraperitoneal inoculation (i.p.)	CD8 + T cell	[61]
RH-Δcps (a virulent *T. gondii* strains)	---	Pancreatic Cancer	Pan02 cells	C57BL/6: mice lacking CD8a, MyD88, IFN-g, and IL12p35.	intraperitoneal inoculation (i.p.)	MyD88 pathway CD8 + T cell IL12 and IFNγ	[96]
RH-Δompdc (a virulent *T. gondii* strains)	---	Breast cancer	4T1	BALB/c	Intrathecal injection (i.t.)	IL-12 and IFN-g	[103]
RH-Δcps (a virulent *T. gondii* strain)	---	Melanoma ovarian cancer Lewis Lung carcinoma	B16F10 cells	C57BL/6	i.p.	IL-12, IFN-g, CXCR3	[102]

leading to both local and distant tumor regression. The combination of ΔGRA17 and anti-PD-L1 rendered initially resistant B16-F10 melanoma more susceptible to immunotherapy [91, 147, 148].

Interestingly, ΔGRA17 administration led to the regression of distant, untreated tumors, an effect linked to enhanced innate and adaptive immune responses, increased immune cell infiltration, and the upregulation of immune-related signaling pathways [149, 150]. Despite this systemic response, no evidence of ΔGRA17 tachyzoites was found in uninjected distant tumors, raising the possibility that these immune effects were mediated by indirect mechanisms, such as migration to draining lymph nodes, rather than direct parasite dissemination [91].

Additionally, ΔGRA17 therapy was shown to induce significant immune cell infiltration into both treated and untreated tumors, contributing to tumor regression. This immune activation was characterized by the upregulation of IFN-γ and TNF-α, suppression of immunosuppressive

pathways, and downregulation of Foxp3 in CD4 + T cells and PD-1 in CD8 + T cells. These changes in the tumor microenvironment led to enhanced survival rates and, importantly, no recurrence of cancer following ΔGRA17 treatment [91].

Another innovative approach being investigated involves combining *T. gondii* with chimeric antigen receptor (CAR)-T cells to improve specificity and enhance antitumor effects [19]. Studies have shown that *T. gondii* infection can inhibit the growth of various cancers, including Lewis lung carcinoma [60], Sarcoma-180 (Pyo, Jung et al., 2010), and fibrosarcoma [151]. These findings highlight the potential of *T. gondii* as an immunotherapeutic agent, given its ability to modulate immune responses and infect diverse cell types [152]. While the promise of *T. gondii*-based therapies is clear, further research is essential to fully understand the underlying mechanisms of action and address potential safety concerns, particularly the risk of toxicity associated with live parasitic agents [88].

Effects of *T. gondii* on Cancer development

Changes in gene expression are a hallmark of cancer cells [153, 154]. These changes result from genetic and epigenetic processes that occur at physiological, cellular, and molecular levels. The physiological level involves disruptions to cellular homeostasis, such as mitogenesis, compensatory cell division, evasion of apoptosis, inflammation, and angiogenesis. These alterations disrupt the balance of physiological pathways within cells and tissues. At the cellular level, changes include altered signaling pathways, DNA repair gene expression, and post-translational protein modifications. Molecular changes include DNA adduct formation, strand breaks, gene mutations, chromosomal aberrations, aneuploidy, and altered methylation patterns [153, 154]. While factors like hormones, medications, chemicals, and chronic stimuli contribute to cancer development, infectious agents, including parasites, are also implicated. Understanding the complex interactions between parasites and cancer may offer therapeutic insights [153, 155].

Though most research on the link between parasites and cancer has focused on worms, some protozoa, including *T. gondii*, have been associated with an increased risk of some cancers. *T. gondii* is particularly concerning due to its ability to affect monocyte-driven diseases in humans and mammals, with studies suggesting a link between *T. gondii* infection and somecancers, such as brain cancer, lung cancer, cervical cancer, among others [156] (Fig. 2; Table 2). Despite a significant correlation between *T. gondii* prevalence and brain tumors, definitive evidence linking *T. gondii* to the direct induction of such tumors remains elusive.

Barriers to clinical application of *T. gondii* in oncology

To date, no Phase I or II clinical trials have tested live or attenuated strains of *T. gondii* in cancer patients; all evidence comes from mouse models or in vitro studies. Several challenges must be overcome for clinical application. First, safety is crucial, as even attenuated strains pose risks for immunocompromised patients, requiring thorough toxicity and biodistribution studies. Second, the use of various parasite strains highlights the need for a single, well-characterized GMP-grade line. Third, optimal dosing parameters need to be defined in larger animal models, focusing on dose, delivery route, and scheduling, while monitoring parasite stability. Fourth, human genetic and immunological diversity exceeds that of inbred mice, leading to varied responses. Finally, the immunosuppressed status of cancer patients complicates balancing safe parasite persistence with immune activation, necessitating strategies that enhance efficacy and minimize risk.

Conclusion and future perspectives

Parasites, much like other symbiotic organisms, can exert both beneficial and harmful effects on the body. *T. gondii*, in particular, has shown promise as a potential therapeutic agent due to its antiangiogenic properties, ability to stimulate immune responses and reverse tumor-associated immunosuppression. It activates immune cells and promotes cytokine secretion, including TNF-α and IFN-γ, which contribute to inhibiting tumor growth, suggesting its suitability for cancer immunotherapy. However, in certain situations, such as weakened immune systems, immunosuppressive drug use, genetic enzyme defects, or chemotherapy, *T. gondii* can become pathogenic and harmful to its host.

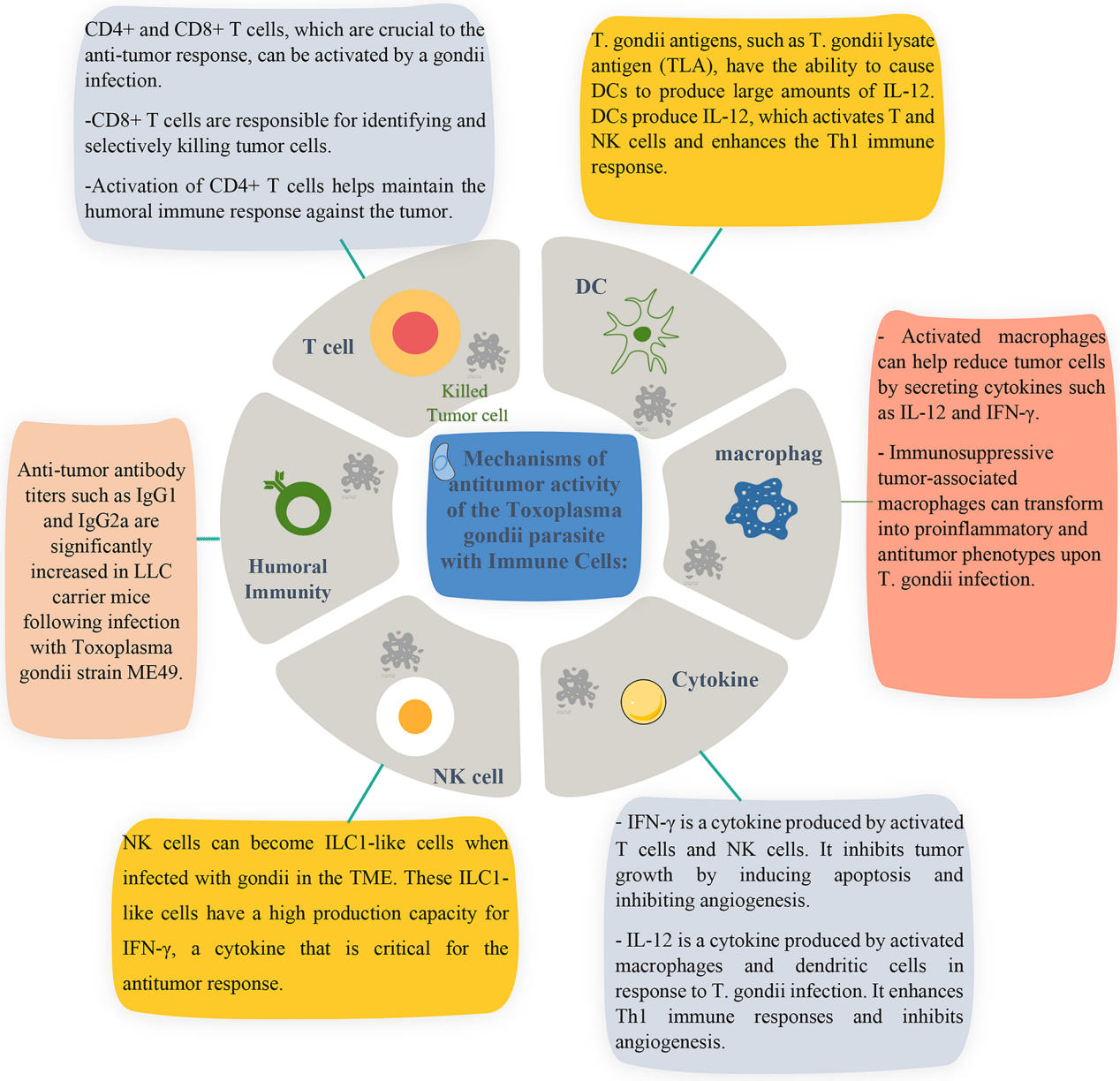

Fig. 2 Mechanisms of the Antitumor Activity of *Toxoplasma gondii* Involving Immune Cells

Recent years have seen growing interest in harnessing *T. gondii* for cancer treatment, largely due to its ability to manipulate the host's immune system and enhance antitumor responses. While the preliminary results from animal studies are promising, significant research is still required to fully elucidate the mechanisms through which *T. gondii* exerts its antitumor effects and to explore its potential for clinical applications. Major challenges remain, including the lack of clinical trials in humans and the inherent risks associated with administering live parasites, such as potential immunodeficiency and infection.

Future research should focus on overcoming these challenges by identifying parasite-derived compounds that specifically target cancer cells with minimal harm to healthy tissues. The development of genetically modified or attenuated strains of *T. gondii* may also offer safer alternatives to leverage its antitumor properties while minimizing the associated risks. Ultimately, continued

Table 2 *T. gondii* and cancer development

Cancer type	Relationship with Toxoplasma gondii	Reference
Brain Cancer	Inflammatory and anti-apoptotic responses are produced due to the cyst in contact.	[157]
Primary Intraocular B-cell Lymphoma	*Toxoplasma gondii* increases inflammation by promoting lymphoma development	[158]
Squamous Carcinoma	In one case, parasite tachyzoites were detected in bronchoalveolar lavages.	[159]
Melanoma, Lung Cancer	Suppression of neovascularization through induction of hypoxia and avascular necrosis.	[60]
Anaplastic Large Cell Lymphoma	Active toxoplasmosis has been shown to play a role in the etiology of this lymphoma.	[160]
Lung, Cervical, Brain, Endometrial Cancer	A seroprevalence relationship has been established.	[161]
Melanoma	Activation of CD8 + and NK cells in APC, with increased expression of MHC-I and MHC-II.	[102]
Fibrosarcoma	An increase in the activity of cytotoxic T cells has been observed.	[162]

exploration of *T. gondii* and its immune-modulating capabilities could pave the way for innovative cancer therapies that balance efficacy with safety.

Acknowledgements
Not applicable.

Author contributions
E.A and P.A conceptualized the manuscript. P.A, AS.P, and K.HN developed the tables and figures. P.A, A.K collected data from the literature. P.A and A.K drafted the initial manuscript. AS.P, K.HN and E.A reviewed and revised the manuscript. E.A supervised the manuscript and provided mentorship. All authors read and approved the final manuscript.

Funding
This review did not receive any external funding.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 6 January 2025 / Accepted: 10 June 2025
Published online: 18 June 2025

References

1. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis. 2009;30 7:1073–81.
2. Tominaga O, Nita ME, Nagawa H, Fujii S, Tsuruo T, Muto T. Expressions of cell cycle regulators in human colorectal cancer cell lines. Jpn J Cancer Res. 1997;88 9:855–60.
3. Rupnarain C, Dlamini Z, Naicker S, Bhoola K. Colon cancer: genomics and apoptotic events. 2004.
4. Bishnupuri KS, Luo Q, Murmu N, Houchen CW, Anant S, Dieckgraefe BK. Reg IV activates the epidermal growth factor receptor/Akt/AP-1 signaling pathway in colon adenocarcinomas. Gastroenterology. 2006;130(1):137–49.
5. Karin M. NF-κB and cancer: mechanisms and targets. Molecular carcinogenesis: published in Cooperation with the university of Texas MD Anderson Cancer center. 2006;45 6:355–61.
6. Maeda K, Nishiguchi Y, Kang S-M, Yashiro M, Onoda N, Sawada T, et al. Expression of thrombospondin-1 inversely correlated with tumor vascularity and hematogenous metastasis in colon cancer. Oncol Rep. 2001;8(4):763–6.
7. Whisenant J, Bergstand E. Anti-angiogenic strategies in Gastrointestinal malignancies. Curr Treat Options Oncol. 2005;6:411–21.
8. Schimanski CC, Schwald S, Simiantonaki N, Jayasinghe C, Gönner U, Wilsberg V, et al. Effect of chemokine receptors CXCR4 and CCR7 on the metastatic behavior of human colorectal cancer. Clin Cancer Res. 2005;11 5:1743–50.
9. Vennervald B, Polman K. Helminths and malignancy. Parasite Immunol. 2009;31:11686–96.
10. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
11. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin. 2021;71:3209–49.
12. Chon SY, Champion RW, Geddes ER, Rashid RM. Chemotherapy-induced alopecia. J Am Acad Dermatol. 2012;67(1):e37–47.
13. Fillon M. Lung cancer radiation May increase the risk of major adverse cardiac events. Cancer J Clin. 2019;69(6):435–7.
14. Tan S, Li D, Zhu X. Cancer immunotherapy: pros, cons and beyond. Biomed Pharmacother. 2020;124:109821.
15. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint Blockade. Science. 2018;359 6382:1350–5.
16. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science. 2018;359 6382:1361–5.
17. Callejas BE, Martínez-Saucedo D, Terrazas LI. Parasites as negative regulators of cancer. Biosci Rep. 2018;38 5:BSR20180935.
18. Shahrivar F, Sadraei J, Pirestani M, Ahmadpour E. Enhancement of apoptosis in Caco-2, Hep-G2, and HT29 cancer cell lines following exposure to Toxoplasma gondii peptides. Drug Target Insights. 2024;18:70.
19. Ding H, Wu S, Jin Z, Zheng B, Hu Y, He K, et al. Anti-Tumor effect of parasitic protozoans. Bioengineering. 2022;9:8.
20. Wang P, Li S, Zhao Y, Zhang B, Li Y, Liu S, et al. The GRA15 protein from Toxoplasma gondii enhances host defense responses by activating the interferon stimulator STING. J Biol Chem. 2019;294 45:16494–508.
21. Chakraborty S, Roy S, Mistry HU, Murthy S, George N, Bhandari V, et al. Potential sabotage of host cell physiology by apicomplexan parasites for their survival benefits. Front Immunol. 2017;8:298145.
22. Huang H, Yang W, Hu J, Jiang Y, Wang J, Shi C, et al. Antitumour metastasis and the antiangiogenic and antitumour effects of a Eimeria stiedae soluble protein. Parasite Immunol. 2021;43 6:e12825.
23. Qin L, Chen C, Chen L, Xue R, Ou-Yang M, Zhou C, et al. Worldwide malaria incidence and cancer mortality are inversely associated. Infect Agents cancer. 2017;12:1–8.
24. Dupont CD, Christian DA, Hunter CA. Immune response and immunopathology during toxoplasmosis. In: Seminars in immunopathology. 2012;793–813. Springer.
25. Fox BA, Bzik DJ. Avirulent uracil auxotrophs based on disruption of orotidine-5′-monophosphate decarboxylase elicit protective immunity to Toxoplasma gondii. Infect Immun. 2010;78 9:3744–52.
26. Jones JL, Kruszon-Moran D, Wilson M, McQuillan G, Navin T, McAuley JB. Toxoplasma gondii infection in the united states: Seroprevalence and risk factors. Am J Epidemiol. 2001;154(4):357–65.

27. Jones JL, Dubey J. Foodborne toxoplasmosis. Clin Infect Dis. 2012;55:6845–51.
28. de Barros RAM, Torrecilhas AC, Marciano MAM, Mazuz ML, Pereira-Chioccola VL, Fux B. Toxoplasmosis in human and animals around the world. Diagnosis and perspectives in the one health approach. Acta Trop. 2022;231:106432.
29. Grigg ME, Bonnefoy S, Hehl AB, Suzuki Y, Boothroyd JC. Success and virulence in Toxoplasma as the result of sexual recombination between two distinct ancestries. Science. 2001;294 5540:161–5.
30. Hunter CA, Sibley LD. Modulation of innate immunity by Toxoplasma gondii virulence effectors. Nat Rev Microbiol. 2012;10:11766–78.
31. Hassan MA, Olijnik A-A, Frickel E-M, Saeij JP. Clonal and atypical Toxoplasma strain differences in virulence vary with mouse sub-species. Int J Parasitol. 2019;49(1):63–70.
32. Hoffmann S, Batz MB, Morris JG Jr. Annual cost of illness and quality-adjusted life year losses in the united States due to 14 foodborne pathogens. J Food Prot. 2012;75 7:1292–302.
33. Torgerson PR, Mastroiacovo P. The global burden of congenital toxoplasmosis: a systematic review. Bull World Health Organ. 2013;91:501–8.
34. Safarpour H, Cevik M, Zarean M, Barac A, Hatam-Nahavandi K, Rahimi MT, et al. Global status of *Toxoplasma gondii* infection and associated risk factors in people living with HIV. AIDS. 2020;34 3:469–74. https://doi.org/10.1097/QAD.0000000000002424
35. Flegr J, Stříž I. Potential Immunomodulatory effects of latent toxoplasmosis in humans. BMC Infect Dis. 2011;11:1–7.
36. Majidiani H, Dalimi A, Ghaffarifar F, Pirestani M, Ghaffari AD. Computational probing of Toxoplasma gondii major surface antigen 1 (SAG1) for enhanced vaccine design against toxoplasmosis. Microb Pathog. 2020;147:104386.
37. Ahmadpour E, Babaie F, Kazemi T, Mehrani Moghaddam S, Moghimi A, Hosseinzadeh R, et al. Overview of apoptosis, autophagy, and inflammatory processes in *Toxoplasma gondii* infected cells. Pathogens. 2023;12(2):253. https://doi.org/10.3390/pathogens12020253
38. Payne TM, Molestina RE, Sinai AP. Inhibition of caspase activation and a requirement for NF-κB function in the Toxoplasma gondii-mediated Blockade of host apoptosis. J Cell Sci. 2003;116 21:4345–58.
39. Keller P, Schaumburg F, Fischer SF, Häcker G, Groß U, Lüder CG. Direct Inhibition of cytochrome c-induced caspase activation in vitro by Toxoplasma gondii reveals novel mechanisms of interference with host cell apoptosis. FEMS Microbiol Lett. 2006;258 2:312–9.
40. Goebel S, Gross U, Lüder CG. Inhibition of host cell apoptosis by Toxoplasma gondii is accompanied by reduced activation of the caspase cascade and alterations of Poly (ADP-ribose) Polymerase expression. J Cell Sci. 2001;114:193495–505.
41. Simbulan-Rosenthal CM, Rosenthal DS, Iyer S, Boulares AH, Smulson ME. Transient Poly (ADP-ribosyl) ation of nuclear proteins and role of Poly (ADP-ribose) Polymerase in the early stages of apoptosis. J Biol Chem. 1998;273 22:13703–12.
42. Hong SJ, Dawson TM, Dawson VL. Nuclear and mitochondrial conversations in cell death: PARP-1 and AIF signaling. Trends in Pharmacological sciences. 2004;25(5):259–64.
43. Seyedeh MS, Nahid S, Nahid M, Shima DP, Morteza Y, Hossein YD. Low titer of antibody against Toxoplasma gondii May be related to resistant to cancer. J Cancer Res Ther. 2015;11 2:305–7. https://doi.org/10.4103/0973-1482.144638.
44. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998;392:6673245–52.
45. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. Nat Rev Cancer. 2012;12:4265–77.
46. Cella M, Engering A, Pinet V, Pieters J, Lanzavecchia A. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. Nature. 1997;388 6644:782–7.
47. Curiel TJ, Cheng P, Mottram P, Alvarez X, Moons L, Evdemon-Hogan M, et al. Dendritic cell subsets differentially regulate angiogenesis in human ovarian cancer. Cancer Res. 2004;64 16:5535–8.
48. Scarlett UK, Cubillos-Ruiz JR, Nesbeth YC, Martinez DG, Engle X, Gewirtz AT, et al. In situ stimulation of CD40 and toll-like receptor 3 transforms ovarian cancer–infiltrating dendritic cells from immunosuppressive to immunostimulatory cells. Cancer Res. 2009;69 18:7329–37.
49. Pepper M, Dzierszinski F, Wilson E, Tait E, Fang Q, Yarovinsky F, et al. Plasmacytoid dendritic cells are activated by Toxoplasma gondii to present antigen and produce cytokines. J Immunol. 2008;180 9:6229–36.
50. Fox B, Sanders K, Bzik D. Non-replicating Toxoplasma gondii reverses tumor-associated immunosuppression. Oncoimmunology. 2013;2 11:e26296.
51. Chen J, Liao W, Peng H. Toxoplasma gondii infection possibly reverses host immunosuppression to restrain tumor growth. Front Cell Infect Microbiol. 2022;12:959300.
52. Mantovani A, Sica A, Locati M. New vistas on macrophage differentiation and activation. Eur J Immunol. 2007;37(1):14–6.
53. Wei Z, Zhang X, Yong T, Bie N, Zhan G, Li X, et al. Boosting anti-PD-1 therapy with metformin-loaded macrophage-derived microparticles. Nat Commun. 2021;12 1:440.
54. Mantovani A, Sica A, Locati M. Macrophage polarization comes of age. Immunity. 2005;23 4:344–6.
55. Moghaddami R, Mahdipour M, Ahmadpour EJTM. Disease I. Inflammatory pathways of *Toxoplasma gondii* infection in pregnancy. Travel Med Infect Dis. 2024;62:102760. https://doi.org/10.1016/j.tmaid.2024.102760
56. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. J Clin Investig. 2012;122(3):787–95.
57. Zhu L, Zhao Q, Yang T, Ding W, Zhao Y. Cellular metabolism and macrophage functional polarization. Int Rev Immunol. 2015;34 1:82–100.
58. Stout RD, Suttles J. Functional plasticity of macrophages: reversible adaptation to changing microenvironments. J Leukoc Biol. 2004;76(3):509–13.
59. Sica A, Invernizzi P, Mantovani A. Macrophage plasticity and polarization in liver homeostasis and pathology. Hepatology. 2014;59 5:2034–42.
60. Kim J-O, Jung S-S, Kim S-Y, Kim TY, Shin D-W, Lee J-H et al. Inhibition of Lewis lung carcinoma growth by Toxoplasma gondii through induction of Th1 immune responses and Inhibition of angiogenesis. J Korean Med Sci. 2007;22 Suppl:S38.
61. Baird JR, Fox BA, Sanders KL, Lizotte PH, Cubillos-Ruiz JR, Scarlett UK, et al. Avirulent Toxoplasma gondii generates therapeutic antitumor immunity by reversing immunosuppression in the ovarian cancer microenvironment. Cancer Res. 2013;73 13:3842–51.
62. Fox BA, Sanders KL, Rommereim LM, Guevara RB, Bzik DJ. Secretion of Rhoptry and dense granule effector proteins by nonreplicating Toxoplasma gondii uracil auxotrophs controls the development of antitumor immunity. PLoS Genet. 2016;12 7:e1006189.
63. Hafez EN, Youssef HM, El-Kabany HA. Vaccination with gamma radiation-attenuated Toxoplasma gondii protects against ovarian infiltration in mice-bearing Ehrlich Ascites carcinoma. Int J Radiat Biol. 2020;96 6:814–22.
64. Li Y, Zhang Y, Xia N, Zhou T, Shen B. Antitumor effects of a Toxoplasma mutant lacking lactate dehydrogenases. Parasitol Res. 2021;120:3335–9.
65. Sasai M, Pradipta A, Yamamoto M. Host immune responses to Toxoplasma gondii. Int Immunol. 2018;30 3:113–9.
66. Zhu S, Lu J, Lin Z, Abuzeid AM, Chen X, Zhuang T, et al. Anti-tumoral effect and action mechanism of exosomes derived from Toxoplasma gondii-infected dendritic cells in mice colorectal cancer. Front Oncol. 2022;12:870528.
67. Chen M, Yang P, Xin Z, Chen J, Zou W, Zhou L, et al. Toxoplasma gondii gra5 deletion mutant protects hosts against Toxoplasma gondii infection and breast tumors. Front Immunol. 2023;14:1173379.
68. Song Y, Yuan H, Yang X, Yang Z, Ren Z, Qi S, et al. The opposing effect of acute and chronic Toxoplasma gondii infection on tumor development. Parasites Vectors. 2024;17(1):1–14.
69. Fol M, Karpik W, Zablotni A, Kulesza J, Kulesza E, Godkowicz M, et al. Innate lymphoid cells and their role in the immune response to infections. Cells. 2024;13(4). https://doi.org/10.3390/cells13040335.
70. Gazzinelli RT, Hieny S, Wynn TA, Wolf S, Sher A. Interleukin 12 is required for the T-lymphocyte-independent induction of interferon gamma by an intracellular parasite and induces resistance in T-cell-deficient hosts. Proceedings of the National Academy of Sciences. 1993;90(13):6115-9.
71. Mahmoudzadeh S, Dizaji Asl K, Nozad Charoudeh H, Rahbarghazi R, Ahmadi M, Heidarzadeh M, et al. Toxoplasma gondii suppress human cord blood cell differentiation to the NK cell population. Immun Inflamm Dis. 2024;12 6:e1329. https://doi.org/10.1002/iid3.1329
72. Mahmoudzadeh S, Nozad Charoudeh H, Marques CS, Bahadory S, Ahmadpour EJPR. The role of IL-12 in stimulating NK cells against *Toxoplasma gondii* infection: a mini-review. Parasitol Res. 2021;120(7):2303–9. https://doi.org/10.1007/s00436-021-07204-w
73. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, et al. Innate production of TH2 cytokines by adipose tissue-associated c-Kit + Sca-1 + lymphoid cells. Nature. 2010;463 7280:540–4.
74. Kamiya T, Chang Y-H, Campana D. Expanded and activated natural killer cells for immunotherapy of hepatocellular carcinoma. Cancer Immunol Res. 2016;4 7:574–81.

75. Vivier E, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate lymphoid cells: 10 years on. Cell. 2018;174 5:1054–66.
76. Goldszmid RS, Bafica A, Jankovic D, Feng CG, Caspar P, Winkler-Pickett R, et al. TAP-1 indirectly regulates CD4 + T cell priming in Toxoplasma gondii infection by controlling NK cell IFN-γ production. J Exp Med. 2007;204 11:2591–602.
77. Goldszmid RS, Caspar P, Rivollier A, White S, Dzutsev A, Hieny S, et al. NK cell-derived interferon-γ orchestrates cellular dynamics and the differentiation of monocytes into dendritic cells at the site of infection. Immunity. 2012;36 6:1047–59.
78. Klose CS, Flach M, Möhle L, Rogell L, Hoyler T, Ebert K, et al. Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. Cell. 2014;157(2):340–56.
79. Raskov H, Orhan A, Christensen JP, Gögenur I. Cytotoxic CD8 + T cells in cancer and cancer immunotherapy. Br J Cancer. 2021;124(2):359–67.
80. Chen Y, Yu D, Qian H, Shi Y, Tao Z. CD8 + T cell-based cancer immunotherapy. J Translational Med. 2024;22(1):394.
81. Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll D, Levitsky H. The central role of CD4(+) T cells in the antitumor immune response. J Exp Med. 1998;188 12:2357–68. https://doi.org/10.1084/jem.188.12.2357.
82. Sanders KL, Fox BA, Bzik DJ. Attenuated Toxoplasma gondii therapy of disseminated pancreatic cancer generates long-lasting immunity to pancreatic cancer. Oncoimmunology. 2016;5 4:e1104447.
83. Eissa MM, Gaafar MR, Younis LK, Ismail CA, El Skhawy N. Prophylactic antineoplastic activity of Toxoplasma gondii RH derived antigen against Ehrlich solid carcinoma with evidence of shared antigens by comparative Immunoblotting. Infect Agents Cancer. 2023;18:1.
84. Zhang H, Xie W, Zhang Y, Dong X, Liu C, Yi J, et al. Oncolytic adenoviruses synergistically enhance anti-PD-L1 and anti-CTLA-4 immunotherapy by modulating the tumour microenvironment in a 4T1 orthotopic mouse model. Cancer Gene Ther. 2022;29 5:456–65.
85. Astaneh M, Dashti S, Esfahani ZT. Humoral immune responses against cancer-testis antigens in human malignancies. Hum Antib. 2019;27:4237–40.
86. Rudnick SI, Lou J, Shaller CC, Tang Y, Klein-Szanto AJ, Weiner LM, et al. Influence of affinity and antigen internalization on the uptake and penetration of Anti-HER2 antibodies in solid tumors. Cancer Res. 2011;71 6:2250–9.
87. Xia L, Oyang L, Lin J, Tan S, Han Y, Wu N, et al. The cancer metabolic reprogramming and immune response. Mol Cancer. 2021;20:1–21.
88. Zheng Z, Lu X, Zhou D, Deng X-f, Liu Q-x, Liu X-b, et al. A novel enemy of cancer: recent investigations into protozoan anti-tumor properties. Front Cell Infect Microbiol. 2024;13:1325144. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10808745/pdf/fcimb-13-1325144.pdf.
89. Suzuki Y, Kobayashi A. Antitumor effect of intralesional injection with formalin-fixed Toxoplasma gondii organisms on Lewis lung carcinoma in Toxoplasma-infected mice. Cancer Lett. 1985;25:3247–54.
90. Hafez EN, Moawed FS, Abdel-Hamid GR, Elbakary NM. Gamma radiation-attenuated Toxoplasma gondii provokes apoptosis in Ehrlich Ascites carcinoma-bearing mice generating long-lasting immunity. Technol Cancer Res Treat. 2020;19:1533033820926593.
91. Zhu Y-C, Elsheikha HM, Wang J-H, Fang S, He J-J, Zhu X-Q, et al. Synergy between Toxoplasma gondii type I ΔGRA17 immunotherapy and PD-L1 checkpoint Inhibition triggers the regression of targeted and distal tumors. J Immunother Cancer. 2021;9:11.
92. Chen M, Yang P, Xin Z, Chen J, Zou W, Zhou L, et al. Toxoplasma gondii gra5 deletion mutant protects hosts against Toxoplasma gondii infection and breast tumors. Front Immunol. 2023;14:1173379. https://doi.org/10.3389/fimmu.2023.1173379.
93. Fox BA, Bzik DJ. De Novo pyrimidine biosynthesis is required for virulence of Toxoplasma gondii. Nature. 2002;415 6874:926–9.
94. Gigley JP, Fox BA, Bzik DJ. Cell-mediated immunity to Toxoplasma gondii develops primarily by local Th1 host immune responses in the absence of parasite replication. J Immunol. 2009;182(2):1069–78.
95. Dupont CD, Christian DA, Selleck EM, Pepper M, Leney-Greene M, Harms Pritchard G, et al. Parasite fate and involvement of infected cells in the induction of CD4 + and CD8 + T cell responses to Toxoplasma gondii. PLoS Pathog. 2014;10:4e1004047.
96. Sanders KL, Fox BA, Bzik DJ. Attenuated Toxoplasma gondii stimulates immunity to pancreatic cancer by manipulation of myeloid cell populations. Cancer Immunol Res. 2015;3 8:891–901.
97. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol. 2010;11 10:889–96.
98. Goldszmid RS, Dzutsev A, Trinchieri G. Host immune response to infection and cancer: unexpected commonalities. Cell Host Microbe. 2014;15 3:295–305.
99. Shaw MH, Freeman GJ, Scott MF, Fox BA, Bzik DJ, Belkaid Y, et al. Tyk2 negatively regulates adaptive Th1 immunity by mediating IL-10 signaling and promoting IFN-γ-dependent IL-10 reactivation. J Immunol. 2006;176 12:7263–71.
100. Wilson DC, Matthews S, Yap GS. IL-12 signaling drives CD8 + T cell IFN-γ production and differentiation of KLRG1 + effector subpopulations during Toxoplasma gondii infection. J Immunol. 2008;180 9:5935–45.
101. Jordan KA, Wilson EH, Tait ED, Fox BA, Roos DS, Bzik DJ, et al. Kinetics and phenotype of vaccine-induced CD8 + T-cell responses to Toxoplasma gondii. Infect Immun. 2009;77 9:3894–901.
102. Baird JR, Byrne KT, Lizotte PH, Toraya-Brown S, Scarlett UK, Alexander MP, et al. Immune-mediated regression of established B16F10 melanoma by intratumoral injection of attenuated Toxoplasma gondii protects against Rechallenge. J Immunol. 2013;190:1469–78.
103. Xu L-Q, Yao L-J, Jiang D, Zhou L-J, Chen M, Liao W-Z, et al. A uracil auxotroph Toxoplasma gondii exerting Immunomodulation to inhibit breast cancer growth and metastasis. Parasites Vectors. 2021;14(1):601.
104. Shen B, Brown K, Long S, Sibley LD. Development of CRISPR/Cas9 for efficient genome editing in *Toxoplasma gondii*. In Vitro Mutagenesis: Methods Protocols. 2017; 47(12): 79–103
105. Shen Y, Zheng B, Sun H, Wu S, Fan J, Ding J, et al. A live attenuated RHΔ Ompdc Δ Uprt mutant of Toxoplasma gondii induces strong protective immunity against toxoplasmosis in mice and cats. Infect Dis Poverty. 2023;12 03:51–66.
106. Le Roux D, Djokic V, Morisse S, Chauvin C, Doré V, Lagrée A-C, et al. Evaluation of immunogenicity and protection of the Mic1-3 knockout Toxoplasma gondii live attenuated strain in the feline host. Vaccine. 2020;38 6:1457–66.
107. Hedhli D, Dimier-Poisson I, Judge JW, Rosenberg B, Mévélec MN. Protective immunity against Toxoplasma challenge in mice by coadministration of T. gondii antigens and Eimeria profilin-like protein as an adjuvant. Vaccine. 2009;27 16:2274–81.
108. Pollard TD, Almo S, Quirk S, Vinson V, Lattman EE. Structure of actin binding proteins: insights about function at atomic resolution. Annu Rev Cell Biol. 1994;10(1):207–49.
109. Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS, et al. TLR11 activation of dendritic cells by a protozoan profilin-like protein. Science. 2005;308 5728:1626–9.
110. Pyo K-H, Lee Y-W, Lim SM, Shin E-H. Immune adjuvant effect of a Toxoplasma gondii profilin-like protein in autologous whole-tumor-cell vaccination in mice. Oncotarget. 2016;7 45:74107.
111. Miyahara K, Yokoo N, Sakurai H, Igarashi I, Sakata Y, Yoshida Y, et al. Antitumor activity of Toxoplasma lysate antigen against methylcholanthrene-induced tumor-bearing rats. J Vet Med Sci. 1992;54(2):221–8.
112. Miyahara K, Honda U, Tose S, Sakurai H, Igarashi I, Saito A, et al. Therapeutic effects of Toxoplasma lysate antigen on 20-methylcholanthrene-induced balb/c mouse tumors. J Vet Med Sci. 1992;54(1):7–12.
113. Yang MP, Goitsuka R, Ono K, Suzuki N, Hasegawa A. Effect of Toxoplasma lysate antigen (TLA) on feline cytotoxicity against FeLV positive lymphoma cells. Nihon Juigaku Zasshi Japanese J Veterinary Sci. 1990;52(4):735–42. https://doi.org/10.1292/jvms1939.52.735.
114. Pyo K-H, Jung B-K, Chai J-Y, Shin E-H. Suppressed CD31 expression in sarcoma-180 tumors after injection with Toxoplasma gondii lysate antigen in balb/c mice. Korean J Parasitol. 2010;48 2:171.
115. Pyo K-H, Jung B-K, Xin C-F, Lee Y-W, Chai J-Y, Shin E-H. Prominent IL-12 production and tumor reduction in athymic nude mice after Toxoplasma gondii lysate antigen treatment. Korean J Parasitol. 2014;52 6:605.
116. Mirzaei R, Arab S, Motamedi M, Amari A, Hadjati J. The opposite effects of DNA and protein components of listeria monocytogenes and *Toxoplasma gondii* on immunologic characteristics of dendritic cells. Iran J Allergy Asthma Immunol. 2015;14(3):313–20.
117. Stryiński R, Łopieńska-Biernat E, Carrera M. Proteomic insights into the biology of the most important foodborne parasites in Europe. Foods. 2020;9 10:1403.
118. Rezaei F, Sharif M, Sarvi S, Hejazi SH, Aghayan S, Pagheh AS, et al. A systematic review on the role of GRA proteins of Toxoplasma gondii in host immunization. J Microbiol Methods. 2019;165:105696.
119. Wu X, Sun L, Zhang L, Liu Z-Q, Luo Q, Zhang L-X. Impact of Toxoplasma gondii on the proliferation and apoptosis of tumor cell lines. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za zhi = chinese. J Parasitol Parasitic Dis. 2012;30 2:157–9.

120. Jiao YuMeng JY, Zhang Li ZL, Ge YiYue GY, Liang YueJin LY, Wang Yong WY. Effects of excreted/secreted antigens of Toxoplasma gondii on CD4 + CD25 + Foxp3 + T cells and NK cells of melanoma-bearing mice. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi. 2011;23(3):301–6.
121. Mercier C, Dubremetz J-F, Rauscher B, Lecordier L, Sibley LD, Cesbron-Delauw M-F. Biogenesis of nanotubular network in Toxoplasma parasitophorous vacuole induced by parasite proteins. Mol Biol Cell. 2002;13 7:2397–409.
122. Lopez J, Bittame A, Massera C, Vasseur V, Effantin G, Valat A, et al. Intravacuolar membranes regulate CD8 T cell recognition of membrane-bound Toxoplasma gondii protective antigen. Cell Rep. 2015;13 10:2273–86.
123. Fox BA, Guevara RB, Rommereim LM, Falla A, Bellini V, Pètre G, et al. Toxoplasma gondii parasitophorous vacuole membrane-associated dense granule proteins orchestrate chronic infection and GRA12 underpins resistance to host gamma interferon. MBio. 2019;10(4). https://doi.org/10.1128/mbio.00589– 19.
124. Lockyer EJ, Torelli F, Butterworth S, Song O-R, Howell S, Weston A, et al. A heterotrimeric complex of Toxoplasma proteins promotes parasite survival in interferon gamma-stimulated human cells. PLoS Biol. 2023;21 7:e3002202.
125. Dou Z, McGovern OL, Di Cristina M, Carruthers VB. Toxoplasma gondii ingests and digests host cytosolic proteins. MBio. 2014;5(4):01188–14. https://doi.org/10.1128/mbio.
126. Kim J-S, Lee D, Kim D, Mun S-J, Cho E, Son W, et al. Toxoplasma gondii GRA8-derived peptide immunotherapy improves tumor targeting of colorectal cancer. Oncotarget. 2020;11 1:62.
127. Torrens-Mas M, Oliver J, Roca P, Sastre-Serra J. SIRT3: oncogene and tumor suppressor in cancer. Cancers. 2017;9 7:90.
128. Sousa B, Pereira J, Paredes J. The crosstalk between cell adhesion and cancer metabolism. Int J Mol Sci. 2019;20:8.
129. Kim Y-R, Kim J-S, Yun J-S, Kim S, Kim SY, Jang K, et al. Toxoplasma gondii GRA8 induces ATP5A1–SIRT3-mediated mitochondrial metabolic resuscitation: A potential therapy for sepsis. Exp Mol Med. 2018;50(3):e464–e.
130. Bougdour A, Durandau E, Brenier-Pinchart M-P, Ortet P, Barakat M, Kieffer S, et al. Host cell subversion by Toxoplasma GRA16, an exported dense granule protein that targets the host cell nucleus and alters gene expression. Cell Host Microbe. 2013;13 4:489–500.
131. Seo S-H, Kim S-G, Shin J-H, Ham D-W, Shin E-H. Toxoplasma gra16 inhibits nf-κb activation through pp2a-b55 upregulation in non-small-cell lung carcinoma cells. Int J Mol Sci. 2020;21 18:6642.
132. Chakraborty C, Sharma AR, Sharma G, Lee S-S. The interplay among mirnas, major cytokines, and cancer-related inflammation. Mol Therapy-Nucleic Acids. 2020;20:606–20.
133. Habanjar O, Bingula R, Decombat C, Diab-Assaf M, Caldefie-Chezet F, Delort L. Crosstalk of inflammatory cytokines within the breast tumor microenvironment. Int J Mol Sci. 2023;24 4:4002.
134. Odell AV, Tran F, Foderaro JE, Poupart S, Pathak R, Westwood NJ, et al. Yeast three-hybrid screen identifies TgBRADIN/GRA24 as a negative regulator of Toxoplasma gondii bradyzoite differentiation. PLoS ONE. 2015;10:3e0120331.
135. Braun L, Brenier-Pinchart M-P, Yogavel M, Curt-Varesano A, Curt-Bertini R-L, Hussain T, et al. A Toxoplasma dense granule protein, GRA24, modulates the early immune response to infection by promoting a direct and sustained host p38 MAPK activation. J Exp Med. 2013;210 10:2071–86.
136. Fox BA, Rommereim LM, Guevara RB, Falla A, Hortua Triana MA, Sun Y, et al. The Toxoplasma gondii Rhoptry Kinome is essential for chronic infection. MBio. 2016;7(3). https://doi.org/10.1128/mbio.00193– 16.
137. Etheridge RD, Alaganan A, Tang K, Lou HJ, Turk BE, Sibley LD. The Toxoplasma pseudokinase ROP5 forms complexes with ROP18 and ROP17 kinases that synergize to control acute virulence in mice. Cell Host Microbe. 2014;15 5:537–50.
138. Payne SN, Emmerich PB, Davis NM, Deming DA, Knoll LJ. Novel murine pancreatic tumor model demonstrates immunotherapeutic control of tumor progression by a Toxoplasma gondii protein. Infect Immun. 2021;89:12.
139. Li Y, Poppoe F, Chen J, Yu L, Deng F, Luo Q, et al. Macrophages polarized by expression of ToxoGRA15II inhibit growth of hepatic carcinoma. Front Immunol. 2017;8:137.
140. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell. 2015;161(2):205–14.
141. Wei SC, Duffy CR, Allison JP. Fundamental mechanisms of immune checkpoint Blockade therapy. Cancer Discov. 2018;8 9:1069–86.
142. Pardoll DM. The Blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64.
143. Spranger S, Spaapen RM, Zha Y, Williams J, Meng Y, Ha TT et al. Up-regulation of PD-L1, IDO, and Tregs in the melanoma tumor microenvironment is driven by CD8 + T cells. Science translational medicine. 2013;5(200):200ra116-200ra116.
144. Herbst RS, Soria J-C, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515 7528:563–7.
145. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol. 2015;16:8.
146. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017;168:4707–23.
147. Gnjatic S, Bronte V, Brunet LR, Butler MO, Disis ML, Galon J, et al. Identifying baseline immune-related biomarkers to predict clinical outcome of immunotherapy. J Immunother Cancer. 2017;5:1–18.
148. Heinhuis K, Ros W, Kok M, Steeghs N, Beijnen J, Schellens J. Enhancing antitumor response by combining immune checkpoint inhibitors with chemotherapy in solid tumors. Ann Oncol. 2019;30(2):219–35.
149. Teng MW, Ngiow SF, Ribas A, Smyth MJ. Classifying cancers based on T-cell infiltration and PD-L1. Cancer Res. 2015;75 11:2139–45.
150. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint Blockade in cancer therapy. Nat Rev Cancer. 2016;16 5:275–87.
151. Darani HY, Shirzad H, Mansoori F, Zabardast N, Mahmoodzadeh M. Effects of Toxoplasma gondii and Toxocara canis antigens on WEHI-164 fibrosarcoma growth in a mouse model. Korean J Parasitol. 2009;47 2:175.
152. Kochanowsky JA, Koshy AA. Toxoplasma gondii. Curr Biol. 2018;28 14:R770–1.
153. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. Nat Rev Drug Discovery. 2015;14:9.
154. Jacqueline C, Tasiemski A, Sorci G, Ujvari B, Maachi F, Missé D, et al. Infections and cancer: the Fifty shades of immunity hypothesis. BMC Cancer. 2017;17:1–11.
155. Ali MI, Abd El Wahab WM, Hamdy DA, Hassan A. Toxoplasma gondii in cancer patients receiving chemotherapy: Seroprevalence and interferon gamma level. J Parasitic Dis. 2019;43:464–71.
156. Lun Z-R, Lai D-H, Wen Y-Z, Zheng L-L, Shen J-L, Yang T-B, et al. Cancer in the parasitic protozoans trypanosoma brucei and Toxoplasma gondii. Proc Natl Acad Sci. 2015;112(29):8835–42.
157. Thomas F, Lafferty KD, Brodeur J, Elguero E, Gauthier-Clerc M, Missé D. Incidence of adult brain cancers is higher in countries where the protozoan parasite Toxoplasma gondii is common. Biol Lett. 2012;8(1):101–3.
158. Herbort CP, Tuaillon N, Buggage RR, Egwuagu CE, Chan C-C. Detection of Toxoplasma gondii DNA in primary intraocular B-cell lymphoma. Mod Pathol. 2001;14 10:995–9.
159. Lu N, Liu C, Wang J, Ding Y, Ai Q. Toxoplasmosis complicating lung cancer: a case report. Int Med Case Rep J. 2015; 8:37–40.
160. Sayyahfar S, Karimi A, Gharib A, Fahimzad A. Association of systemic anaplastic large cell lymphoma and active toxoplasmosis in a child. Iran J Cancer Prev. 2015;8:4.
161. Cong W, Liu G-H, Meng Q-F, Dong W, Qin S-Y, Zhang F-K, et al. Toxoplasma gondii infection in cancer patients: prevalence, risk factors, genotypes and association with clinical diagnosis. Cancer Lett. 2015;359(2):307–13.
162. Motamedi M, Arab S, Moazzeni SM, Khamis Abadi M, Hadjati J. Improvement of a dendritic cell-based therapeutic cancer vaccine with components of Toxoplasma gondii. Clin Vaccine Immunol. 2009;16 10:1393–8.

Publisher's note