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REVIEW

Targeting pattern recognition receptors for cancer therapy: Mechanisms and strategies

Dengjie Ouyang[a,b,c,†], Ruixian Chen[a,b,†], Haitong Xie[a,b], Xiwen Yang[a,b], Qintong Li[d,*], Jie Chen[a,b,*]

[a]*Department of General Surgery, West China Hospital, Sichuan University, Chengdu 610041, China*
[b]*Breast Center, West China Hospital, Sichuan University, Chengdu 610041, China*
[c]*Institutes for Breast Health Medicine, West China Hospital, Sichuan University, Chengdu 610041, China*
[d]*Departments of Obstetrics & Gynecology and Pediatrics, West China Second University Hospital, Key Laboratory of Birth Defects and Related Diseases of Women and Children, Ministry of Education, Development and Related Diseases of Women and Children Key Laboratory of Sichuan Province, Sichuan University, Chengdu 610041, China*

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Abstract Pattern recognition receptors (PRRs) play a crucial role in immune responses, acting as primary sensors for microbial and host-derived signals. PRRs, which include Toll-like receptors (TLRs), retinoic acid-inducible gene 1-like receptors, nucleotide-binding oligomerization domain-like receptors, C-type lectin receptors, and various cytoplasmic DNA sensors, are essential for initiating immune responses that regulate both inflammation and tumor immunity. Recent studies have highlighted their dual roles in cancer, where they can either suppress or promote tumor progression by influencing the tumor microenvironment and modulating responses to immunotherapy. In the context of cancer, PRRs not only activate immune cells but also contribute to immune evasion mechanisms within tumors. Therapeutically, targeting PRRs represents a promising approach for cancer treatment, with related drugs showing potential to enhance the efficacy of existing immunotherapies. Numerous PRR-based agents, particularly TLR agonists, are currently under clinical investigation for their ability to augment antitumor immunity and overcome resistance to immune checkpoint inhibitors. This review examines the molecular mechanisms by which PRRs influence cancer, with a focus on recent advancements in PRR-targeted therapies and their integration with contemporary immunotherapeutic strategies.

*Corresponding authors.
E-mail addresses: liqintong@scu.edu.cn (Qintong Li), chenjiedoctor@wchscu.cn (Jie Chen).
†These authors made equal contributions to this work.
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1. Introduction

Innate immune disorders are implicated in at least one-third of cancers, driving the initiation and perpetuation of a chronic inflammatory state within the tumor microenvironment (TME). This inflammation underpins nearly every stage of cancer formation and contributes to chemotherapy resistance[1,2]. Indeed, "tumor inflammation" is now considered a hallmark of cancer[3]. Immunity serves as the host's first line of defense against invasive microorganisms, and its significance in tumorigenesis is underscored by the growing number of cancers, such as stomach, liver, colorectal, pancreatic, and cervical cancers, that are causally linked to microbiota, bacterial, or viral infections[4-6].

Innate immunity also influences cancer progression by modulating adaptive antitumor cellular immunity, such as activating natural killer (NK) cells and cytotoxic T cells through antigen-presenting dendritic tumor cells[7]. However, despite the clear association between innate immunity and cancer, the molecular structures of innate immunomodulators that drive cancer initiation and progression remain poorly defined. Moreover, no targeted innate immune therapeutics have been used in cancer treatment. By contrast, adaptive immunity-based therapies, particularly immune checkpoint inhibitors (ICIs) targeting programmed cell death 1 (PD-1), programmed death ligand 1 (PD-L1), and cytotoxic t-lymphocyte associated protein 4 (CTLA4), have been utilized in an increasing number of cancers, albeit with varying degrees of success[8].

The discovery of pattern recognition receptors (PRRs) as pivotal sensors for microbial and host tissue damage revolutionized our understanding of how innate immunity orchestrates inflammatory responses[9]. PRRs encompass several families, including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLRs), C-type lectin receptors (CLRs), and other cytoplasmic DNA sensors[10-16]. Their role as critical regulators of immune responses to microbial infections and tissue damage has been widely recognized[17]. Over the past decade, preclinical and clinical studies have rapidly expanded, implicating PRRs in nearly all solid and liquid cancers. These studies demonstrate that PRRs not only coordinate inflammatory responses in immune cells but also exert dual tumor-suppressive and tumor-promoting effects in non-immune cells, such as epithelial cells[9,13,18-20].

The activation of PRRs triggers intracellular signaling cascades that drive the expression of inflammatory mediators, facilitating numerous processes. These include the release and recruitment of chemokines, cytokines, and hormones; the development of an inflammatory microenvironment; prolonged inflammation; stimulation of innate immunity; transition to adaptive immune responses; preservation of host microecological homeostasis; and removal of mutated or damaged cells[21,22] (Fig. 1). Moreover, immune and non-immune functions of PRRs may occur in parallel or independently, depending on the type of cancer. Collectively, these findings have fueled global research into the molecular and cellular mechanisms through which PRRs contribute to cancer.

This study aims to consolidate the scientific principles underpinning the use of PRRs in cancer treatment and explore therapeutic strategies to enhance their clinical efficacy in combination with existing immunotherapy approaches. As previously discussed, PRRs are expressed across various cell types in both non-tumor and tumor tissues. Developing drugs targeting PRRs and their pathways may induce abnormal inflammatory responses in normal tissues. Despite this potential for side effects, which are addressed in this review, the substantial therapeutic benefits justify continued exploration of PRR-targeted drug development to optimize efficacy and minimize risks.

2. Targeting TLRs for cancer immunotherapy

2.1. TLRs

TLRs represent a critical class of proteins within the Type I integral membrane glycoprotein family[23]. The human TLR family includes 10 receptors, which are categorized into two groups: surface receptors anchored to the plasma membrane (TLR1, 2, 4, 5, 6, and 10) and intracellular receptors localized within endosomal compartments (TLR3, 7, 8, and 9)[24,25]. TLRs recognize diverse components based on their location and type. Surface TLRs primarily detect membrane-associated components. For example, TLR2 forms heterodimers with TLR1 or TLR6 to recognize lipoproteins or lipopeptides, while TLR2/6 complexes also identify peptidoglycans[26]. Other pathogen-associated molecular patterns (PAMPs) recognized by TLRs include lipopolysaccharide (LPS) *via* TLR4 and flagellin *via* TLR5. TLR10 can form homodimers with TLR1, TLR2, or TLR6, or heterodimers with TLR6[27,28]. However, the functions and recognition patterns of TLR10 remain incompletely understood. Recent studies have identified human immunodeficiency virus (HIV)-gp41 as a ligand for TLR10, leading to interleukin (IL)-8 induction and nuclear factor (NF)-κBα activation[29,30]. Conversely, intracellular TLRs are specialized to detect self-derived nucleic acids in disease states or nucleic acids from pathogens, including double-stranded RNA (dsRNA; TLR3), single-stranded RNA (ssRNA; TLR7 and TLR8), and CpG DNA (TLR9)[24,31].

Upon detecting endogenous molecules released from damaged or dying cells (damage-associated molecular patterns, DAMPs) and conserved structures of pathogens (PAMPs), TLRs are activated as the first line of defense against cancer, viruses, bacteria, parasites, and other danger signals[32,33]. Through this mechanism, TLRs play critical roles in recognizing invading threats and inducing innate immune responses. They also act as a bridge between innate and adaptive immune responses[34]. Upon TLR pathway activation, innate immune cells not only directly combat pathogens but also transmit pathogen information to the adaptive immune system. Antigen-presenting cells (APCs) present antigen fragments to T cells, activating both T and B cells in lymph nodes[35], which initiates targeted responses through antibody production and cytotoxic reactions against specific pathogens.

2.2. An overview of TLR signaling

Binding with specific ligands triggers the dimerization of TLRs, bringing their two Toll/IL-1 receptor (TIR) domains into

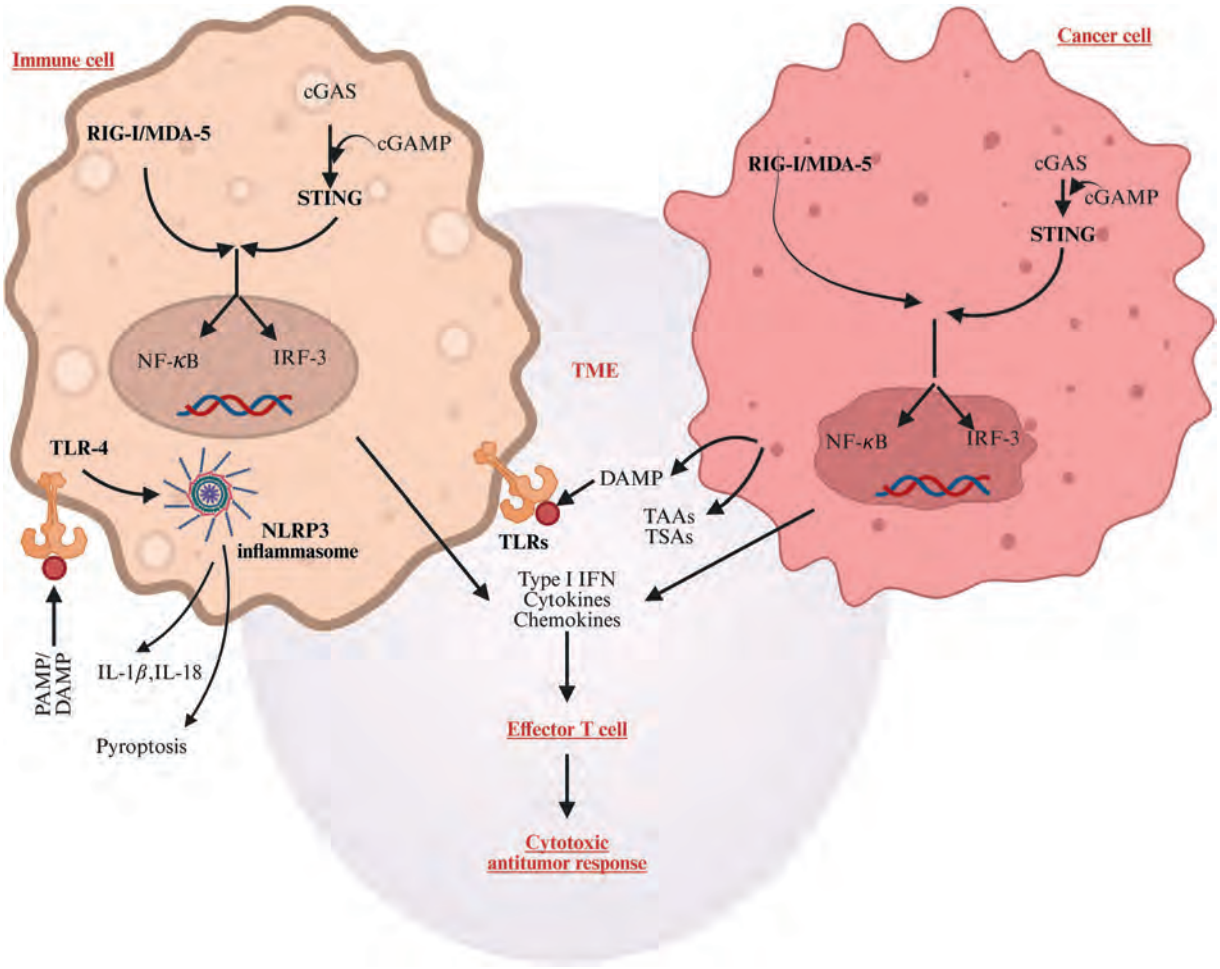

Figure 1 Interactions between PRRs and TME. A varied array of PRRs identifies DAMPs, PAMPs, or TAAs, triggering the activation of innate immune cells in the TME. Tumor cells can also manifest these sensors. Various PRRs, such as cGAS/STING, RIG-I, and TLRs, facilitate the transcription of proinflammatory genes through IRF-3 and NF-κB. Such mechanisms lead to the generation of Type I IFN, cytokines, and chemokines, bolstering a cytotoxic antitumor reaction facilitated by effector T cells and NK cells. Besides facilitating pyroptosis, the NLRP3 inflammasome induces a proinflammatory reaction *via* IL-1β and IL-18 pathways. PRRs, pattern recognition receptors; DAMPs, molecular patterns linked to damage; PAMPs, pathogen-related molecular patterns; TAAs, tumor-related antigens; TME, tumor microenvironment; cGAS/STING, cyclic GMP-AMP (cGAMP) synthase/stimulator of IFN gene; RIG-I, retinoic acid-inducible gene 1; TLRs, toll-like receptor; IRF-3, interferon regulatory factor 3; NF-κB, nuclear factor-κB; IFN, interferon.

proximity. Subsequently, various adaptor proteins, such as TIR domain-containing adapter protein (TIRAP), TIRAP-inducing IFN-β (TRIF)-related adaptor molecule (TRAM), myeloid differentiation primary response-88 (MYD88), and TRIF, are recruited to the active TLRs, initiating downstream signaling pathways[35,36]. Notably, the dimerized TIR domains interact with TIRAP and TRAM, facilitating the recruitment of MYD88 and TRIF, respectively[37]. These primary TLR signaling pathways ultimately induce pro-inflammatory effects, chemotaxis, and T-cell activation.

Most TLR signaling pathways are initiated by MYD88, a universal adaptor protein for all TLRs except TLR3. MYD88 contains an N-terminal death domain (DD) and a C-terminal TIR domain[38]. Upon ligand stimulation, MYD88 binds to the intracellular TIR domain of TLRs and recruits IL-1 receptor-associated kinases (IRAKs) through DD-DD interactions[39]. These kinases form a complex with IRAK family members, known as the Myddosome[40]. Activated IRAK1 associates with tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), catalyzing the synthesis of Lys63 (K63)-linked polyubiquitin chains. This initiates a cascade of events involving the mitogen-activated protein kinase (MAPK) and pro-inflammatory NF-κB signaling pathways[24,41].

The polyubiquitination of TRAF6 and IRAK1 is critical for activating downstream IκB kinase (IKK) or TGF-β-activated kinase 1 (TAK1)[42,43]. The RING domain of TRAF6 binds with the Ubc13/Uev1A complex of the E2 enzyme, mediating Lys63-linked auto-ubiquitination of TRAF6 and subsequent activation of IKK[44]. The activated IKK complex phosphorylates the NF-κB inhibitor IκBα, leading to its proteasomal degradation. This permits NF-κB to translocate into the nucleus, promoting the expression of pro-inflammatory cytokines[45]. Furthermore, IKK activation stimulates downstream interferon (IFN) regulatory factors (IRFs), such as IRF5 and IRF7, which are closely linked to type I/II IFN production[46].

In parallel, TAK1 activation occurs through TAK1-binding proteins (TAB2 and TAB3), which phosphorylate MAPK kinases 4/7 (MKK4/7) and MKK3/6. This activation engages MAPK family members, including c-Jun N-terminal kinases (JNKs) and

P38, collectively known as the JNK-p38 pathway[47,48]. MAPK pathway stimulation drives activator protein-1 (AP-1) transcriptional responses, regulating the production of immune factors and chemokines in conjunction with NF-κB[49,50] (Fig. 2).

Interactions between TLRs and TRIF require TRAM, a peripheral membrane protein with an N-terminal myristoylation motif that monitors dimerized TLRs to form the complex known as the Triffosome[51]. Upon TIR dimerization, TRAF-associated NF-κB activator (TANK-binding kinase 1 (TBK1)), which regulates pathways related to innate immunity, tumorigenesis, energy homeostasis, and autophagy, is activated by TRIF−TRAM complexes in a TRAF3-dependent manner[52,53]. TRAF3 acts as a crucial link between TLR adaptors and downstream regulatory kinases essential for IRF activation[54]. TBK1 activation significantly enhances the expression of IFNs and IFN-stimulated genes (ISGs)[55]. Additionally, similar glycolytic responses are observed when TBK1 is activated within Myddosomes[56].

Two key regulators of IFN gene expression, IRF3 and IRF7, are phosphorylated upon TBK1 activation. When phosphorylation occurs, a specific pLxIS motif in TRIF recruits IRF3[57], regulating IFN signaling. TBK1 activation also triggers IRF7 responses, leading to subsequent IFN production[58,59] (Fig. 2).

Activated NF-κB and AP-1 translocate to the nucleus, promoting the transcription of pro-inflammatory genes. Pro-inflammatory cytokines are transported *via* the bloodstream to target sites, recruiting immune cells. On one hand, TLR activation induces immune cells, such as dendritic cells (DCs) and macrophages, to release cytokines like IL-12 and TNF-α, activating NK cells and $CD8^+$ T cells[60]. Certain TLRs can also induce tumor cell apoptosis under specific conditions. For example, TLR3 activation triggers endogenous IFN signaling, resulting in tumor cell death[61].

Additionally, TLR activation drives DC maturation and enhances tumor antigen (TA) presentation to T cells through major histocompatibility complex (MHC) class II (MHC-II) and co-stimulatory molecules, eliciting a specific immune response and blocking regulatory T-cell (Treg) suppression in an IL-6-dependent manner[62]. On the other hand, chronic TLR activation in the TME can result in sustained inflammation, releasing factors such as IL-6, IL-10, and TNF-α, which promote tumor cell proliferation, angiogenesis, and invasion[63,64].

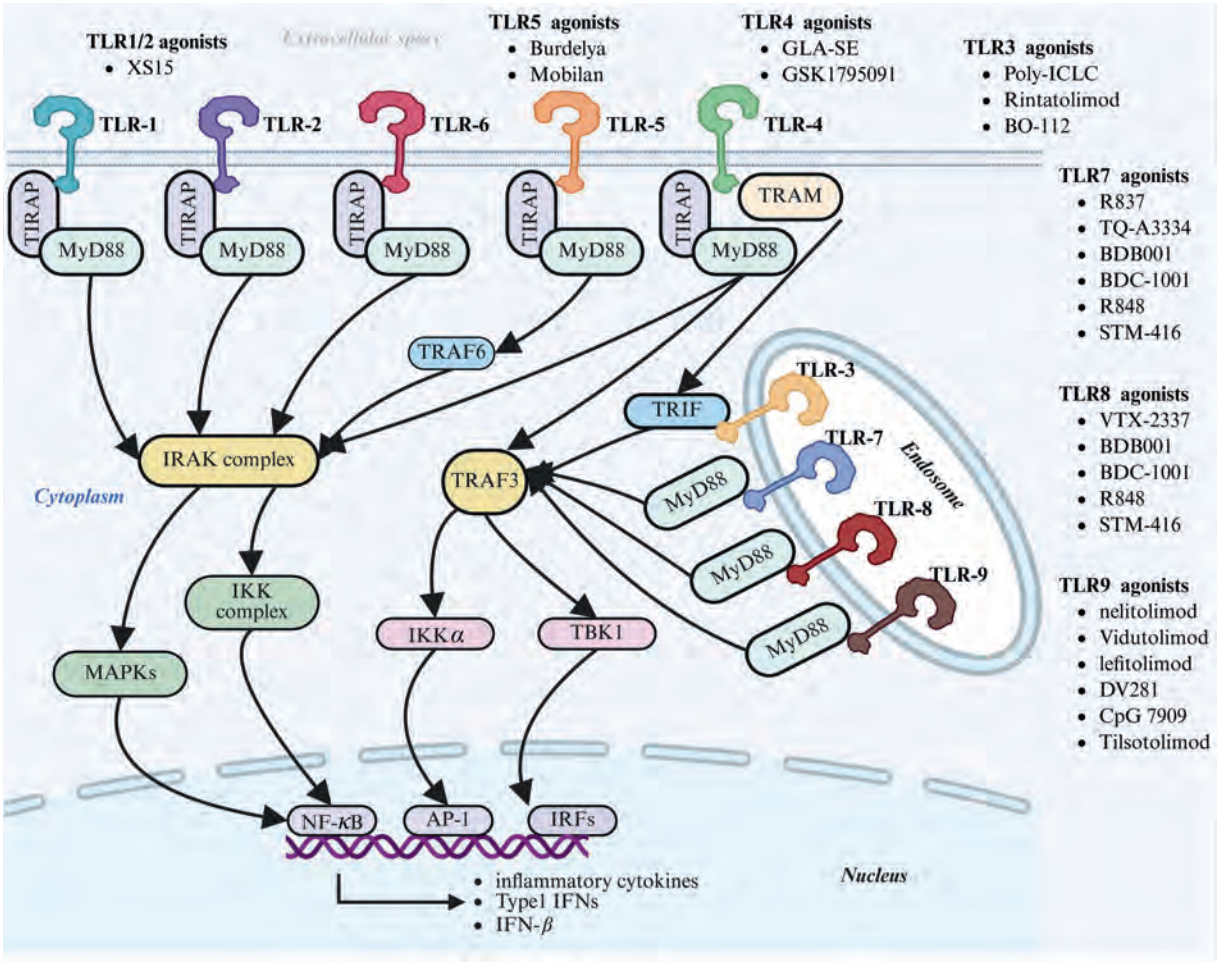

Figure 2 TLR signaling pathway. TLRs recognize PAMPs and recruit MYD88 and TRAF6, leading to the polyubiquitination of IRAK1 and activation of the IKK complex. This results in the degradation of IκBα, allowing NF-κB to translocate into the nucleus. Concurrently, the MAPK pathway is activated, promoting the release of cytokines and the induction of inflammatory responses. TLR, toll-like receptor; PAMP, pathogen-associated molecular pattern; TIRAP, Toll/IL-1 receptor domain-containing adapter protein; MYD88, myeloid differentiation primary response-88; TRAF, tumor necrosis factor receptor-associated factor; TRIF, TIRAP-inducing interferon-β; TRAM, TRIF-related adaptor molecule; IRAK, interleukin-1 receptor-associated kinase; IκB, inhibitor of NF-κB; IKK, IκB kinase; MAPK, mitogen-activated protein kinase; TBK1, TANK-binding kinase 1; NF-κB, nuclear factor-κB; AP-1, activator protein-1; IRF, interferon regulatory factor.

Certain TLR activations, such as TLR4, may induce macrophage polarization toward the M2 phenotype[65]. M2 macrophages secrete IL-10, which suppresses antitumor immune responses, thereby fostering tumor growth. Furthermore, TLR and IRAK4 activation enhance tumor cell resistance to chemotherapy, likely by promoting survival pathway proteins through TLR signaling[66,67]. The anti-apoptotic effects of NF-κB activation also support tumor cell survival and mediate resistance to cytotoxic T lymphocyte (CTL)-induced cell death[68,69].

2.3. *TLR expression in different cell types of the TME*

2.3.1. *TLR expression and function in immune cells*

TLR-mediated activation and maturation are critical processes for APCs, including DCs, B cells, and macrophages[70,71]. DCs act as immune sentinels, detecting cell stress, tumors, or pathogens[72]. Upon antigen recognition, DCs present peptides to T cells and produce cytokines (*e.g.*, Type I IFN, TNF-α, IL-1, IL-6, IL-12), co-stimulatory signals (*e.g.*, CD40, CD80, CD86), and chemokines (*e.g.*, CCR2, CCR5, CCR7) to regulate T cell activation and migration, shaping diverse immune responses[70,73,74]. DCs recognize "danger signals" from invaders and damaged host cells by combining PAMPs or DAMPs with TLRs[75], triggering rapid inflammatory responses to pathogens or presenting tumor-associated antigens to T cells to mediate cancer immunity[76].

Various stimuli trigger different DC subsets to activate distinct signaling pathways, releasing specific cytokines that subsequently determine and activate particular T-helper (Th) cell subsets[45,77]. Plasmacytoid DCs (pDCs) express high levels of TLR7 and TLR9[78], while CD11c$^+$ myeloid DCs selectively express TLR1, TLR2, and TLR3[79]. Monocytes preferentially express TLR1, TLR2, TLR4, TLR5, and TLR8, but these receptors are gradually lost during differentiation into mature DCs, with TLR3 expression gained[80]. Stimulation of TLR1/2 on DCs induces the secretion of inflammatory cytokines, such as IL-1β, into the perivascular microenvironment[81,82]. TLR2 also controls mDC infiltration into tumors and clonal expansion of antitumor T cells[83]. TLR3 and TLR9 enhance IL-12β expression in DCs, supporting Th1 differentiation[84,85], while multiple TLRs synergistically promote IL-12 production, amplifying immune cell effects[86].

TLR4-stimulated DCs can cross-present antigens *via* DC-SIGN, triggering signaling that increases CD8$^+$ T cell activation[87]. The chemokine-dependent migration of DCs to medullary thymic epithelial cells (mTECs) is pivotal for antigen presentation by DCs[88,89]. TLR9−MYD88 signaling in mTECs plays a key role in CD14$^+$ monocyte-derived DC(moDC) recruitment and Treg generation[90]. Recent studies reveal that TLR9 activation triggers amplification of DC3, a state of tumor-associated DCs, and reduces PD-L1 expression, enhancing antitumor DC function and promoting tumor rejection[91,92]. Cytosine-guanine (CpG) binding to TLR9 activates human and mouse immune cells, inducing DC maturation, strong Th1-type immunity, pathogen clearance, and tumor cytotoxicity[93]. Additionally, TLR7/8 agonists enhance oxidative phosphorylation and glutamine metabolism in pDCs, increasing IFN-α production and boosting T cell responses[94]. However, the TME can impair pDCs through the cyclic GMP−AMP (cGAMP) synthase (cGAS)−TLR7/9−STING signaling pathways, reducing IFN-α and C−X−C motif chemokine ligand 10 (CXCL10) production[95].

Macrophages, classified into M1 and M2 types, are defined by their responses to IFNγ and TLR activation (M1) or IL-4/IL-13 (M2)[96]. The polarization of macrophages into M1 or M2 phenotypes is crucial for inflammation and host defense[97]. Macrophages contribute to nearly every aspect of organismal biology, including development, homeostasis, repair, and immune responses to pathogens. Macrophages undergo autophagy in response to various immune and inflammatory signals, often mediated by TLRs[98], regulating cytokine production and transcription (*e.g.*, IL-1β)[99,100]. This process also regulates MHC-I and -II molecule processing and transport to the cell surface, facilitating T cell responses[101].

M1-like macrophages with antitumor activity typically exhibit high HLA-DR (MHC-II) expression. *In vivo*, CpG activates TLR9 and anti-IL-10 receptor antibodies, redirecting tumor-associated macrophages (TAMs) from M2 to M1 phenotypes. This reprogramming modifies the TME, reducing tumor sizes[102-104]. Conversely, cathepsin K (CTSK), a metastasis-related secretory protein, binds TLR4 through a mechanistic target of rapamycin kinase (mTOR)-dependent pathway, stimulating M2 TAM polarization[105]. CTSK-stimulated M2 TAMs secrete cytokines like epidermal growth factor receptor (EGFR), IL-10, and IL-17, promoting tumor cell invasion and metastasis *via* the NF-κB pathway[106,107]. Tumor cells also induce MIR182 expression in macrophages through TGFβ signaling, directly inhibiting TLR4, suppressing NF-κB activation, and further polarizing TAMs to the M2 phenotype[108].

B cells, a critical component of humoral immunity, are classified into diverse subtypes based on several phenotypic surface markers[109]. These cells exhibit diverse functions, participating in immune surveillance or suppression depending on their differentiation stages, environmental cues, or spatial context[110,111]. The interplay between TLR and B cell receptor signaling pathways coordinates innate and adaptive immune responses in healthy B cells, boosting NF-κB activation[112]. Chemotherapy-induced DNA fragments activate the stimulator of IFN genes (STING) pathway, increasing the expression of MHC-I molecules in cancer cells *via* an IFN-dependent mechanism[113]. Concurrently, innate-like B cells are triggered *via* the TLR9 signaling pathway, promoting follicular helper and Th1 T cells through the ICOSL−ICOS axis, which enhances cytotoxic T cell activity in tertiary lymphoid organ-like structures[113]. Additionally, TLR7-deficient B cells display impaired antigen-presenting function, leading to downregulated expression of both non-classical and classical MHC-I molecules. This subsequently reduces the activation of cytotoxic CD8$^+$ T cells[114].

Mature APCs stimulate and regulate T cell functions directly or indirectly[115]. Human CD4$^+$ and CD8$^+$ T cells isolated from peripheral blood and spleen express abundant mRNA levels of TLR1, TLR2, TLR3, TLR4, TLR5, TLR7, and TLR9[78,116]. T cell regulation by TLRs varies depending on the subtype and specific TLR involved[117]. CD4$^+$ T cells primarily recognize antigens presented by APCs, generating responses that regulate the activity of other immune cells, while CD8$^+$ T cells play a crucial role in antitumor immunity by recognizing and eliminating cancerous cells[118,119]. CD4$^+$ T cells also facilitate CD8$^+$ T cell proliferation, differentiation, and memory formation[120]. Activation of TLR2 on CD4$^+$ T cells enhances T-cell receptor (TCR)-triggered Th1 responses, promoting the secretion of various chemokines and cytokines[121]. Similarly, TLR2/MYD88-dependent signaling in CD8$^+$ T cells improves survival and clonal expansion. TLR2 activation allows CD8$^+$ T cells to generate memory cells efficiently, even with weak TCR signals, reducing their dependence on APC co-stimulatory signals[45,122]. During inflammation or immune damage, the number of TLR4$^+$CD4$^+$ T cells increases in

peripheral blood mononuclear cells (PBMCs), highlighting a role in allergy and immune regulation[123]. TLR4-deficient $CD4^+$ naïve T cells exhibit impaired differentiation into Th17 cells, with the TLR4−RELA−MIR30A pathway regulating Th17 cell differentiation by directly binding to regulatory elements of the MIR30a gene[124]. Additionally, activating TLR3, TLR7, and TLR9 on $CD4^+$ and $CD8^+$ T cells promotes cytokine release (*e.g.*, IL-2, IL-8, IFN-γ) and enhances their effector functions[78,125-127].

Tregs, a highly immunosuppressive subset of T lymphocytes, are essential for maintaining immune homeostasis and facilitating tumor immune escape[128]. Various Treg subtypes expressing diverse antigens are present in tumor sites[129-132]. TLR8 is highly expressed on Tregs, and its activation by a ligand reverses their immunosuppressive function[133]. Activation of TLR8 signaling in $CD4^+$ Tregs alters glucose metabolism, reversing their suppressive functions and increasing naïve $CD4^+$ T cell proliferation[134]. TLR9 agonists can directly affect $CD4^+$ T cell subsets, specifically inhibiting immune suppression by $CD4^+CD25^+$ Tregs[135]. However, TLR4 binding to ligands such as lipopolysaccharide (LPS) induces Treg proliferation, enhancing their suppressive function[136].

These interconnected roles of TLRs in DCs, macrophages, B cells, T cells, and Tregs highlight their central role in orchestrating immune responses with significant implications for tumor immunity.

2.3.2. TLR expression and function in tumor cells

TLRs act as a double-edged sword in tumor regulation and are expressed not only in immune cells but also in various tumor cells[137]. Mutations in TLRs influence the composition and structure of immune and tumor cells within the TME, significantly impacting tumor occurrence, proliferation, and prognosis[138]. Specific TLR activation can promote tumor proliferation or induce chronic inflammation, rendering the microenvironment more immunosuppressive[139]. Alterations in the microbiota and TLR activation have been observed in various tumors, suggesting that these receptors may detect microbial disturbances, disrupt immune tolerance, and influence tumor growth[140].

Glioma, the most common primary malignant tumor of the central nervous system (CNS), exhibits elevated expression of TLR1, TLR2, TLR4, TLR5, TLR6, and TLR9 in brain tumor cell lines and tissues, including gliomas and astrocytomas (AST). The mRNA and protein levels of TLR2 and TLR9 correlate positively with glioma WHO histological grade and poor clinical outcomes. Similarly, TLR4 expression increases with advancing tumor stages, mediating pathways that regulate tumor cell proliferation, migration, immune evasion, and resistance to TNF-α therapy[141]. TLR9 expression is also elevated in glioma stem cells (GSCs), supporting their proliferation and maintenance. Activation of TLR9 in GSCs mediates downstream signaling, activating the signal transducer and activator of transcription 3 (STAT3) pathway to promote GSCs growth[142].

Cells within the TME of brain tumors, such as microglia, express TLR2. The TLR2−MHC-I axis aids in $CD8^+$ T cell proliferation and activation, enabling rapid immune responses and regulating inflammation-related activities, including phagocytosis[143]. In contrast, TLR4 expression is lower in glioblastoma (GB) compared to AST. Chemotherapy-resistant GB cells and TAMs exhibit impaired TLR4 expression and function[144].

Medulloblastoma, an embryonal brain tumor primarily affecting children, exhibits differential expression of TLR7, TLR8, and TLR9 across its subtypes. TLR7 is a prognostic factor for pediatric medulloblastoma, with higher expression linked to improved overall survival (OS)[145].

Several TLRs play essential roles in the development and progression of head and neck cancers. High expression of TLR1-5 and TLR9 in immune cells correlates with poor clinicopathological indicators and prognosis in patients with head and neck squamous cell carcinoma (HNSCC)[146]. TLR2 demonstrates a direct oncogenic role in head and neck cancers. In hypopharyngeal squamous cell carcinoma, downregulation of the m6A demethylase AlkB homolog 5 (ALKBH5) increases m^6A modification of TLR2, promoting tumorigenesis and progression[147]. In oral squamous cell carcinoma (OSCC), TLR2 activation reduces the mRNA levels of caspase recruitment domain-containing protein 10 (CARD10). Knockdown of CARD10 may confer resistance to cisplatin-induced apoptosis *via* the MIR146A pathway[148].

TLR3 is overexpressed in head and neck cancers, shielding cells from cisplatin-induced DNA damage and contributing to cisplatin resistance through upregulation of cytokines and chemokines, including IFNβ and CCL5[149]. TLR4 and MYD88 expression are significantly elevated in OSCC, where the TLR4−MYD88−NF-κB signaling pathway induces epithelial−mesenchymal transition (EMT), facilitating metastasis by enabling cancer cells to detach, invade lymphatic and blood vessels, and lose epithelial adhesion[150]. TLR7 activation in OSCC is associated with poor prognosis and cisplatin resistance[151,152]. Additionally, TLR9 facilitates OSCC invasion, proliferation, migration, and immune evasion through the PARP1/PD-L1 pathways[153].

Esophageal cancer (EC) is a lethal malignancy with a poor prognosis, with over 600,000 new cases reported globally in 2020[154]. Various TLRs, including TLR1-4, TLR6, TLR7, and TLR9, are overexpressed in EC[155,156]. Notably, high TLR3 protein and mRNA expression correlate with a better prognosis, particularly in advanced esophageal squamous cell carcinoma (ESCC)[157]. By contrast, TLR4 expression and activation *via* the MYD88-dependent signaling pathway promote tumor proliferation in esophageal tumors[158]. TLR4 expression in ESCC is linked to lymph node metastasis, and LPS-induced TLR4 activation increases NF-κB activity and expression of IL-8 and COX-2, promoting proliferation[159].

High TLR6 expression in advanced thoracic ESCC following curative esophagectomy is associated with improved 5-year OS and disease-specific survival rates[160]. Conversely, high TLR9 expression is linked to poor differentiation, high proliferation rates, and widespread metastasis in esophageal adenocarcinoma[161]. Researchers have also discovered that TLR-related lncRNAs (AP000696.1, LINC00689, LINC00900, and AP000487.1) are significantly associated with OS in patients with EC. These lncRNA markers may stratify patients with EC and represent promising prognostic biomarkers[162].

Lung cancer remains the leading cause of cancer-related deaths worldwide[163]. The role of TLRs in the lung TME offers novel insights for targeted therapies. TLR2 plays a crucial role in the initial stages of lung tumorigenesis by activating intrinsic cell cycle arrest mechanisms and the pro-inflammatory senescence-associated secretory phenotype, hindering the progression of early lung cancer and enhancing patient survival rates[164]. TLR3 expression in early-stage non-small cell lung cancer (NSCLC) is associated with a favorable prognosis, and its activation induces tumor cell apoptosis through caspase-3 activity[165].

By contrast, TLR4 activation, which regulates NSCLC development *via* the TLR4/ERK/PD-L1 pathway, correlates with poorer

OS and disease-free survival (DFS)[166]. TLR4 stimulation triggers autophagy through the TRAF6−BECN1 signaling axis by ubiquitinating BECN1, increasing migration and invasion of lung cancer cells[167]. Postoperative infectious complications elevate the risk of lung tumor metastasis, as gram-negative *Escherichia coli* pneumonia promotes NSCLC liver metastasis through TLR4 activation[168].

TLR7/8 are moderately to highly expressed in human NSCLC, and their activation triggers inflammatory mediator release and transcriptional modulation of genes associated with cell survival, angiogenesis, and immune evasion, contributing to chemoresistance[169]. Furthermore, cancer stem cells (CSCs) are critical in lung tumor recurrence and chemoresistance. Lysosomal mtDNA serves as a ligand for TLR9, and the TLR9-dependent NOTCH1−AMPK pathway drives lung CSC proliferation[170].

Breast cancer (BC) is the most common cancer among women, with its incidence rising steadily each year[154]. TLRs play a crucial role in the composition and pathogenesis of the TME in BC, influencing its initiation, progression, and various other aspects. BC CSCs are resistant to chemotherapy and contribute to tumor recurrence. TLR2 is overexpressed in breast CSCs, promoting their self-renewal[171]. TLR2 stimulation activates the MYD88/NF-κB and AKT pathways, promoting the secretion of cytokines and growth factors like TGF-β and IL-6. These factors autocrinally activate the STAT3 and SMAD3 pathways, enhancing CSC survival, self-renewal, and invasion[172,173]. Additionally, TLR2 has been confirmed to be linked to chemoresistance in BC. The absence or pharmacological inhibition of TLR2 increases the sensitivity of BC cell lines to doxorubicin. High TLR2 expression in patients with BC undergoing chemotherapy is associated with a lower recurrence-free survival (RFS) tendency[174].

Moreover, TLR3 functions as both an inducible and suppressor gene in BC, inhibiting the proliferation of human BC cells at various stages of the disease through downregulation of the EGFR/phosphoinositide 3-kinase (PI3K)/AKT pathway[175]. Researchers suggest that higher expression levels of TLR4 and TLR7 are indicative of a poorer prognosis[176]. TLR4 is responsible for the production of pro-inflammatory cytokines by BC cells, contributing to the formation of a tumor-promoting inflammatory microenvironment. Paclitaxel, acting as a ligand, activates TLR4, thereby enhancing BC metastasis[177]. Reduced TLR5 expression in triple-negative BC (TNBC) enhances vascular endothelial growth factor (VEGF) receptor expression and angiogenesis, leading to increased TNBC cell proliferation[178]. TLR5-4T1 cells exhibit greater lung metastatic potential compared to TLR5$^+$ 4T1 cells. Downregulation of TLR5 increases TNBC invasiveness and EMT *via* the SOX2 pathways, suggesting TLR5 as a potential biomarker for monitoring TLR5-positive TNBC[179]. In ER-negative BC cell lines, TLR9 expression has been reported to promote migration, invasion, and other aggressive tumor behaviors[180].

Liver cancer ranks as the third leading cause of cancer deaths worldwide. Hepatocellular carcinoma (HCC) accounts for 80% of liver cancer cases and has a poor prognosis, with a median survival of only 6 to 10 months[181]. TLRs and their downstream signaling pathways play significant roles in the pathogenesis and treatment of HCC. HBV-induced S100A9 activates the RAGE/TLR4−ROS signaling pathway, leading to the formation of numerous neutrophil extracellular traps (NETs), which in turn promote the growth and metastasis of liver cancer cells[182]. TLR4 activity can also modulate the expression of the DNA repair protein KU70, thereby helping to prevent HCC development and progression[183]. In HCC tissues, *TLR3* mRNA and protein levels are notably reduced compared to adjacent non-cancerous tissues. This decrease in TLR3 expression allows transforming hepatocytes to evade TLR3-triggered apoptosis, thereby facilitating HCC progression and resulting in a poorer prognosis[184]. Research has identified TLR3 inhibition as an independent risk factor affecting both OS and RFS in patients with HCC[185]. Additionally, high TLR5 levels in liver cancer cells are associated with poorer 5-year OS and disease-specific survival rates[186]. Furthermore, TLR7 and TLR9 expression are upregulated in human HCC tissues, and their activation significantly enhances the *in vitro* proliferation of liver cancer cells[187].

Since patients with early-stage gastric cancer (GC) often exhibit no symptoms, treatment of GC remains challenging. Multiple TLRs are expressed in human GC cells. TLR2 expression is associated with enhanced cancer cell growth and promotes the proliferation of human GC cells through the regulation of six anti-apoptotic genes (BCL2A1, BCL2, BIRC3, CFLAR, IER3, TNFAIP3) and two tumor suppressor genes (PDCD4, TP53INP1)[188]. The NET/TLR2/COX2 axis contributes to GC progression and distant metastasis[189]. Another study identified TLR3 as a prognostic marker for GC, with elevated expression indicating a poorer prognosis[190]. TLR4 is overexpressed in GC cells and contributes to their invasiveness. The interaction between LPS and TLR4 promotes cell proliferation without affecting apoptosis[191]. Notably, the spread of GC to distant sites is a major factor contributing to poor prognosis. Researchers have shown that in a metastatic microenvironment model, TLR4 signaling cascades enhance GC adhesion and peritoneal dissemination[192]. Activation of TLR5 by flagellin induces IL-8 production in GC cells, enhancing tumor cell proliferation and playing a crucial role in GC progression[193]. TLR9 has been reported to be upregulated in patients with GC with *Helicobacter pylori* infection and in those with gastritis. *In vitro* models show that TLR9 knockout inhibits tumor development by reducing gastric mucosal inflammation and cell proliferation, suggesting that TLR9 is involved in the onset and progression of GC[194].

Colorectal cancer (CRC) ranks as the second leading cause of cancer-related deaths[195]. Currently, various immunotherapy strategies, including new targets and novel combination therapies, are being tested for their effectiveness in CRC[196]. The expression of TLR2, TLR3, TLR4, TLR5, and TLR9 has been confirmed in CRC[140,197]. Evidence shows that TLR2 exhibits varying levels of activation or expression under different clinicopathological conditions[198]. Strong TLR2 activity is associated with a better CRC prognosis[199]; however, it can also promote CRC tumorigenesis by enhancing the secretion of cytokines such as IL-6 and IL-17A[198]. TLR3-induced secretion of cytokines and chemokines like CCL2, CCL5, and IL-8 may inhibit CRC lymph node metastasis by modulating stromal cells, including macrophages, T cells, and neutrophils[200]. TLR4-positive CRC cells exhibit greater chemotherapy resistance and enhanced cancer stemness compared to TLR4-negative cells[201]. The thrombospondin 2/TLR4 axis elevates HIF-1α protein levels. Blocking HIF-1α with small interfering RNA (siRNA) can reverse the enhanced glycolytic activity and the upregulation of glycolytic enzymes in CRC cells, thereby promoting CRC progression[202]. Additionally, MIR155 can enhance the TLR4 signaling pathway by targeting and downregulating the negative regulators suppressor of cytokine signaling 1 and Src homology 2 domain-containing inositol-5′-phosphatase 1, thereby promoting proliferation in CRC[203]. Moreover, a study validated that high TLR5 expression in CRC tissues is associated with a better prognosis, while TLR7 showed no prognostic

value[204]. TLR6 is aberrantly downregulated in CRC, leading to an increased proportion of cells in the S and G2/M phases, reduced apoptosis, and inhibited CRC development and progression[205].

Ovarian cancer (OC) is a common and highly heterogeneous tumor affecting women[206]. Due to its high invasiveness and tendency to metastasize, OC has a low survival rate, highlighting the need for new treatment strategies[207]. Elevated levels of IL-4, IL-10, TGF-β, and VEGF are observed in OC ascites and other clinical samples. These factors suppress the activity of macrophages and DCs, reducing their phagocytic capabilities[208,209]. TLR2-4, TLR5, and TLR9 are moderately to highly expressed in neoplastic ovarian epithelial cells[180,210]. Patients with TLR4/MYD88-positive OCs tend to relapse more quickly[211]. TLR4 is closely associated with paclitaxel chemoresistance, and blocking TLR4 can increase chemotherapy sensitivity[212]. One theory suggests that OC chemoresistance is due to TLR4 overexpression, which leads to upregulation of androgen receptors and transactivation of paclitaxel-resistant genes[213]. Microbiota and inflammatory factors may also contribute to cancer progression. Recent research suggests that activation of the TLR4/NF-κB pathway promotes OC progression through Hedgehog (Hh) signaling, which can be influenced by gut microbiota (GM) in the human body[214]. TLR9 activation in OC cells is associated with signal transduction that leads to disease invasiveness and poor clinical outcomes[180,215]. Researchers have also found elevated levels of TLR9 ligands (hypomethylated DNA) in the serum of patients with distant OC metastases, suggesting that this signaling cascade is linked to the invasive activity of OC[215].

2.3.3. *TLR expression and function in epithelial cells*

TLRs are widely expressed in epithelial and endothelial cells of normal non-tumor tissues, including the respiratory tract[216], gastrointestinal tract[216,217], skin[218], and others. The gastrointestinal tract is a crucial immune organ in the body, and the expression, regulation, and function of TLRs in the intestinal epithelial lineage are essential for maintaining immune homeostasis. TLR1−TLR9 are expressed in various cell types within the gut, including intestinal epithelial cells (IECs), as well as other stromal and parenchymal non-immune cells[219,220]. The expression of TLRs is strongly influenced by bacteria. Compared to germ-free mice, the expression of TLR1, TLR2, TLR5, and TLR9 is significantly upregulated in mice infected with specific pathogens[221]. Additionally, TLR1, TLR2, TLR4, and TLR5 exhibit the highest expression levels in the colon, which correlates with increased bacterial load in the distal gastrointestinal tract[221,222]. It has also been observed that TLR4 is expressed in both small intestinal and colonic IECs, as well as in colonic organoids, whereas no functional response to TLR4 ligands was detected in the organoids, suggesting the presence of other mechanisms that specifically suppress TLR4 signaling[219]. TLRs are expressed in most IEC lineages and, by recognizing microorganisms, initiate a cascade of responses that regulate IEC proliferation, enhance epithelial barrier function[223], shape immune responses[224], and help maintain gut homeostasis while limiting the cascade of inflammatory signaling[225,226].

Mucus production is closely related to the presence of bacteria and the synthesis of mucin 2 (MUC2). Activation of TLR1, TLR2, TLR4, and TLR5 promotes MUC2 secretion and inhibits bacterial colonization, while animals lacking these TLRs exhibit intestinal inflammation and microbial dysbiosis[227]. Additionally, TLRs enhance the synthesis of antimicrobial peptides, further strengthening the intestinal immune defense and protecting the epithelium from microbial invasion[228]. Epithelial TLRs regulate immune responses through multiple mechanisms, including attracting immune cells to migrate into the lamina propria[219], upregulating pIgR to facilitate transcytosis of IgA from the lamina propria into the intestinal lumen, and triggering IgA secretion by B cells in a T cell-independent manner[224]. After ligand recognition and the production of inflammatory mediators, IECs can further limit their response by upregulating the expression of molecules that inhibit TLR downstream signaling pathways[229,230], including TOLLIP, PPARγ, TNFAIP3, and others.

2.4. *TLRs for cancer immunotherapy*

TLRs are upregulated in various cancer types and play distinct roles in different cancer subtypes, highlighting their potential as key regulators in cancer immunity. They induce the release of pro-inflammatory cytokines and anti-apoptotic factors, recruit immune cells, and promote cell proliferation within the TME[176,197,231]. The activation of TLR signaling balances pro-tumor and antitumor activities through interactions between immune cells and tumor cells. TLR agonists have become major research focuses due to their ease of clinical application in cancer treatment. Current research is heavily focused on developing TLR-related drugs, particularly agonists, due to their immunostimulatory properties and promising results when combined with other anticancer therapies[232]. However, for chronic inflammation-driven tumors, TLR antagonists may effectively suppress tumor growth by inhibiting TLR signaling and reducing inflammatory factors in the TME. Here, we elaborate on drugs targeting TLRs based on different receptors (Table 1).

2.4.1. *TLR agonist*

2.4.1.1. TLR1/2. Rammensee et al.[233] developed a novel water-soluble synthetic Pam3Cys derivative named XS15, which, with a single injection, can bind to TLR1/2 and induce robust *in vitro* proliferation of functional $CD8^+$ and Th1 $CD4^+$ cells. Currently, a Phase I clinical trial is evaluating the safety and efficacy of its use as an immunomodulator in addition to standard postoperative radiotherapy and temozolomide chemotherapy in patients with newly diagnosed HLA-A2-positive, MGMT-methylated GB (NCT04842513). Another Phase I study aims to evaluate the immunogenicity, safety, toxicity, and preliminary efficacy of a peptide vaccine incorporating XS15 in patients with acute myeloid leukemia (AML) who have achieved complete remission following first-line treatment (NCT06252584).

2.4.1.2. TLR3. TLR3 agonists demonstrate antitumor potential by activating the adaptive immune system, promoting cytokine release by immune cells, and activating T cells to enhance anticancer efficacy[234]. Poly-ICLC, a dsRNA composed of synthetic polyinosinic-polycytidylic acid (pIC) (poly I:C), functions by mimicking viral genetic material and acts as an effective TLR3 ligand[235]. Poly-ICLC binding to TLR3 induces the activation of APCs, such as DCs, resulting in the secretion and expression of IL-12 and co-stimulatory molecules like CD80/CD86, which initiate subsequent T cell responses. It also induces systemic IL-15 production *via* the melanoma differentiation-associated gene 5 (MDA5)/IFN-I pathway, making it a potential vaccine adjuvant for amplifying activated $CD8^+$ T cells[236].

Poly-ICLC has been evaluated in multiple clinical trials to validate its safety and efficacy in tumors, especially in combination with other conventional treatments or immunotherapies. For

Table 1 The drugs targeting TLRs based on different receptors.

Drug	Target	Phase	Disease	Treatment	NCT number	Status
XS15	TLR1/2	I	Acute myeloid leukemia	Combination with multi-peptide vaccination	NCT06252584	Not yet recruiting
		I	Chronic lymphocytic leukemia	Combination with multi-peptide vaccination	NCT04688385	Active, not recruiting
		I	Glioblastoma multiforme of the brain	Combination with multi-peptide vaccination	NCT04842513	Recruiting
Poly-ICLC	TLR3	I	Malignant pleural mesothelioma	Monotherapy	NCT04525859	Recruiting
		I	Hepatocellular carcinoma	Combination with nivolumab	NCT05281926	Recruiting
		I	Metastatic solid tumors	Combination with CDX-301, radiation therapy, CDX-1140	NCT04616248	Recruiting
		I	Advanced melanoma	Combination with ultrasound ablation (FUSA)	NCT06472661	Not yet recruiting
		I	Pancreatic cancer	Combination with peptide vaccine, durvalumab, and tremelimumab	NCT06564623	Not yet recruiting
		I	Advanced solid tumors	Combination with FUSA (+/− PD-1 blockade)	NCT04116320	Active, not recruiting
		I	Prostate cancer	Monotherapy	NCT03262103	Completed
		I	Melanoma	Combination with an experimental vaccine (MELITAC 12.1), lipopolysaccharide, and montanide ISA-51	NCT01585350	Completed
		I/II	Metastatic colon cancer	Combination with pembrolizumab	NCT02834052	Completed
		I/II	Non-hodgkin's lymphoma, metastatic breast cancer, and head and neck squamous cell carcinoma	Combination with Flt3L, radiation, and pembrolizumab	NCT03789097	Recruiting
		I/II	Low-grade b-cell lymphoma	Combination with Flt3L and radiation	NCT01976585	Completed
		II	Low-grade gliomas with neurofibromatosis type 1	Monotherapy	NCT04544007	Recruiting
		II	Prostate cancer	Monotherapy	NCT06343077	Recruiting
		II	Advanced solid tumor	Monotherapy	NCT01984892	Terminated
Rintatolimod	TLR3	I	Breast cancer	Combination with celecoxib, recombinant interferon alfa-2b	NCT03599453	Completed
		I/II	Metastatic pancreatic cancer	Combination with durvalumab	NCT05927142	Recruiting
		I/II	Metastatic triple negative breast cancer	Combination with celecoxib, interferon alpha 2b, and pembrolizumab	NCT05756166	Recruiting
		I/II	Breast cancer	Combination with HER-2/neu peptide vaccine and sargramostim	NCT01355393	Completed

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Table 1 (*continued*)

Drug	Target	Phase	Disease	Treatment	NCT number	Status
		I/II	Ovary cancer	Combination with interferon-alpha	NCT02432378	Recruiting
		II	Metastatic carcinoma in the liver	Combination with celecoxib and recombinant interferon alfa-2b	NCT03403634	Completed
		II	Hla-a2+ refractory melanoma	Combination with polarized dendritic cell (aDC1) based therapy, interferon alpha-2, and celecoxib	NCT04093323	Recruiting
		II	Pancreatic cancer	Monotherapy	NCT05494697	Recruiting
BO-112	TLR3	I	Solid tumor	Monotherapy (+/− anti-PD1 treatment)	NCT02828098	Completed
		I	Hepatocellular carcinoma	Combination with pembrolizumab	NCT04777708	Terminated
		I	Soft tissue sarcoma	Combination with nivolumab	NCT04420975	Active, not recruiting
		II	Melanoma	Combination with pembrolizumab	NCT04570332	Active, not recruiting
		II	Colorectal cancer, gastric cancer, esophageal cancer	Combination with pembrolizumab	NCT04508140	Terminated
		II	Basal cell carcinoma	Monotherapy	NCT06422936	Recruiting
Bacillus Calmette-Guerin (BCG)	TLR2/4	I/II	Bladder cancer	Combination with durvalumab	NCT03317158	Recruiting
		I/II	Bladder cancer	Combination with atezolizumab	NCT02792192	Terminated
		I/II	Metastatic breast cancer	ConvitVax is composed of autologous tumor cells, BCG D1331, and formalin	NCT06023277	Not yet recruiting
		III	Urinary bladder neoplasm	Monotherapy (+/− nivolumab)	NCT04149574	Terminated
		III	Bladder cancer	Monotherapy (+/− sasanlimab)	NCT04165317	Active, not recruiting
		III	Bladder cancer	Monotherapy (+/− durvalumab)	NCT03528694	Active, not recruiting
		III	Bladder cancer	Monotherapy (+/− atezolizumab)	NCT03799835	Active, not recruiting
GLA-SE	TLR4	I	Soft tissue sarcoma	Combination with therapy	NCT02180698	Completed
		I	Melanoma	Combination with peptide vaccine	NCT02320305	Completed
		I	Merkel cell carcinoma	Monotherapy	NCT02035657	Completed
		I	Melanoma, ovarian cancer, sarcoma, non-small cell lung cancer, breast cancer	Combination with recombinant NY-ESO-1 antigen	NCT02015416	Completed
GSK1795091	TLR4	I	Solid tumor	Combination with either GSK3174998, GSK3359609, or pembrolizumab	NCT03447314	Completed
		I	Healthy subject	Monotherapy	NCT02798978	Completed
Entolimod (CBLB502)	TLR5	I	Solid tumor	Monotherapy	NCT01527136	Completed
		II	Colorectal cancer	Monotherapy	NCT02715882	Unknown status
Mobilan	TLR5	I	Prostate cancer	Monotherapy	NCT02654938	Completed
		I/II	Prostate cancer	Monotherapy	NCT02844699	Unknown status
Imiquimod	TLR7	I	Oral cancer	Monotherapy	NCT04883645	Active, not recruiting

Table 1 (*continued*)

Drug	Target	Phase	Disease	Treatment	NCT number	Status
		I	Metastatic breast cancer	Combination with pembrolizumab, GM-CSF, and cryotherapy	NCT03982004	Terminated
		I	Renal cell carcinoma	Vaccination cocktail with adjuvant GM-CSF and imiquimod	NCT05641545	Recruiting
		I	Cervical intraepithelial neoplasia	Combination with topical fluorouracil	NCT03196180	Active, not recruiting
		I	Advanced solid tumor	Combination with TRK-950	NCT03872947	Recruiting
		I	Brain tumor	Combination with dendritic cells	NCT01171469	Completed
		II	Advanced breast cancer	Combination with abraxane	NCT00821964	Completed
		III	Basal cell carcinoma	Monotherapy	NCT05212246	Not yet recruiting
		III	Basal cell carcinoma	With prior curettage	NCT02242929	Unknown status
		III	Lentigo maligna melanoma (head or neck)	Monotherapy followed by surgery	NCT01720407	Completed
		Not applicable	Cervical intraepithelial neoplasia	Monotherapy	NCT02917746	Unknown status
TQ-A3334	TLR7	I/II	Non-small cell lung cancer	Monotherapy (+/− anlotinib)	NCT04273815	Unknown status
		I/II	Non-small cell lung cancer	Monotherapy (+/− anlotinib)	NCT06507891	Terminated
LHC165	TLR7	I	Solid tumor	Monotherapy (+/− PDR001)	NCT03301896	Terminated
NJH395	TLR7	I	Non-breast her2+ advanced cancer	Monotherapy	NCT03696771	Completed
BNT411	TLR7	I/II	Solid tumor, small cell lung cancer	Monotherapy (+/− atezolizumab, carboplatin, and etoposide)	NCT04101357	Terminated
RO7119929	TLR7	I	Advanced or metastatic hepatocellular carcinoma, biliary tract cancer, or solid tumors with hepatic metastases	Monotherapy	NCT04338685	Completed
SHR2150	TLR7	I/II	Solid tumor	Combination with chemotherapy and PD-1 or CD47 antibody	NCT04588324	Unknown status
BDB001	TLR7/8	I	Solid tumor	Combination with pembrolizumab	NCT03486301	Active, not recruiting
		I	Solid tumor	Combination with atezolizumab	NCT04196530	Active, not recruiting
		II	Solid tumor	Combination with atezolizumab and radiotherapy	NCT03915678	Recruiting
		II	Solid tumor	Monotherapy	NCT04819373	Completed
BDB018	TLR7/8	I	Solid tumor	Monotherapy (+/− pembrolizumab)	NCT04840394	Active, not recruiting
BDC-1001	TLR7/8	I/II	Solid tumor	Monotherapy (+/− nivolumab)	NCT04278144	Active, not recruiting
		II	Breast cancer	Monotherapy (+/− pertuzumab)	NCT05954143	Active, not recruiting
Resiquimod (R848, S28463)	TLR7/8	I	Melanoma	Combination with vaccine therapy	NCT00470379	Completed

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Table 1 (*continued*)

Drug	Target	Phase	Disease	Treatment	NCT number	Status
		I	Melanoma	Combination with MART-1 antigen and Gag:267-274 peptide vaccine	NCT01748747	Completed
		I/II	Cutaneous T cell lymphoma	Monotherapy	NCT01676831	Completed
		I/II	Nodular basal cell carcinoma	Monotherapy	NCT01808950	Terminated
		II	Melanoma	Combination with gp100 (g209-2M) and MAGE-3	NCT00960752	Completed
STM-416	TLR7/8	I	Prostate cancer	Monotherapy	NCT06450106	Not yet recruiting
		I/II	Bladder cancer	Monotherapy	NCT05710848	Recruiting
VTX-2337 (Motolimod)	TLR8	I	Squamous cell cancer of the head and neck	Combination with cetuximab	NCT01334177	Completed
		I	Solid tumor	Combination with cyclophosphamide	NCT02650635	Terminated
		I	Solid tumors or lymphoma	Monotherapy	NCT00688415	Completed
		I	Ovarian epithelial, fallopian tube, or peritoneal cavity cancer	Combination with doxorubicin or pactilaxel	NCT01294293	Completed
		I	Squamous cell carcinoma of the head and neck	Combination with nivolumab	NCT03906526	Terminated
		I	Head and neck squamous cell carcinoma	Monotherapy (+/− nivolumab)	NCT04272333	Terminated
		I	Squamous cell carcinoma of the head and neck	Combination with cetuximab plus nivolumab	NCT02124850	Terminated
		I/II	B Cell lymphoma	Combination with radiotherapy	NCT01289210	Terminated
		I/II	Ovarian cancer	Combination with durvalumab and pegylated liposomal doxorubicin (PLD)	NCT02431559	Completed
		II	Epithelial ovarian, fallopian tube or primary peritoneal cancer	Combination with pegylated liposomal doxorubicin (PLD)	NCT01666444	Completed
		II	Squamous cell of head and neck	Combination with cisplatin or carboplatin + 5-FU + cetuximab	NCT01836029	Completed
SD-101 (nelitolimod)	TLR9	I	Advanced malignancy	Combination with MK-1966	NCT02731742	Terminated
		I	Pancreatic adenocarcinoma	Combination with anti-PD-1	NCT05607953	Recruiting
		I	Solid neoplasm	Combination with BMS-986178	NCT03831295	Completed
		I	Uveal melanoma in the liver	Monotherapy (+/− checkpoint blockade)	NCT04935229	Active, not recruiting
		I	Pancreatic adenocarcinoma	Combination with nivolumab and radiation therapy	NCT04050085	Completed
		I	B-cell non-hodgkin lymphoma	Combination with BMS 986178 and radiation therapy	NCT03410901	Active, not recruiting
		I	Lymphoma	Combination with local radiation	NCT01745354	Completed

Table 1 (*continued*)

Drug	Target	Phase	Disease	Treatment	NCT number	Status
		I/II	Hepatocellular carcinoma, intrahepatic cholangiocarcinoma	Monotherapy (+/− checkpoint blockade)	NCT05220722	Active, not recruiting
		I/II	Head neck cancer, head neck cancer	Combination with pembrolizumab	NCT02521870	Terminated
		I/II	B-cell lymphoma	Combination with localized low-dose radiation	NCT02266147	Terminated
		I/II	Lymphoma	Combination with ibrutinib and radiation	NCT02927964	Completed
		I/II	Lymphoma	Combination with ibrutinib and radiation	NCT02254772	Completed
		II	Prostatic neoplasm	Combination with stereotactic body radiation therapy (SBRT) and pembrolizumab	NCT03007732	Active, not recruiting
CMP-001 (Vidutolimod)	TLR9	I	Melanoma	Monotherapy (+/− pembrolizumab)	NCT02680184	Completed
		I	Non small cell lung cancer	Combination with atezolizumab with or without radiation therapy	NCT03438318	Completed
		I	Metastatic colorectal cancer	Combination with nivolumab, ipilimumab, and radiosurgery	NCT03507699	Completed
		I/II	Solid neoplasm	Combination with INCAGN01949	NCT04387071	Terminated
		II	Melanoma	Combination with nivolumab	NCT04698187	Completed
		II	Squamous cell carcinoma of head and neck	Combination with pembrolizumab	NCT04633278	Completed
		II	Triple negative breast cancer	Combination with stereotactic body RadioTherapy (SBRT)	NCT04807192	Recruiting
		II	Melanoma	Combination with nivolumab	NCT04401995	Active, not recruiting
		II	Advanced or metastatic cancer	Combination with cemiplimab	NCT04916002	Active, not recruiting
		II/III	Melanoma	Combination with nivolumab	NCT04695977	Completed
MGN1703 (lefitolimod)	TLR9	I	Advanced solid malignancy	Combination with ipilimumab	NCT02668770	Active, not recruiting
		II	Small cell lung cancer	Monotherapy	NCT02200081	Completed
		II	Advanced colorectal carcinoma	Monotherapy	NCT01208194	Completed
		III	Metastatic colorectal cancer	Monotherapy	NCT02077868	Unknown status
DV281	TLR9	I	Non-small cell lung cancer	Combination with nivolumab	NCT03326752	Completed
CpG 7909/ELI-002	TLR9	I	Pancreatic ductal adenocarcinoma and solid tumor	Monotherapy	NCT04853017	Active, not recruiting
		I/II	Pancreatic ductal adenocarcinoma and solid tumor	Monotherapy	NCT05726864	Recruiting
Tilsotolimod (IMO-2125)	TLR9	I	Advanced cancer	Combination with nivolumab and ipilimumab	NCT04270864	Active, not recruiting

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Table 1 (*continued*)

Drug	Target	Phase	Disease	Treatment	NCT number	Status
		I	Advanced solid tumor	Combination with ABBV-368 plus nab-paclitaxel, and ABBV-181	NCT04196283	Completed
		I	Solid tumor	Monotherapy	NCT03052205	Completed
		II	Solid tumor	Combination with nivolumab and ipilimumab	NCT03865082	Active, not recruiting
		II	Malignant melanoma	Monotherapy	NCT04126876	Recruiting
		II	Metastatic melanoma	Combination with ipilimumab or pembrolizumab	NCT02644967	Completed
		III	Metastatic melanoma	Combination with ipilimumab	NCT03445533	Terminated

Tlr: toll-like receptor.

example, a Phase I/II clinical trial has demonstrated that a novel *in situ* vaccine combining Flt3 ligands (Flt3L), radiotherapy, and poly-ICLC to reduce systemic tumor burden in patients with advanced indolent non-Hodgkin lymphoma and enhance anti-tumor $CD8^+$ T cell responses (NCT01976585)[237]. Another Phase I study investigates the effects of an experimental vaccine, MELITAC 12.1, in combination with LPS (endotoxin), poly-ICLC, and Montanide ISA-51 in patients with melanoma (NCT01585350), which have proven to be safe and effective vaccine adjuvants capable of supporting durable $CD8^+$ T cell responses[238].

Combining poly I:C with chimeric antigen receptor (CAR)-T-cell therapy could overcome tumor immune suppression and improve solid tumor treatment. A study explored the synergy between EGFRvIII-targeted CAR-T cells and poly I:C in colon and BC models. Poly I:C significantly enhanced CAR-T activity by boosting IL-2 and IFN-γ production and improving cytotoxicity, which inhibited tumor growth *in vivo*. Poly I:C also reduced myeloid-derived suppressor cells (MDSCs) in peripheral blood and spleen, mitigating their suppressive impact on CAR-T cell proliferation and function[239]. Another study developed a pIC nanoscale coordination polymer (pIC@NCP) as a promising strategy to reactivate CTLs in combination with anti-PD-1/PD-L1 ICIs[240,241]. pIC@NCP significantly increased the percentage of DCs and macrophages in tumor-draining lymph nodes and tumors, promoting antigen cross-presentation from APCs to T cells. This approach also upregulated PD-L1 expression on tumor cells[242].

Rintatolimod, a promising TLR3 agonist, inhibits pancreatic cancer cell function while enhancing antitumor immune responses[243]. Most clinical trial protocols investigating rintatolimod's safety and efficacy involve its use in combination with other drugs. A Phase I/II study evaluated the clinical efficacy of a regimen combining intraperitoneal cisplatin, intraperitoneal rintatolimod, and oral celecoxib (a COX2 blocker) in patients with advanced OC. This combination was found to be safe, well-tolerated, and beneficial for the chemotaxis of CTLs (NCT02432378) [244]. Another Phase I trial is investigating the efficacy of chemokine modulation therapy (celecoxib, recombinant IFN$\alpha 2\beta$, and rintatolimod) for treating TNBC with metastatic spread (NCT03599453). Among six patients included in the study, one achieved a clinical response, and three exhibited stable disease (SD)[245].

BO-112, a nanocomplex form of poly I:C, shows potential for local and systemic tumor reduction[246]. BO-112 is undergoing multiple clinical trials for diverse tumors (NCT04570332, NCT04777708, NCT04508140, etc.).

Although TLR3 agonists have demonstrated the ability to enhance tumor-specific immune responses in mice and patients, their systemic toxicity and narrow therapeutic window, as seen with poly I: C and its derivatives, significantly limit their clinical application[247]. Optimizing the dosage or combining these agents with other therapies may improve their efficacy in cancer treatment.

2.4.1.3. TLR2/4. The TLR2/4 agonist Bacillus Calmette-Guérin (BCG) has been approved by the US Food and Drug Administration (FDA) for use as a monotherapy in cancer treatment[248]. Preclinical studies confirm that intratumoral BCG therapy alters the cellular composition of the TME, increasing inflammatory macrophages, neutrophils, and $CD8^+$ T lymphocytes, indicative of tumor remission[249,250]. Numerous clinical trials are investigating BCG in combination with immunotherapeutic agents (NCT05943106, NCT05580354, NCT04149574, etc.).

Proteins produced by *Akkermansia muciniphila* activate TLR2/4, promoting the release of IL-10 and IL-12, which enhance IFN-γ production[251,252]. The presence of *A. muciniphila* in the gut correlates with improved efficacy of ICIs in patients with lung cancer[252]. In patients with advanced NSCLC receiving second-line anti-PD-1/PD-L1 therapy, a higher abundance of *A. muciniphila* in the gut is associated with better disease stability and immune responses, suggesting that GM-TLR interactions may enhance ICI efficacy[253].

2.4.1.4. TLR4. TLR4, TLR4 plays a central role in cancer growth and progression, driving the development of various modulators with therapeutic potential. Glycopyranosyl lipid A in stable-emulsion formulation (GLA-SE), a TLR4 agonist, is a well-established adjuvant for hepatitis B and human papillomavirus vaccines[254]. GLA-SE also activates DCs, inducing a stronger Th1 $CD4^+$ cell response than other TLR agonists[255]. It selectively induces chemokine expression, such as CXCL10 and MCP3, on monocytes, endothelial cells, and fibroblasts, thereby activating and recruiting inflammatory cells, including DCs[256]. This activation triggers downstream signaling cascades, leading to cytotoxic responses[257].

In rat models of breast and colon cancer, GLA-SE administration increased the number and cytotoxicity of NK and T cells in lungs and lymph nodes shortly after treatment, rapidly activating immune responses and promoting cytotoxic cell migration to critical sites[258]. This suggests its potential application during the perioperative period in patients with cancer. A Phase I trial evaluated the safety of weekly GLA-SE injections in combination with palliative radiation for metastatic sarcoma, demonstrating local lesion control in all patients, one case of complete remission, and an increase in tumor-infiltrating lymphocytes (TILs; NCT02180698)[259]. Another Phase I multicenter study investigated the safety and immune response of IDC-G305, an immunomodulator composed of recombinant NY-ESO-1 antigen and GLA-SE, in patients with melanoma, OC, sarcoma, BC, or NSCLC (NCT02015416), showing robust T cell responses and potential clinical benefits[260].

The TLR4 agonist GSK1795091 is an aminoalkyl glucosaminide 4-phosphate that has shown acceptable tolerance and a dose-dependent increase in TNF-α and neutrophils during cancer treatment[261]. A Phase I, open-label study evaluated the safety and efficacy of GSK1795091 in participants with advanced solid tumors in combination with immunotherapies (NCT03447314). Researchers observed elevated levels of IL-10, IL1RN, IL-6, and MCP-1 upon drug administration, with approximately one-third of the patients reporting partial responses or SD[262]. However, during the dose-escalation phase, researchers modified the manufacturing process of GSK1795091 to enable large-scale production. Subsequent pharmacodynamic (PD) and pharmacokinetic (PK) analyses revealed that the modified formulation significantly reduced bioactivity, leading to the discontinuation of GSK1795091 and early termination of the study[262].

2.4.1.5. TLR5. *In vitro* testing conducted by Burdelya and colleagues on engineered flagellin derivatives for NF-κB activation identified Entolimod/CBLB502 as the most effective TLR5 agonist. This agent features the complete N-terminal and C-terminal domains (CTDs) of flagellin connected by a flexible linker[263]. Preclinical studies have confirmed its antitumor activity. In highly refractory 4T1 BC mouse models, intratumoral administration of CBLB502 in combination with CTLA4 and PD-1 antibodies improved survival rates. Mice with long-term survival exhibited immune memory upon tumor rechallenge and showed a unique cytokine profile, including CSF3 and CXCL5[264]. Wang et al. developed a novel therapy to optimize CAR-T/NK cells for treating antigenically diverse solid tumors. They engineered CAR133-NK92 cells to secrete CBLB502 (CAR133-i502-NK92), enabling CAR133-dependent elimination of CD133-positive colon cancer cells while leveraging CBLB502's immune effects to target CD133-negative colon cancer cells[265]. Entolimod/CBLB502 has also been evaluated in Phase I/II studies for safety analysis in cancer treatment (NCT02715882 and NCT01527136), warranting further exploration.

Additionally, Mett et al.[266] developed Mobilan, an adenovirus-based vector for intratumoral delivery that of self-activating TLR5 signaling. Structurally similar to Entolimod, preclinical studies demonstrated that Mobilan injections into primary tumors in transgenic prostate adenocarcinoma mouse models significantly mobilized neutrophils and NK cells into the tumor, effectively inhibiting tumor invasiveness.

2.4.1.6. TLR7. The potent TLR7 agonist imiquimod (R837, Aldara, UGN-201) is a synthetic imidazoquinoline. Preclinical studies in neu transgenic mice, a model for human HER-2-positive BC, demonstrated that the imiquimod-TLR7 combination activates DCs *in vivo*, enhancing their antigen processing and presentation capabilities *via* type I IFN secretion. This induces type I cytokines and TILs[267]. A subsequent Phase II clinical trial evaluated imiquimod in combination with traditional chemotherapy in patients with advanced BC (NCT00821964). The trial achieved an overall response rate of 72%, suggesting that T cell exhaustion or systemic immunosuppression could serve as biomarkers for selecting responsive patients[268]. The TOPIC-3 study, a multicenter, open-label, non-randomized, controlled trial, confirmed the safety and feasibility of topical imiquimod for treating high-grade cervical intraepithelial neoplasia (NCT02917746)[269]. Additionally, a Phase III randomized controlled trial evaluated curettage followed by imiquimod as a less invasive alternative to surgical excision for nodular basal cell carcinoma (NCT02242929). While 86.3% of patients in the curettage and imiquimod group remained recurrence-free after 12 months compared to 100% in the surgical group, the combination remains a viable treatment option[270].

Other TLR7 agonists are in development, showing significant potential. TQ-A3334, an oral TLR7 agonist that induces cytokines like IFN-α and IP-10, is being evaluated for safety, tolerance, and efficacy in patients with NSCLC (NCT06507891 and NCT04273815)[271]. LHC165, which enhances cytokine release and promotes effector T-cell activity, demonstrated acceptable safety and preliminary antitumor activity in a Phase I/Ib trial with or without spartalizumab (NCT03301896)[272]. NJH395, a TLR7 agonist conjugated with an anti-HER2 monoclonal antibody, increased tumor-infiltrating cytotoxic T cells. In a clinical trial, 50% of patients achieved SD after 3 weeks of treatment (NCT03696771)[273,274].

The intravenous administration of BNT411 is under evaluation in a Phase I/IIa study, both as a monotherapy and in combination with atezolizumab, carboplatin, and etoposide for chemotherapy-naïve patients with extensive-stage small cell lung cancer (NCT04101357)[275]. As a novel TLR7 agonist, RO7119929 may reprogram the immune microenvironment and stimulate $CD8^+$ TILs in liver tumors[276]. A Phase I study in patients with advanced liver cancers reported dose-dependent cytokine release syndrome as the primary risk factor (NCT04338685)[277]. SHR2150 is being tested in a Phase I/II trial for efficacy and dosing in patients with unresectable or metastatic solid tumors (NCT04588324).

The combination of TLR7 agonists with CAR-T cells effectively restores their immune function, offering a novel approach to enhancing immunotherapy for solid tumors. Low et al. developed a targeted strategy to selectively rejuvenate exhausted CAR-T cells without affecting other immune cells[278]. By conjugating fluorescein with TLR7-3 (a synthetic hydroxymethylated derivative targeting TLR7)[279], researchers created a selective conjugate (fluorescein-TLR7) that specifically targets CAR-T cells engineered to bind fluorescein. The results demonstrated that fluorescein-TLR7 reversed CAR-T cell exhaustion, enhanced cytotoxicity against $CD19^+$ tumor cells, and increased IFN-γ secretion. To minimize systemic immune activation, the conjugate was designed to release the TLR7 agonist through disulfide bond reduction, ensuring precise delivery to CAR-T cells. Another study reported that a folate-targeted TLR7 agonist (FA-TLR7-1A) selectively reprogrammed TAMs and MDSCs from an immunosuppressive to a pro-inflammatory phenotype. This reprogramming enhanced CAR-T cell therapy efficacy in 4T1 solid tumors without inducing systemic immune activation, as it did not affect similar myeloid cells in healthy tissues[280].

However, like TLR3 agonists, systemic administration of TLR7 agonists may activate multiple cell receptors, leading to

severe side effects, including potentially fatal cytokine storms[281]. This significantly limits their clinical applicability.

2.4.1.7. TLR7/8. Among all TLRs, TLR7/8 stands out for its potent immunoregulatory capabilities, with its agonists playing a crucial role in modulating signaling pathways linked to cancer progression. BDB001 is an intravenous TLR7/8 dual agonist immunomodulator that reprograms and activates plasmacytoid and myeloid DCs, inducing antitumor activity. A Phase I open-label dose-escalation study assessed BDB001 as a single agent and in combination with pembrolizumab in patients with advanced solid tumors (NCT03486301). Results confirmed that BDB001 combined with pembrolizumab was tolerable without dose-limiting toxicities, and sustainable clinical responses were observed in several refractory solid tumors[282]. AGADIR, a multicenter Phase II study (NCT03915678), evaluated the combination of atezolizumab, BDB001, and radiotherapy in multiple solid tumors. Initial results for patients with advanced pancreatic cancer revealed a partial response in 9.5% of cases, SD in 28.5%, and disease progression in 62.0%, leading to a disease control rate of 38.0%[283]. An improved analogue of BDB001, the TLR7/8 agonist BDB018, has been developed to enhance immune efficacy while ensuring safety and is gradually entering clinical trials[284].

BDC-1001, an immune-stimulating antibody conjugate from the Boltbody™ platform, features a trastuzumab biosimilar conjugated to a proprietary TLR7/8 dual agonist *via* a non-cleavable linker[285]. This agent targets HER2-expressing tumor cells with a trastuzumab analog, triggering phagocytosis through FcγR binding, while internalized TLR7/8 agonists activate myeloid APCs to mobilize T cells for tumor cell destruction[286]. A Phase I/II clinical trial (NCT04278144) evaluated BDC-1001 as a single agent or in combination with nivolumab in HER2-expressing advanced malignancies. Encouraging clinical activity was observed in heavily pretreated patients, whether used alone or in combination[287].

Resiquimod (R848, S28463), an imidazoquinoline derivative of imiquimod, has demonstrated effective antitumor activity and immunomodulatory potential[288]. A study by Ye et al.[289] found that combining R848 with stereotactic body radiotherapy in pancreatic tumors enhanced $CD8^+$ T cells, reduced Tregs, promoted APC maturation, and increased IFN-γ and granzyme B levels, improving antitumor effects and reversing the immunosuppressive microenvironment. Researchers have also developed an adamantane-modified derivative of resiquimod that retains macrophage polarization activity while significantly reducing systemic side effects in a mouse MC38 tumor model[290]. To further reduce systemic side effects, Lu et al.[291] developed R848-toco by conjugating R848 with α-tocopherol in a nano-suspension using tocopherol-modified hyaluronic acid. This system enhanced localized immune responses and inhibited tumor growth in head and neck cancer mouse models by recruiting $CD8^+$ T cells.

In addition to preclinical studies, resiquimod (R848) was tested in a Phase II trial (NCT00960752) to assess its effectiveness as an adjuvant for gp100 and MAGE-3 vaccines in patients with HLA-$A0201^+$ metastatic melanoma. Results indicated that resiquimod increased type I IFN levels at the vaccination site by activating plasmacytoid and myeloid DCs, contributing to melanoma metastasis regression[292]. Despite these promising findings, further research is required to guide the development and application of TLR7/8 agonists.

STM-416 is a novel TLR7/8 agonist currently undergoing a first-in-human Phase I/II study. This study involves single-treatment dose escalation and expansion for patients with bladder cancer undergoing transurethral resection of bladder tumor without perioperative intravesical chemotherapy (NCT05710848). The trial aims to determine the safety and tolerability of STM-416.

2.4.1.8. TLR8. VTX-2337 (Motolimod) is a synthetic small-molecule TLR8 agonist derived from a 2-aminobenzazepine core structure[293]. *In vitro* experiments have shown that VTX-2337 stimulates NK cells to produce IFN-γ, enhances their cytotoxic effects, and strengthens the efficacy of rituximab and trastuzumab[293]. The safety and maximum tolerated dose of VTX-2337 have been confirmed in a Phase I ascending-dose study in adults with advanced solid tumors or lymphoma[294].

In a Phase II clinical trial comparing the OS of patients with OC treated with VTX-2337 and pegylated liposomal doxorubicin (PLD) *versus* PLD alone (NCT01666444), the combination showed an efficacy rate of 15.4% and a disease control rate of nearly 70%[295]. Additionally, a Phase I trial (NCT01334177) assessing the safety and efficacy of VTX-2337 combined with cetuximab for locally advanced squamous cell carcinoma of the head and neck (SCCHN) revealed that VTX-2337 activates TLR8 and inflammasomes, complements FcγRIII stimulation, and enhances NK cell responsiveness, potentially improving clinical outcomes[296].

A subsequent Phase II study (NCT01836029) in patients with SCCHN evaluated the efficacy of VTX-2337 combined with standard-of-care (SOC) therapies compared to SOC alone. However, given the association between excessive TLR8 activation and autoimmune diseases[297], the benefits and risks of using TLR8 agonists in cancer therapy should be carefully weighed. Developing more specific and personalized receptor activation strategies remains crucial.

2.4.1.9. TLR9. SD-101 (nelitolimod) is a synthetic TLR9 agonist composed of class C CpG oligodeoxynucleotides (CpG-ODNs). In preclinical models of TSA breast adenocarcinoma and MCA38 colon cancer resistant to anti-PD-1 therapy, SD-101 has been shown to overcome resistance, promoting the infiltration and activation of $CD8^+$ T cells[298]. Numerous clinical trials have been conducted to evaluate its performance.

A Phase Ib/II trial (NCT02521870) combining intratumoral SD-101 with intravenous pembrolizumab for metastatic melanoma and recurrent HNSCC demonstrated good tolerability and significant immune activation, with increased $CD8^+$ T cells, NK cells, DCs, and B cells, and an ORR of 78% in anti-PD-1 therapy-naïve patients[299]. A multicenter Phase I/II study (NCT02266147) combining SD-101 with localized low-dose radiation therapy for untreated low-grade B-cell lymphoma showed favorable safety and structural changes in the TME, including a decrease in tumor cells and an increase in $CD8^+$ and $CD4^+$ effector T cells[300].

In liver-involved metastatic uveal melanoma, a Phase I/Ib study (NCT04935229) explored the efficacy and toxicity of pressure-enabled hepatic artery infusion of SD-101 alone or with ICIs. Among patients treated with SD-101 and ICI, 57.1% exhibited a reduction in circulating tumor cells[301]. A recent preclinical study further supports this administration method, demonstrating that pressure-enabled drug delivery of narlimumab in a mouse model of liver metastasis reduced hepatic MDSCs and increased M1-like macrophages, enhancing antitumor immunity[302].

CMP-001 (Vidutolimod) consists of an immune-active TLR9 agonist, CpG-A ODN, encapsulated within a bacteriophage capsid protein. Lemke-Miltner et al.[303] demonstrated in a preclinical study that intratumoral delivery of CMP-001 stimulates pDCs to secrete cytokines such as IFN-α, enhancing antitumor T-cell responses in B-cell lymphoma mouse models, with further enhancement when combined with anti-PD-1 therapy. Another preclinical study showed that CMP-001 could stimulate a strong immune response within the peritoneal cavity in C57BL/6 mice with peritoneal invasion by pancreatic cancer cells, improving survival rates and reducing disease progression[304].

Treatment options for advanced melanoma resistant to anti-PD-1 therapy are limited. A treatment extension study (NCT02680184) assessing the safety and efficacy of CMP-001, administered alone or in combination with pembrolizumab, reported an objective response rate (ORR) of 23.5% for the combination therapy and 11.5% for CMP-001 monotherapy[304]. In this trial, researchers also evaluated biomarkers through RNA and whole-exome sequencing, as well as immunohistochemistry after tumor biopsies. It was found that patients with high baseline levels of PD-L1, $CD8^+$ T cells, or immune-validated transcriptional features exhibited weaker responses to CMP-001 combined with pembrolizumab[305]. Additionally, a Phase I study (NCT03438318) evaluated CMP-001 combined with atezolizumab in patients with refractory NSCLC, finding that the regimen was manageable in terms of safety and led to disease stabilization in heavily pretreated PD-1/PD-L1 patients with NSCLC, with or without radiotherapy[306].

MGN1703 (lefitolimod) is a TLR9 agonist composed of covalently closed DNA molecules. It exhibits immunomodulatory properties capable of activating various components of PBMCs, significantly increasing the expression of activation markers on immune cells and inducing the release of cytokines and chemokines such as IP-10, IFN-α, MIP-1-α, MIP-1-β, IL-6, and IFN-γ[307]. In a mouse model of renal cell carcinoma, treatment with MGN1703 resulted in a 92% survival rate, significantly higher than that of the control group[308]. The Phase II IMPULSE study (NCT02200081) assessed MGN1703 as maintenance treatment in patients with extensive small cell lung cancer who had at least a partial response to first-line therapy. Lefitolimod did not significantly affect the primary outcome of OS in the target population[309]. A Phase I clinical trial (NCT02668770) validated the highest tolerable dose of MGN1703 combined with anti-CTLA4 therapy ipilimumab in patients with advanced tumors. Preliminary flow cytometry analysis of tumor biopsies revealed the induction of $CD8^+$ T cell proliferation and the presence of memory CD8 phenotypes ($CD45RO^+$)[310].

DV281 is a synthetic oligonucleotide designed with optimized CpG motifs to mimic microbial DNA, inducing immune-activating effects such as the functional maturation of pDCs and the production of type I IFNs[311]. A Phase I dose-escalation and expansion trial investigated the safety and preliminary efficacy of inhaled DV281 combined with nivolumab for NSCLC (NCT03326752). Among 26 patients with advanced NSCLC, 80.7% had undergone prior anti-PD-1/PD-L1 therapy, and 50% achieved SD, demonstrating the therapy's potential[312].

CpG 7909 is a well-tolerated synthetic CpG-ODN featuring four unmethylated motifs, serving as a potent immune-activating agent. It stimulates immune responses in lymph nodes, boosts tumor-specific $CD8^+$ T cells in peripheral blood, and prolongs relapse-free survival[313]. Furthermore, it promotes the recruitment of conventional DCs (cDCs; cDC1) and $CD14^+$ APCs to the injection site and draining lymph nodes, creating favorable conditions for T cell priming[314]. Recent studies have reported significant findings regarding CpG 7909. Researchers at Memorial Sloan Kettering Cancer Center (MSKCC) demonstrated encouraging early results for a vaccine, ELI-002 2P, which combines amphiphilic modified peptides of G12D and G12R mutant KRAS with a CpG oligonucleotide adjuvant (Amph-CpG-7909). Among patients, 84% achieved the expected immune response, 84% showed a reduction in circulating tumor DNA, and 24% exhibited a complete disappearance of tumor DNA[315].

Tilsotolimod, developed by Idera Pharmaceuticals, is a promising TLR9 agonist granted Fast Track designation by the US FDA for combination use with ipilimumab in treating anti-PD-1 antibody-refractory melanoma. In a Phase Ib trial, monotherapy was well-tolerated, with 12 patients (34%) achieving SD (NCT03052205)[316]. A Phase I/II trial reported a best ORR of 22%, a disease control rate of 71%, and a median OS of 21 months (NCT02644967)[317].

Pixatimod is a unique TLR9 agonist that activates DCs. It enhances TA presentation to T cells, overcoming ICI resistance in "cold" tumors and eradicating cancer cells[318]. An open-label, multicenter, dose-escalation Phase I study evaluated pixatimod combined with nivolumab in advanced solid tumors (NCT05061017). In patients with microsatellite-stable (MSS) metastatic colorectal cancer (mCRC), the ORR was 12%, and the disease control rate was 44%. Responding patients exhibited elevated IP-10 levels, a higher IP-10-to-IL-8 ratio, and increased $CD4^+$ and $CD8^+$ effector memory T cells[319]. A Phase II trial (NCT05061017) is currently investigating pixatimod combined with nivolumab in patients with PD-1-refractory or resistant melanoma, NSCLC, and MSS mCRC.

Combining CAR-T therapy with TLR9 agonists presents a promising, universal strategy for synergistic immunotherapy in solid tumors. Researchers developed CpG ODN nanoadjuvants by encapsulating CpG ODN, which was then mixed with tumor cell lysates (TCLs) and neoantigens derived from mouse melanoma to create an antitumor vaccine. This nanostrategy effectively delivered CpG ODN to mouse bone marrow-derived DCs (BMDCs), significantly promoting DC maturation, cellular immunity, cytokine secretion, and memory T cell responses. Additionally, the TCL vaccine induced anti-melanoma antibodies that collaborated with CD16-CAR-T cells *via* the antibody-dependent cellular cytotoxicity pathway, enhancing targeted antitumor effects[320].

Other studies have demonstrated interactions between TLR9 agonists and the GM. Intratumoral administration of CpG-ODN induces significant antitumor effects in mice by promoting TNF and IL-12 secretion[103]. The gut colonization of *Alistipes shahii* (gram-negative) and *Lactobacillus fermentum* (gram-positive), respectively, enhances or diminishes TNF production triggered by CpG-ODN[321]. The presence of gram-negative bacteria correlates with improved responses to CpG-ODN, facilitating tumor necrosis and modifying the TME to support $CD8^+$ T cell-mediated tumor cell destruction[322]. Preclinical and clinical studies suggest that individual differences in GM composition may account for the variability in the success of ICI therapies[323,324]. Sequencing and metabolomic analyses have shown that reduced diversity or richness of fecal microbiota correlates with lower immunotherapy response rates and poorer survival outcomes[325]. Additionally, the accumulation of specific gut bacterial species or strains is associated with improved ICI therapeutic efficacy and enhanced antitumor T cell immunity. The modulation of GM by TLRs may also contribute to the enhanced efficacy of ICIs. In a neoadjuvant

clinical trial (NCT03618641) combining CpG-ODN with anti-PD1 blockade, prolonged progression-free survival (PFS) was associated with an enriched abundance of gram-negative bacteria, including Bacteroidetes, Proteobacteria, and Vibrionaceae, in the feces.

2.4.2. TLR antagonist

Atractylenolide I, a natural sesquiterpene lactone, inhibits TLR4 and the MYD88-dependent NF-κB signaling pathway in ovarian tumors[326], reducing the secretion of pro-inflammatory factors such as IL-6, TGF-β1, and VEGF[327]. This reversal of immunosuppressive signals in the TME activates T lymphocytes[328], effectively suppressing the progression of epithelial OC. CXC195, another TLR4 inhibitor, exhibits significant antiproliferative effects in LPS-induced human liver cancer cells by suppressing the TLR4 pathway and reducing the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6[329], ultimately slowing tumor progression. Zhou et al.[330] evaluated the anti-osteosarcoma activity of paeonol, which acts by inhibiting the activation of the TLR4/MAPK/NF-κB pathway. *In vitro* studies demonstrated its ability to induce dose-dependent apoptosis in osteosarcoma cells, suppressing their proliferation, invasion, and migration. In animal models, paeonol (200 mg/kg) significantly slowed tumor growth and reduced TLR4 expression in mice, further confirming its anticancer potential[328,330].

TAK-242 (Resatorvid) binds to the TIR domain of TLR4, blocking its interaction with downstream adaptors such as TRAM and TIRAP[331,332]. It demonstrates significant antitumor activity in breast and OCs by regulating NF-κB and P53-dependent apoptotic gene expression and reducing the activity of matrix metalloproteinases (MMPs)[140,333,334]. Additionally, TAK-242 enhances the sensitivity of cancer cells to doxorubicin and cisplatin, improving the efficacy of combination therapies[335]. Post-treatment with TAK-242 results in the downregulation of EMT-related genes, including ZEB1, SNAIL1, and SNAIL2, underscoring its anti-metastatic potential[140,332]. Moreover, GM and pathogenic bacteria are key drivers of inflammation and colitis-associated colorectal cancer (CAC). A study found that bacterial activation of TLR4 on colon-resident immune cells triggers inflammation and promotes tumor growth, while blocking TLR4 signaling with TAK-242 in mouse models significantly reduces the incidence and progression of colorectal tumors by inhibiting macrophage infiltration[336].

Echinacea purpurea polysaccharide (EPP) exerts immunomodulatory, anti-inflammatory, and antitumor effects, particularly in HCC[337]. EPP treatment significantly reshapes GM, increasing the abundance of propionate- and butyrate-producing bacteria such as *Coprococcus*, *Clostridium*, and *Roseburia*. These changes enhance intestinal tight junction protein expression, repair the intestinal barrier, and control LPS leakage. Reduced LPS leakage inhibits the TLR4/NF-κB pathway, leading to the downregulation of pro-inflammatory cytokines (*e.g.*, IL-6, TNF-α) and migration factors (*e.g.*, MMP-2)[338]. Ultimately, EPP disrupts HCC cell survival in mice by restoring GM homeostasis and modulating the immune response, thereby inhibiting tumor proliferation and inducing apoptosis[338].

A humanized anti-TLR2 antibody, OPN-301, inhibits inflammation-driven tumorigenesis and growth in GC models by downregulating CXCL2 and TNF-α expression[339]. IMO-8400 targets the TLR7, TLR8, and TLR9 signaling pathways, inducing tumor cell apoptosis and reducing tumor growth in B-cell lymphoma models with the MYD88 L265P mutation[340,341]. While it demonstrated some efficacy and safety in Phase I and II clinical trials across various B-cell lymphoma types[340], its Phase I trials in lymphoma (NCT02252146) and Waldenström's macroglobulinemia (NCT02092909) were terminated due to unsatisfactory results[339].

Overall, experimental and clinical data indicate that TLR antagonists are less effective than agonists in targeting aggressive cancers. TLR agonists enhance tumor immunity by activating immune cells and factors, boosting TAMs and cytotoxic cells to target cancer cells. However, their systemic activation can disrupt normal tissue or cause excessive and chronic inflammation, which may lead to side effects or cancer promotion. Therefore, careful control of dosage and treatment duration is essential to avoid immune dysregulation and achieve beneficial outcomes.

3. Targeting RLRs for cancer immunotherapy

3.1. RLRs

RLRs function as intracellular PRRs, identifying pathogenic RNA types produced by RNA viruses, DNA viruses, and certain bacterial infections. The RLR family includes RIG-I (or DDX58), MDA5 (also known as IFIH1), and laboratory of genetics and physiology 2 (LGP2, or DEAH-box 58 (DHX58)). RIG-I and MDA5 both possess a pair of N-terminal tandem caspase activation and recruitment domains (CARDs) essential for transmitting signals, two core Rec A domains (Hel-1 and Hel-2) with DExH-box-type RNA helicase function, and a CTD working in tandem with the helicase domain to identify immunostimulatory RNAs[342,343].

While RIG-I and MDA5 have comparable structures and functions, their preferred RNA ligands vary. RIG-I shows a preference for attaching to short dsRNAs, whereas MDA5 identifies dsRNAs or RNA aggregates[344,345]. In uninfected cells, an autoinhibitory structure conceals the CARDs of RIG-I and MDA5, hindering subsequent signal transduction[346]. Post-viral infection, viral RNAs attach to both the CTD and helicase domains of RLRs, triggering ATPase activity and resulting in a structural alteration that reveals the CARDs[342,347]. Subsequently, the CARDs in RIG-I and MDA5 engage with mitochondrial antiviral signaling protein (MAVS), resulting in the transcriptional activation of type I/III IFNs and various cytokines. These enhance immune reactions and stimulate the production of proteins that disrupt the life cycle of pathogens[14,342,347].

The expression of IFNs and ISGs through RLRs can lead to both advantageous and harmful impacts on the host[348]. Considering that RLRs are crucial in initiating the host's immune reaction and inhibiting the development of inflammation-related cancers, they present potential focal points for cancer immunotherapy[349,350].

3.2. An overview of RLRs signaling

Identifying RNA and triggering the innate immune response through RLRs serves as a strategy to combat viral or bacterial infections. The traditional RIG-I ligand, known as 5′-tri- or 5′-di-phosphate RNA (5′-pppRNA or 5′-ppRNA), is also produced by RNA polymerase III (RNAPOLIII) upon attaching to AT-rich dsDNA[351,352]. Consequently, both RNA and DNA viruses can potentially trigger RIG-I[353]. Importantly, RIG-I activation can occur through external circular RNA, irrespective of the 5′ triphosphate or dsRNA configuration[354]. Furthermore, the alteration of endogenous dsRNA from adenosine (A) to inosine (I) by adenosine deaminase

targeting RNA 1 hinders MDA5's detection of self-RNA and initiates the MAVS-driven type I IFN reaction[355-357]. However, RLRs are also capable of detecting internal RNA and DNA that are incorrectly located or processed within cells[358-362].

RIG-I's inherent noncoding RNAs include small nucleolar RNA, signal recognition particle RNA, transfer RNA, vault RNA, Y RNA, and RNAs derived from retrotransposons[358,360,361]. Furthermore, the accumulation of mitochondrial dsRNA types in the cytoplasm can trigger an IFN reaction *via* the MDA5−MAVS pathway, particularly when mitochondrial dsRNA undergoes cleavage by RNAase L[362,363].

Although LGP2, RIG-I, and MDA5 share comparable RNA-binding abilities, LGP2 lacks N-terminal CARDs essential for signal transmission. Consequently, LGP2 typically functions to regulate RIG-I and MDA5[364]. LGP2 can potentially inhibit RIG-I signaling *via* various pathways, including disrupting RIG-I's interaction with MAVS[365,366], preventing Dicer from processing long dsRNA[367], stopping viral dsRNA from attaching to RIG-I[368], and curbing TRIM25-driven RIG-I ubiquitination[369]. Conversely, growing research suggests that LGP2 collaborates with MDA5 in enhancing the IFN response[370-372]. LGP2 facilitates the initiation of MDA5 and its transformation into an active form[370]. Depending on the situation, LGP2 may act as both a coactivator and a corepressor for RLRs, functioning to deactivate RIG-I while simultaneously regulating MDA5. However, LGP2 is not essential for the IFN reactions to artificial RNA ligands of MDA5 and RIG-I[373].

Upon RNA activation of RIG-I and MDA5, the revealed RIG-I/MDA5 CARDs engage with the mono CARD domain of MAVS, a protein located in the outer mitochondrial membrane, triggering MAVS oligomerization[374]. The presence of oligomeric MAVS further triggers the activation of TRAF2/3/5/6, leading to the sensitization of TBK1 and the activation of various transcription factors, such as IRF3/7, subsequently promoting the production of IFN and cytokines[375,376]. Furthermore, MAVS activates the IKK complex, which subsequently triggers NF-κB and stimulates the expression of genes associated with inflammation (Fig. 3).

Numerous studies show that pathogens have developed strategies to evade host immune defenses, harm affected tissues, and foster cancer development. In addition to effectors originating from pathogens, cancer cells are capable of deactivating RLR

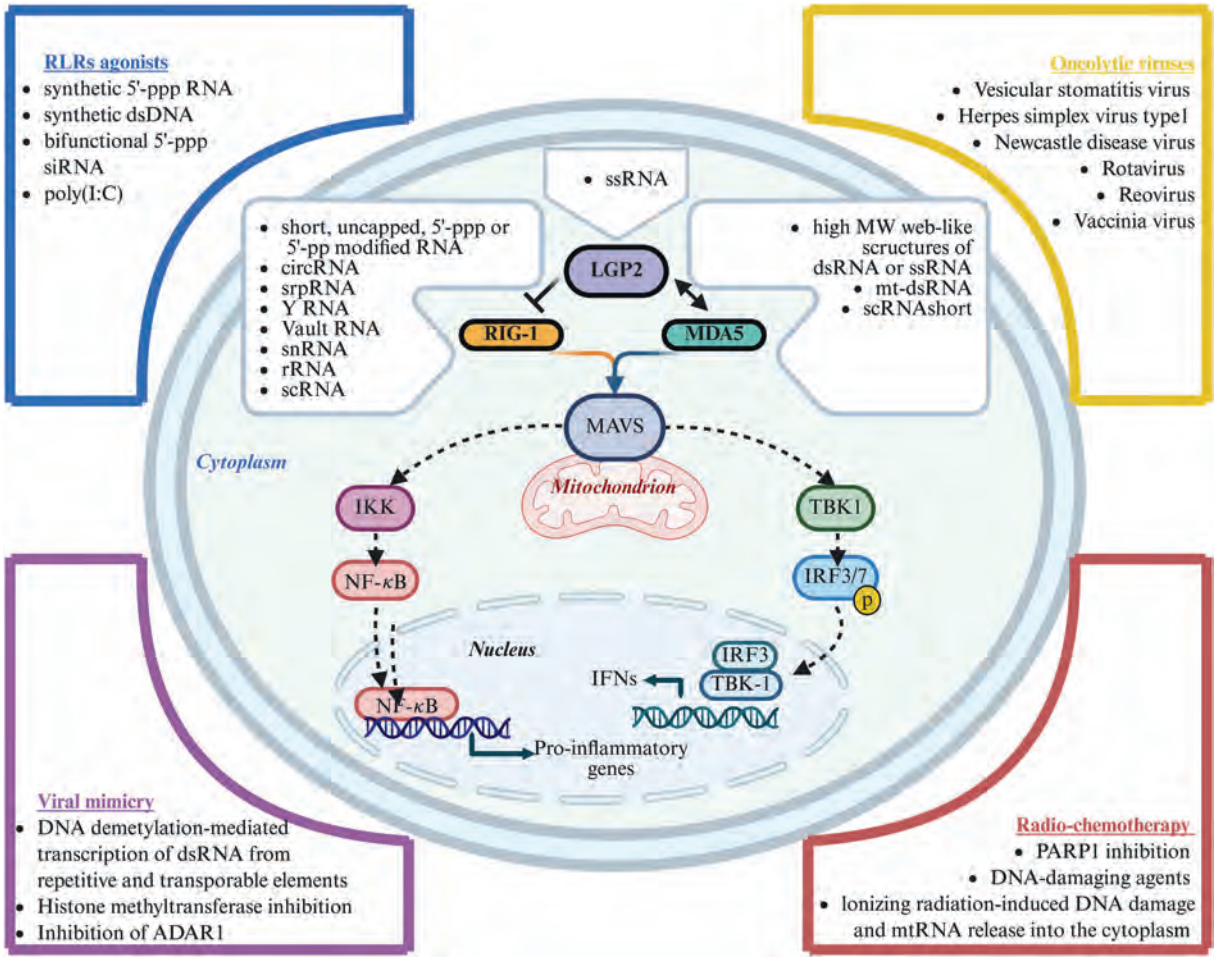

Figure 3 RLR signaling pathway. RIG-I and MDA5 detect viral RNA, leading to the exposure of their CARD domains, which interact with MAVS on the mitochondrial membrane. This triggers downstream signaling pathways that activate NF-κB and IRF3/7, driving the production of type I interferons and enhancing antiviral immune responses. CARD, caspase activation and recruitment domain; LGP2, laboratory of genetics and physiology 2; RIG-I, retinoic acid-inducible gene 1; RLR, RIG-I-like receptor; MDA5, melanoma differentiation-associated gene 5; MAVS, mitochondrial antiviral signaling protein; IκB, inhibitor of NF-κB; IKK, IκB kinase; NF-κB, nuclear factor-κB; TBK1, TANK-binding kinase 1; IFN, interferon; IRF, IFN regulatory factor.

signaling *via* inherent factors. The epigenetic suppression of RIG-I transcription can lead to reduced RIG-I expression in cancers[377,378]. A decrease in RLR levels suppresses innate immunity while simultaneously diminishing the ensuing adaptive immune responses. A weakened immune response in the host exacerbates cancer progression, persisting even after pathogen eradication.

3.3. RLR expression in different types of cells of the TME

3.3.1. RLR expression and function in immune cells

Macrophages play a pivotal role in managing viral infections by inducing ISGs and pro-inflammatory cytokines. However, the cytopathic activity of infected macrophages is deactivated, requiring RLR detection to control viral load[379]. To produce type I IFN and pro-inflammatory cytokines in response to vesicular stomatitis virus (VSV), mouse bone marrow macrophages (BMMs) require MAVS activation through RIG-I and MDA5[380]. Additionally, RIG-I and MDA5 are involved in generating MIP1α, RANTES, CXCL10, and IFNγ in human monocyte-derived macrophages (MDMs) during Middle East respiratory syndrome coronavirus infection[381]. For infections with African swine fever virus, VSV, Sendai virus (SeV), encephalomyocarditis virus (EMCV), and influenza A virus (IAV), porcine alveolar macrophages redundantly depend on RIG-I and MDA5 to induce IFNβ and ISG15, highlighting their concurrent roles in these infections[382,383]. In a mouse model of Ebola virus infection, MAVS is essential for IFNβ secretion and survival[384]. Although the exact function of LGP2 in RLR signaling remains unclear, evidence indicates that LGP2 cooperates with RIG-I and MDA5 to enhance pro-inflammatory cytokine and IFN production in murine and porcine macrophages[373,385].

RLRs traditionally control antiviral responses in macrophages, but they also exhibit distinct functions in viral infection regulation and macrophage activity. In macrophages derived from human monocytes, RIG-I, unlike MDA5, is critical for initiating type I and type III IFNs during H5N1 IAV infection[386]. In murine hepatitis virus (MHV) infection, MDA5-deficient mouse BMMs produce less IFNβ compared to both wild-type and RIG-I-deficient BMMs[387]. These differences stem from distinct RNAs produced by different viruses during infection. Using whole-body genetic double knockouts of MDA5 and RIG-I in mice, studies demonstrate that each RLR plays a unique role in defending against West Nile virus (WNV), affecting virus replication and IFNβ production[388,389]. During WNV infection, RLRs enhance M1 macrophage pro-inflammatory programming and suppress wound-healing functions of M2 macrophages in both mouse and human cells[388]. Overall, RLRs regulate IFN and antiviral gene programming while influencing macrophage polarization.

In APCs, DCs are critical for presenting foreign antigens and initiating adaptive immune responses to eliminate pathogens and establish immune memory. While external inflammation triggered by RLR signaling in epithelial or other cells can affect DC activation, intrinsic RLR signaling is necessary for DC activation, maturation, and antigen presentation. Human moDCs infected with IAV or measles virus require MDA5 and RIG-I to produce type I IFNs, similar to murine BMDCs during rabies and WNV infections[389-392]. MDA5 signaling is crucial in BMDCs for cytokine production during norovirus and EMCV infections[393,394]. Reovirus-, EMCV-, SeV-, and VSV-infected DCs lacking LGP2 show reduced type I IFN production, indicating LGP2's regulatory role in MDA5 and RIG-I pathways[373].

Although human moDCs and murine BMDCs are useful models, studies reveal that distinct DC subsets *in vivo* have unique dependencies on PRR pathways. For instance, introducing Newcastle disease virus (NDV) into murine embryonic fibroblasts and classical dendritic cells (cDCs) activates type I IFN, dependent on RIG-I. However, the absence of RIG-I does not affect IFN production in pDCs. For pDCs, NDV-induced IFN expression relies on RNA sensors TLR7 and TLR9[395]. Interestingly, applying a RIG-I-activating hepatitis C virus (HCV)-derived PAMP to human pDCs induces type III IFN production[396].

Mass spectrometry analysis of splenic DC subsets detected RIG-I and MDA5 predominantly in $CD4^+$ and double-negative ($CD4^-CD8\alpha^-$) cDC subsets, with minimal or no expression in $CD8\alpha^+$ cDCs. This suggests that RLR expression, specific to certain DC subsets, may regulate their activity during acute viral infections[397]. Moreover, variations in the expression and utilization of signal transduction molecules following PRR activation across different DC subsets likely influence their distinct mechanisms of dsRNA detection[398].

Changes in RLR expression over time may allow RIG-I, MDA5, and LGP2 to perform specialized roles within certain cell populations during infections. During lymphocytic choriomeningitis virus (LCMV) infection, RIG-I mediates type I IFN production on the first day, while MAVS-MDA5 drives the subsequent IFN response on the second day[399]. In Zika virus-infected human DCs, RIG-I, MDA5, and LGP2 levels increase, indicating a dynamic role for these RLRs in regulating DC functions and immune responses as the infection progresses[400].

Collectively, these findings underscore the importance of multiple nucleic acid sensors, co-regulatory molecules, and downstream effectors in the spatial and temporal antiviral functions of RLRs. With ongoing research into RLRs' roles in both innate and adaptive immune responses, their functions beyond antiviral defense are becoming evident. Investigating these functions within immune cells will facilitate the development of targeted therapies and enhancers to fine-tune DC activity for optimal adaptive immune activation.

Corroborating this, RLR signaling regulates DC activity and downstream adaptive immunity. For example, RIG-I and MAVS pathways are essential for cytolytic $CD8^+$ T cell production in DCs following IAV infection, in both *in vitro* and *in vivo* settings[390,401]. Specifically, RIG-I signaling in mouse BMDCs is critical for presenting antigens to both $CD4^+$ and $CD8^+$ T cells during IAV infection[401].

RLR signaling also influences $CD4^+$ T cell immunity. During dengue virus infection, human DCs secrete IL-27, polarizing T follicular helper (TFH) cells and facilitating the production of IgM and IgG. This process relies on RIG-I and MDA5, as demonstrated through siRNA-mediated MAVS suppression[402]. The complete removal of MAVS in $CD11c^+$ cells, unlike in B cells, increases viral load while amplifying WNV-specific IgG and neutralizing antibody responses[403]. RLR signaling in DCs is essential for both antiviral responses and the activation of DCs, contributing to robust adaptive immune reactions.

The activation and functionality of T cells in various infectious models depend on both intrinsic and extrinsic RLR expression. RLRs perform distinct roles in activating $CD4^+$ T cells. During IAV infection in mice, RIG-I is necessary for the activation of $CD4^+$ T cells[401]. Similarly, H5N1 IAV infection requires RIG-I for $CD4^+$ T cell activation and Th1 polarization[404]. In the context of dengue virus infection, both RIG-I and MDA5 are essential for Th1 polarization[405]. Interestingly, RLR expression in

DCs appears critical for Th1 polarization in dengue virus infections. Additionally, RIG-I and MDA5 are required in DCs to facilitate $CD4^+$ TFH cell responses during dengue virus infection[402]. TFH cells play a crucial role in germinal centers by aiding B-cell and antibody-mediated responses, suggesting that RIG-I and MDA5 influence B cell and antibody responses during infections.

While RLRs are integral to $CD4^+$ T cell function, their role in regulating $CD8^+$ T cell responses is even more thoroughly studied. RIG-I plays a pivotal role in priming $CD8^+$ T cells during IAV infection, as evidenced by reduced production of IFNγ, TNF, and Granzyme B in mice lacking RIG-I and MAVS[401]. Studies using mixed bone-marrow chimeras and laboratory experiments have confirmed the necessity of RIG-I signaling in both DCs and $CD8^+$ T cells for $CD8^+$ T cell activation and function during IAV infection[401]. MDA5 deficiency impairs effector $CD8^+$ T cell responses in infections caused by WNV and MHV[406]. In WNV infections, MDA5 signaling outside T cells is required to manage CNS infections and activate $CD8^+$ T cells[407]. Similarly, prolonged LCMV infections demonstrate reduced virus-specific $CD8^+$ T cell responses in the absence of MDA5, emphasizing the necessity of MDA5-mediated type I IFN activation to prevent $CD8^+$ T cell depletion[408].

MDA5 also supports memory formation by promoting the survival and longevity of $CD8^+$ T cells during herpesvirus infections[409]. LGP2 plays a regulatory role in T cell immune responses. For instance, WNV-infected mice lacking LGP2 exhibit reduced WNV-specific $CD8^+$ T cell numbers and survival rates. However, it remains unclear whether LGP2 acts directly within $CD8^+$ T cells or through APCs[410].

Regardless of whether RLRs function intrinsically within T cells or are regulated externally by RLR signaling in other cells that modulate T cell immunity, their significant contribution to T cell immunity is undeniable. As a result, RLRs have garnered substantial attention, particularly in cancer-related contexts, for their role in enhancing the cytotoxic antitumor T cell immune response[411-413].

Emerging research sheds light on the mechanisms by which RLRs enhance B cell immunity. Studies demonstrate that using RLR agonists as adjuvants can significantly improve B-cell and antibody responses. For instance, administering a RIG-I agonist RNA alongside H5N1 virus-like particles reduces viral levels while increasing IgG2a responses in mice[404]. Similarly, combining a RIG-I agonist with a DNA vaccine vector for the influenza virus activates IFNβ in cell cultures and raises serum antibody concentrations[414]. Using *in vitro* transcribed defective interfering RNA from SeV as an adjuvant with an H1N1 IAV vaccine increases IgG subtypes, airway IgA levels, and survival rates following a lethal challenge[414].

In addition to boosting antibody levels, RIG-I-targeting adjuvants used with H1N1 IAV vaccination in mice enhance IgG titers, accelerate germinal center formation, and facilitate the rapid generation of virus-specific plasma cells in the bone marrow, compared to vaccination alone[415]. These findings suggest that RIG-I adjuvants can amplify antibody levels and potentially extend the durability of immune responses.

In humans, a specific RIG-I polymorphism is associated with increased IgA seroconversion following rotavirus vaccination, highlighting the role of RLRs in enhancing humoral immunity[416]. Collectively, these studies underscore the critical role of RLRs in modulating B cell immunity, presenting opportunities to leverage RLR pathways for improved protective immunity.

Limited research has been conducted on the function of RLRs in NK cells amidst viral infections. Nonetheless, the impact of artificial agonists on viral detectors like RLRs in NK cells has been research. NK cells, both human and mouse, grown together with DCs and exposed to poly I:C, a man-made dsRNA analogue that activates MDA5, enhance the production of IFNγ by NK cells. The production of IFNγ by NK cells markedly decreases due to the absence of MAVS and relies on DCs[417]. Given that poly I:C activates TLR3, the membrane-bound dsRNA sensor, it's essential to identify the ligand's target that influences NK function. Administering poly I:C to mice resulted in the generation of IFNγ in NK cells, a process reliant on both MDA5 and TLR3[418]. Mice with mixed bone marrow chimeras pinpointed the necessity of MDA5 in the stromal area for the generation of NK cell type I IFN when exposed to poly I:C in a living organism[418]. Similar to findings in mouse models, RLR agonists are capable of stimulating human NK cells as well[417]. NK cells in humans infected with HBV and subjected to poly I:C treatment exhibit reduced concentrations of perforin, Granzyme B, and IFNγ, in contrast to the control group[419]. Infection by HBV led to a decrease in RIG-I levels and a reduction in NF-κB and MAPK signaling pathways[419]. Focusing on activating RIG-I through the treatment of NK cells with 5′-triphosphate ssRNA resulted in a heightened expression of the TNF-related apoptosis-inducing ligand (TRAIL) on the surface, indicating an inherent role of NK cells in RLR[419]. Collectively, these research works reveal the necessity of both RIG-I and MDA5 in the control of NK cell activity.

Primarily located in the skin and mucosal areas, mast cells serve as immune detectors for different virus types. The presence of RLRs in mast cells plays a crucial role in triggering inflammation to fight against the virus during replication. Inhibiting MDA5 and RIG-I through siRNA in human mast cells resulted in a heightened VSV titer during the *in vitro* infection process[419]. Additionally, the production of IFNα, IFNβ, CXCL10, and IL-6 by murine mast cells during VSV infection necessitates the presence of both RIG-I and MDA5[420]. Furthermore, the reduction of MDA5 and RIG-I sensors in the KU812 mast cell line led to a decrease in CXCL10, CCL4, and CCL5 levels when exposed to antibody-augmented dengue virus[421]. Mast cells originating from human cord blood, infected by the dengue virus alongside dengue immune sera, resulted in elevated mRNA levels of MDA5, RIG-I, and type I IFNs[421]. Collectively, these research works endorse a significant and possibly triggerable function of RIG-I and MDA5 in controlling the anti-viral reactions and the synthesis of chemokines/cytokines in mast cells.

Eosinophils play a role in fungal and allergic conditions by secreting chemokines/cytokines, degranulating, and creating extracellular traps[422]. Nonetheless, the question of eosinophils' ability to adapt to challenges in managing viral infections is a developing field of study[423]. Research using mouse models indicates a reduced expression of *RLR* mRNA compared to what is seen in DCs[423]. *In vitro* infection with IAV H1N1 triggered the expression of both DDX58 and IFIH1. Likewise, the expression of RIG-I and MDA5 in human eosinophils is lower than in other PRRs like NOD1 and NOD2, which are recognized for stimulating eosinophils[424,425]. Yet, when exposed to IAV, the expression of RIG-I mRNA can be swiftly triggered, indicating a possible time-dependent function of RIG-I in eosinophils after the receptor's upregulation[426].

Neutrophils swiftly penetrate infected tissues, engulfing pathogens, debris, and cells, and create extracellular traps to assist in eliminating viral infection. Introducing poly I:C into human

neutrophils to stimulate MDA5 leads to an increase in type I IFN, TNF, IL-12, various chemokines/cytokines, and ISGs, showcasing neutrophils' ability to detect and react to viral infections in a standard manner[427]. The generation of IFNβ and TNF by mouse neutrophils amid EMCV infection relies on MDA5[427]. When human neutrophils were exposed to ssRNA40 from HIV-1, there was an increase in RIG-I and MDA5 levels, akin to those found in neutrophils from HIV-1-positive individuals[428,429]. Fascinatingly, the detection of RIG-I and MDA5 in neutrophil secretory vesicles and the attachment of RIG-I to 5′-triphosphate ssRNA on neutrophils' plasma membranes have been noted[430]. Although the significance of this atypical arrangement of RLRs in neutrophils remains unclear, it implies that RLRs might operate distinctively in neutrophils, which could be utilized to adjust their activity during infection or inflammation.

3.3.2. RLR expression and function in tumor cells

A variety of cancer cell types exhibit sensitivity to apoptosis induced by RIG-I. During melanoma treatment, the combined effect of RIG-I activation and 5′-triphosphate-siRNA−induced BCL2 silencing led to extensive apoptosis in cancer cells, necessitating IFN expression and NK cell activation[431]. Conversely, live experiments revealed that 5′-triphosphate-siRNA effectively suppressed BCL2 expression and curtailed lung melanoma cell metastasis[431]. Another research indicated that RIG-I induced melanoma cell death by stimulating the proapoptotic protein Noxa, reliant on MAVS activation but independent of type I IFN activation or P53 pathway activation. Fascinatingly, the same proapoptotic signaling route was operational in benign cells, yet it was salvaged from apoptosis through the targeted increase or activation of the antiapoptotic protein BCL2L, which appears to be impaired in melanoma cells. Furthermore, apoptosis induced by RIG-I effectively reduces melanoma lung metastasis in immunodeficient NOD/SCID mice (deficient in NK and T cells) during *in vivo* engraftment[432]. Additionally, another research found that type I IFNs, unlike IRF3 or IRF7, are not essential for anticancer effects in both neuronal and BC cells[433]. NDV's activation of RIG-I triggered apoptosis in these cells by increasing levels of the proapoptotic protein TRAIL and reducing the levels of antiapoptotic proteins like BCL2, BIRC3, and PRKCE[433]. Nonetheless, when MAVS levels were consistently reduced, IRF3 or IRF7 in IFN-nonresponsive cancer cells showed a decline in anticancer effects by inhibiting genes related to apoptosis and enhancing antiapoptotic genes[433]. IRF-3, a component following the RIG-I/MAVS signaling pathway, played a role in apoptosis triggered by various viruses such as SeV, enocardomyitis virus, and reovirus, all detectable by RIG-I[434,435]. Research indicated that IRF3's expression was directly controlled by a single IRF-element (IRF-E) in response to IRF-E in dRNAs. IRF-3 is recognized as a key controller in the antiviral process for producing type I IFNs[436]. In contrast, the apoptosis triggered by mammalian reovirus relies on IRF-3 instead of type I IFN *via* NOXA expression[437]. Concurrently, the neuronal defense against neurotropic arboviruses (such as Western equine encephalitis virus and St. Louis encephalitis virus) through IRF-3-dependent cell death happened without relying on autocrine or paracrine type I IFN activity[438]. Consequently, the detection of these viruses by RIG-I might lead to the apoptosis of cancer cells in a manner dependent on IRF-3.

Within HNSCC, there was a high expression of RIG-I in both underdeveloped primary cancer tissues and lymph node metastasis, in contrast to normal neighboring tissues[439]. Activating the RIG-I/MAVS pathway was crucial for triggering apoptosis *via* caspase-9. RIG-I was appropriately involved in HNSCC by controlling the activation of the serine/threonine kinase Akt. The stimulation of RIG-I by a small quantity of viral dsRNA resulted in reduced IFNs and proinflammatory cytokines, thereby boosting cell survival reliant on Akt. Conversely, the activation of RIG-I by a larger dose of viral dsRNA triggered apoptosis in cancer cells, along with a decline in Akt activation[439]. Moreover, the apoptosis signaling pathway mediated by RIG-I/MAVS in human primary GB cells was more responsive to RIG-I activation in various primary GB cells than in nonmalignant human cells, evidenced by a rise in CXCL10 secretion[440]. When activated by poly I:C, RIG-I activated pathways leading to apoptosis in human gastric adenocarcinoma cells, and poly I:C stimulation curtailed the development of human gastric adenocarcinoma xenografts in nude mice[441]. Additional research indicated that the activation of the RIG-I/MAVS signaling pathway by nonreplicating SeVs caused prostate cancer cell death by increasing the levels of proapoptotic proteins TRAIL and NILa. Additionally, in immunodeficient (NOD-SCID) mice treated with SeV envelope (also referred to as hemagglutinating virus of Japan envelope) in a living organism, there was a significant reduction in prostate cancer cases[442,443].

The research indicated that triggering the RIG-I/MAVS pathway through non-self-RNA could lead to the demise of cancer cells in both lab and live environments. Concurrently, growing research indicates that activating RIG-I may initiate the activation of adaptive immune cells like CD8 T cells, leading to the immunogenic demise of cancer cells. In the pancreatic cancer mouse model, administering RIG-I ligands effectively suppressed tumors by activating DCs and CD8 T cells[444]. This activation of pancreatic cancer cells *via* RIG-I ligands resulted in natural apoptosis, the movement of calreticulin to the outer cell membrane, and the release of CXCL10 and hazardous molecules like high-mobility group box protein 1 (HMGB1) and heat shock protein 70 (HSP70). The latter played a key role in the absorption and activation of DC antigens, essential for effective T cell activation[445]. Concurrently, there was an increase in the expression of cluster of differentiation 95 (CD95) and MHC-I in pancreatic cells, making them vulnerable to CD95-induced apoptosis and CTL-mediated cell death[445]. In both well-established epithelial OC cell lines and those derived from patients, administering RIG-I agonists led to the immunogenic demise of OC cells by enhancing MHC-I levels and stimulating the production of proinflammatory cytokines like CXCL10, IL-6, TNF-α, and IFN-β[446].

Furthermore, in the intestines, RIG-I was found to hinder CRC progression by controlling GM, a key factor in carcinogenesis. Research indicated that mice lacking RIG-I had a disrupted GM compared to normal (WT) mice, with RIG-I regulating GM by elevating IgA and IL6-dependent STAT3γ[348]. Additionally, in both mouse and human CRC, RIG-I-deficient mice were more prone to sodium/doxane-induced colitis[348]. In these models, RIG-I deficiency led to reduced liver cancer MHC-I expression and decreased the ability of cancer cells to penetrate the extracellular matrix, significantly reducing the extracellular presence of MMP-9 cells, a crucial factor in cancer cell invasion. Elevated levels of MMP-9 have been closely associated with the severity of malignant tumors[447]. Additionally, a lack of RIG-I is known to encourage the development of HCC in mice. Within human HCC tissues, RIG-I expression was found to be lower in males compared to females. RIG-I could serve as an indicator for forecasting the prognosis of HCC and assessing the effectiveness of immunotherapy, such as IFN-α therapy. RIG-I amplified the

IFN−JAK−STAT effector pathway by stimulating STAT1 activation *via* the interruption of SHP1−STAT1 interaction. Individuals suffering from HCC with reduced RIG-I levels experienced diminished survival rates or less effective outcomes from IFN-α treatment[377]. Collectively, these research findings indicate that targeting RIG-I could be a novel approach for cancer treatment, with the activation of RIG-I by RNA ligands potentially resulting in successful cancer therapies. Various research efforts have been directed towards creating dynamic cellular vaccines based on the concept that dying cancer cells contain non-self-RNA capable of triggering RIG-I and prompting the release of type I IFNs along with other immunostimulatory cytokines[448].

Given the comparable structural domains of MDA5 and RIG-I, MDA5 plays a crucial role in antiviral immune responses. Under certain circumstances, MDA5, in contrast to RIG-I, may trigger unique signals and become active by attaching to various RNA ligands[365]. MDA5 shows a preference for identifying extended poly I:C segments, while RIG-I effectively connects to brief 5′ppp-RNA sequence patterns and RNA with dsRNA segments[449,450]. Likewise, MDA5 identifies viral RNA replicas in cancerous cells, initiating the mitochondrial apoptosis process[432,451]. Growing research indicates that abnormal MDA5 expression results in halted growth and cell death in multiple cell types, including cancer cells[452,453]. Within melanoma cell lines, MDA5 triggered a proapoptotic signaling route that operates independently of type I IFNs and P53, yet relies on the proapoptotic protein NOXA, facilitated by MAVS, along with the induction of caspase-9 and APAF-1[432]. Nonmalignant cells were salvaged from apoptosis caused by MDA5 due to their ability to increase BCL2L levels. Consequently, apoptosis specific to melanoma cells might be initiated by the activation of the cytoplasmic MDA5/MAVS pathway, governed by the equilibrium of proapoptotic and antiapoptotic proteins in the BCL2 family[432].

Additionally, TLR3, an RNA-detecting PRR, is vital in the advancement of cancer[454,455]. Elevated TLR3 levels have been linked to a positive prognosis in neuroblastoma (NB). Analogous to TLR3, the presence of MDA5 in human NB tissues also indicated a favorable prognosis[456]. MDA5 may collaborates with TLR3 to inhibit cancer progression. Elevated levels of MDA5 in NB cells led to a notable decrease in cell growth and an increase in cell death caused by poly I:C, a process reliant on MAVS, caspase-9, and caspase-3[456]. Additionally, MDA5 exhibits strong anticancer properties in various other cancers. The stimulation of MDA5 by RNA ligands like poly I:C and 3pRNA significantly triggered apoptosis in primary GB cells, BC, gastric adenocarcinoma, and prostate cancer cell lines, as well as in engraftment mouse models[440,441,443,457].

The stimulation of MDA5 through the cytosolic synthetic dsRNA analog poly I:C resulted in the phosphorylation of the IRF3 transcription factor, the generation of IFN-β, and an increase in MHC-I expression. Agonists of MDA5 triggered the apoptosis of pancreatic cancer cells through both the inherent MDA5/MAVS route and the external CD95-driven apoptosis[458]. Additionally, MDA5 caused significant *in situ* death of tumor cells and notably extended the lifespan of two distinct mouse models of pancreatic cancer, where CD8 T cells were crucial for immunotherapy[458,459]. Furthermore, it was demonstrated that stimulating the RAS/RAF/MEK/ERK pathways, which promote growth, could suppress the proapoptotic effects of MDA5 on human pancreatic and colorectal carcinoma cells[452].

Recent research indicates that activating MDA5 with dsRNA might result in the initiation of apoptosis and the suppression of tumor cell growth. 5-AZA-CdR, a clinical antitumor agent that demethylates DNA, triggered dsRNA production and activated the MDA5/MAVS/IRF7 signaling pathway, thereby promoting the generation of type III IFN over type I IFN. Mimicking retrovirus, 5-AZA-CdR penetrated CRC-initiating cells (CICs) and triggered a potent anti-growth reaction[460]. A brief, low dosage of 5-AZA-CdR markedly decreased the occurrence of colorectal CICs in mouse transplants. The consistent effect of 5-AZA-CdR therapy in reducing sphere-initiating cell counts in primary CRC specimens correlated with heightened dsRNA production[460]. Furthermore, MDA5 amplified the TLR3-linked apoptotic route, unaffected by direct poly I:C activation in hepatoma cells[461]. Enhancing MDA5 triggers the caspase-3/7 pathway, leading to hepatoma cell apoptosis. Concurrently, using poly I:C-liposome to treat hepatoma cells led to growth suppression in mouse engraftments[461]. In summary, MDA5 may be employed to create potential treatments for different cancers by controlling apoptosis and adaptive immunity.

3.3.3. RLR expression and function in epithelial cells

Numerous research findings indicate that the pathways of RIG-I and MAVS not only detect viral pathogens but also contribute to sustaining gut balance and averting intestinal inflammation. Mice devoid of RIG-I naturally manifested a colitis-resembling characteristic, showing heightened vulnerability to dextran sulfate sodium (DSS)-triggered colitis[462] and a greater risk of *E. coli* infection than their wildtype counterparts, owing to reduced bacterial phagocytosis[463]. Mice lacking RIG-I exhibited a spontaneous colitis phenotype linked to diminished expression of the G protein αi2 subunit (GNAI2), shrinkage of Peyer's patches, and excessive activation of T cells similar to those in GNAI2 knockout mice[462]. Studies have shown a decrease in RIG-I levels in the intestinal lining of individuals with Crohn's disease (CD), indicating a potential disruption in the body's natural antiviral defenses and the defensive roles of RIG-I signaling in this condition[464]. By enhancing IgA production and IL-6−STAT3-dependent REG3γ expression, RIG-I inhibits colon tumor growth in mice, thus controlling the microbiome[348,464,465].

Researchers analyzed the characteristics of mice devoid of MYD88, MAVS, or both types of signaling adaptors in the acute DSS-induced colitis framework. Fascinatingly, mice lacking MAVS exhibited more severe colitis than WT mice, with the condition being more severe in MYD88-deficient mice and even more severe in MYD88/MAVS double-knockout mice. This suggests that the two PRR signaling pathways collaborate to safeguard the intestine from harm and inflammation[466]. This model's observed protective impact is facilitated through MAVS signaling in cells that are not hematopoietic. Studies revealed that mice lacking MAVS or RIG-I are more prone to intestinal harm caused by radiation or chemotherapy, leading to more intense graft-*versus*-host disease (GVHD) following allogeneic hematopoietic stem cell transplantation (allo-HSCT)[467]. Administering the RIG-I ligand 5′-triphosphate RNA or cGAS/STING activating DNA systemically somewhat alleviated GVHD in mice post-allo-HSCT, a result counteracted by blocking IFNAR. Research involving intestinal organoid culture revealed that type I IFN directly influences organoid development and the synthesis of the antimicrobial peptide REG3γ[466,467].

The research indicated that introducing fecal RNA into macrophages or epithelial cells triggers the expression of IFN-β, reliant on RIG-I and MAVS[466,467]. Isolating RNA from the feces of antibiotic-treated or germ-free mice significantly diminished

this activity[466], highlighting the critical function of bacterial RNA detection by RIG-I. There remains a possibility that bacteriophages and eukaryotic RNA viruses located in the intestine play a role in the continuous activation of RLR to safeguard the intestinal barrier. The MNV status of their mouse colonies was not disclosed in any of the studies mentioned. It is yet to be determined whether the enteric virome offers protection against HSCT and GVHD, or if it is pathogenic, as indicated by the proliferation of certain enteric viruses in patients with HSCT with GVHD[468].

3.4. RLRs pathway for cancer immunotherapy

Recognizing the essential role of RLRs in triggering IFN reactions and causing immunogenic cell demise, the activation of RIG-I or MDA5 pathways has surfaced as a viable approach in cancer treatment. Immunotherapeutic approaches for cancer, like immune checkpoint blockade, have proven to be highly effective in cancer treatment. Nonetheless, tumors that are not inflamed, known as "immune-cold", show no sensitivity to ICIs. Activating RLRs signaling could enhance the proinflammatory characteristics and prepare the tumor's microenvironment for an ICI response[469]. Furthermore, RIG-I and MDA5 are capable of triggering type I IFN-independent cell death in certain cancer varieties[432]. Consequently, activating RLRs signals could aid in treating "immune-cold" tumors, irrespective of their cause. Growing data from early-stage research indicates the potential of RLRs agonists in treating cancer. RLRs are capable of activation through small RNA, oncolytic viruses, viral imitation, and radio-chemotherapy. Therefore, we describe in this article according to these categories (Table 2).

3.4.1. RLR agonists

Although dsRNA or stem-loop RNA directly stimulates RLRs, its administration depends on the use of synthetic polymers or nanoparticles. Minimal advancements have been made in the treatment of patients with cancer using synthetic RLRs agonists. Liposome serves as a prevalent agent for transporting DNA and RNA into cells[470]. Liposomal vesicles consist of either phospholipids or artificial amphiphiles that are integrated with cholesterol[471]. Administering poly I:C into tumor liposomes triggers the expression of RIG-I/MDA5 and suppresses the proliferation of hepatoma and GC[441,461]. Additionally, microparticles, nanoparticles, and hydrogels serve as typical carriers for siRNA, miRNA, and 5′-pppRNA[472]. The administration of 5′-ppp RNA into tumors through nanoparticles that react to pH changes and destabilize membranes, specifically dimethylaminoethyl methacrylate-*b*-(dimethylaminoethyl methacrylate-*co*-butyl methacrylate-*co*-propylacrylic acid), can withstand the endosomal/lysosomal breakdown of RNA and effectively stimulate RIG-I[473].

Preliminary research indicates that administering nanoparticles systemically, which include carboxylic acid-terminated poly(lactic-*co*-glycolic acid), 5′-ppp dsRNA, and various natural agonists, results in anticancer properties in mouse melanoma models[474]. Systemic administration of RIG-I agonists, as opposed to intratumoral delivery, could prove to be more practical and efficacious in various medical environments. The lipid−calcium−phosphate (LCP) nanoparticle system can also facilitate the systemic administration of RIG-I-activating RNA. The system amalgamates complexes of cationic lipid−protamine−nucleic acids along with calcium phosphate precipitates[475]. In creating complexes of cationic lipid−protamine−nucleic acids, DNA/RNA initially engages with protamine sulfate, a type of cationic polypeptide, followed by incubation with 1,2-dioleoyl-3-trimethylammonium-propane cationic liposomes. This process results in the formation of positively charged nanoparticles, which are subsequently altered by a double-chain phospholipid conjugate of polyethylene glycol (PEG) attached to anisamide[476].

Although the cationic lipid−protamine−RNA complexes maintain their stability in the bloodstream post intravenous injection and effectively transport RNA, the distribution of RNA into the cytoplasm varies across different cells[477]. The issue is resolved through the effective creation of LCP nanoparticles, substituting the central part of cationic lipid−protamine−nucleic acid complexes with tiny calcium phosphate precipitates that trap RNA[478]. Administering LCP nanoparticles, which contain a dual-function 5′-ppp siRNA, intravenously also shows effectiveness against tumors in mouse models of pancreatic adenocarcinoma, without triggering widespread immunomodulation[479].

The efficacy of the conventional liposome in creating a hydrophobic nanoparticle system with negatively charged DNA and RNA *in vivo* is hindered by the harmful effects of positively charged lipids. The team led by Dr. Villus created the ionizable lipid nanoparticles (LNPs) system, notable for its minimal *in vivo* toxicity[480]. Such LNPs consist of a blend of amphipathic phospholipid, ionizable amino lipids, PEG lipids, and cholesterol[480]. Ionizable amino lipids attach themselves directly to nucleic acids, aiding in the escape from endosomes. Amphipathic phospholipid aids in merging LNP with cellular and endosomal membranes[481]. Cholesterol bolsters the steadiness of LNP[482]. Subsequently, mRNA and mRNA vaccines were administered *in vivo* using the LNP system[471,483].

Up to this point, minimal documentation exists regarding the *in vivo* administration of RLR-activating RNA for cancer treatment. Further research is needed to utilize this sophisticated delivery mechanism for cancer treatment through RLR-activating RNA. Furthermore, the advent of extracellular vesicles (EVs) marks a new avenue for the transport of siRNA, peptides, or proteins[484,485]. EVs derived from red blood cells have proven effective in the intratumoral administration of 5′-pppRNA, acting as RIG-I agonists[486]. Additional research is needed to ascertain if engineered EVs are capable of facilitating the intravenous administration of RIG-I agonists to tumors.

The development of CAR-T cells targeting tumors has achieved remarkable success in treating individuals with blood cancers. Yet, the effectiveness of CAR-T treatment in numerous solid tumors continues to be subpar. Engineered CAR-T cells are capable of generating RN7SL1, an unshielded noncoding RNA, and secreting it through EVs[350]. Although RN7SL1 stimulates RLR signaling in CAR-T cells and enhances their functionality, EVs carrying RN7SL1 tend to be more frequently transmitted to immune cells than to tumor cells within the tumor's microenvironment. The process by which RN7SL1-loaded EVs are selectively delivered remains a mystery. When immune checkpoints are obstructed, RN7SL1's activation of RLRs in immune cells boosts cancer-fighting immunity and facilitates tumor inhibition. Utilizing CAR-T cells to introduce RLRs agonists into the tumor's microenvironment presents a hopeful approach for cancer immunotherapy.

Initial trials of MK-4621, a RIG-I oligonucleotide agonist, reveal that injecting MK-4621/jetPEITM directly into tumors, or its combination with pembrolizumab (MK-3475; anti-PD1 mAb), effectively stimulates the RIG-I pathway in cancer sufferers, though no clinical advantages have been observed[487]. The necessity to modify the MK-4621 dosage remains uncertain, as the

Table 2 RLRs agonists in treating cancer.

Drug	Target	Phase	Disease	Treatment	NCT number	Status
MK-4621	RLR	I	Advanced solid tumor	Monotherapy (+/− pembrolizumab)	NCT03739138	Terminated
		I/II	Advanced solid tumor	Monotherapy	NCT03065023	Terminated
CV8102	RLR	I	Advanced melanoma, squamous cell carcinoma of the skin, squamous cell carcinoma of the head and neck, adenoid cystic carcinoma	Monotherapy (+/− SoC anti-PD-1 therapy)	NCT03291002	Unknown status
		I/II	Hepatocellular carcinoma	Combination with IMA970A plus Cyclophosphamide	NCT03203005	Completed
Vesicular stomatitis virus (VSV)	RLR signaling (oncolytic virotherapies)	I	Malignant solid tumor	VSV-IFNβ-sodium iodide symporter (NIS)	NCT02923466	Completed
		I	Myeloma, acute myeloid leukemia or lymphoma	VSV-IFNβ-NIS with or without cyclophosphamide or ipilimumab and nivolumab or cemiplimab	NCT03017820	Recruiting
		I	Endometrial cancer	VSV-IFNβ-NIS with or without ruxolitinib phosphate	NCT03120624	Active, not recruiting
		I	T Cell lymphoma	VSV-IFNβ-NIS with ipilimumab and cemiplimab	NCT06508463	Recruiting
		I	Melanoma	VSV-IFNβ-TYRP1	NCT03865212	Active, not recruiting
VSV-GP	RLR signaling (oncolytic virotherapies)	I	Pancreatic ductal adenocarcinoma	Combination with ATP150/ATP152 and ezabenlimab	NCT05846516	Recruiting
		I	Colorectal cancer	Combination with ATP128 and BI 754091	NCT04046445	Active, not recruiting
Pexa-Vec (JX-594)	RLR signaling (oncolytic virotherapies)	I	Solid tumor	Combination with ipilimumab	NCT02977156	Completed
		I/II	Local progression or metastatic melanoma	Combination with ZKAB001	NCT04849260	Unknown status
		I/II	Soft-tissue sarcoma and breast cancer	Combination with metronomic CP	NCT02630368	Recruiting
		I/II	Colorectal cancer	Combination with immune checkpoint inhibition	NCT03206073	Completed
		I/II	Hepatocellular carcinoma	Combination with nivolumab	NCT03071094	Terminated
Talimogene laherparepvec (T-VEC)	RLR signaling (oncolytic virotherapies)	II	Sarcoma	Combination with pembrolizumab	NCT03069378	Active, not recruiting
		II	Melanoma	Combination with nivolumab	NCT04330430	Active, not recruiting
Pelareorep	RLR signaling (oncolytic virotherapies)	I	Breast cancer	Combination with paclitaxel	NCT05519059	Completed
		I	Breast cancer	Monotherapy (+/− atezolizumab)	NCT04102618	Terminated
		I	Pancreatic adenocarcinoma	Combination with pembrolizumab	NCT02620423	Completed
		II	Breast cancer	Combination with paclitaxel and avelumab	NCT04215146	Active, not recruiting
		II	Breast cancer	Combination with INCMGA00012	NCT04445844	Recruiting
		II	Pancreatic adenocarcinoma	Combination with pembrolizumab	NCT03723915	Terminated

(*continued on next page*)

Table 2 (*continued*)

Drug	Target	Phase	Disease	Treatment	NCT number	Status
		II	Pancreatic cancer	Combination with paclitaxel/carboplatin	NCT01280058	Completed
		II	Pancreatic adenocarcinoma	Combination with gemcitabine	NCT00998322	Completed
		II	Breast cancer	Combination with paclitaxel	NCT01656538	Completed
		II	Non-small cell lung cancer	Combination with docetaxel or pemetrexed	NCT01708993	Completed
Azacitidine	RLR signaling (imitation of viruses)	I/II	Leukemia	Combination with avelumab	NCT02953561	Terminated
		II	Leukemia	Combination with nivolumab and ipilimumab	NCT02397720	Completed
		II	Colorectal cancer	Combination with pembrolizumab	NCT02260440	Completed
Decitabine	RLR signaling (imitation of viruses)/CIITA	I/II	Leukemia	Combination with pembrolizumab	NCT02996474	Completed
		I/II	Malignancy	Combination anti-PD-1 antibody	NCT02961101	Unknown status
		II	Hodgkin lymphoma	Combination with SHR-1210	NCT03250962	Recruiting
Guadecitabine (SGI-110)	RLR signaling (imitation of viruses)	I	Melanoma	Combination with ipilimumab	NCT02608437	Unknown status
		I	Solid tumor	Combination with pembrolizumab	NCT02998567	Recruiting
Vorinostat	HDAC	I/II	Lung cancer	Combination with pembrolizumab	NCT02638090	Active, not recruiting
		II	Squamous cell carcinoma	Combination with pembrolizumab	NCT04357873	Active, not recruiting
		II	Breast cancer	Combination with pembrolizumab and tamoxifen	NCT04190056	Terminated
		II	Prostate cancer	Combination with ^{177}Lu-PSMA-617	NCT06145633	Recruiting
Entinostat	HDAC/CIITA	I	Breast cancer	Combination with capecitabine	NCT03473639	Completed
		I	Fallopian tube cancer	Combination with olaparib	NCT03924245	Terminated
		I	Prostate cancer	Combination with enzalutamide	NCT03829930	Terminated
		I/II	Non-small cell lung cancer, melanoma, and colorectal cancer	Combination with pembrolizumab	NCT02437136	Completed
		I/II	CNS tumor, solid tumor	Combination with nivolumab	NCT03838042	Recruiting
		II	Uveal melanoma	Combination with pembrolizumab	NCT02697630	Completed
		II	Bladder cancer	Combination with pembrolizumab	NCT03978624	Active, not recruiting
		II	Melanoma	Combination with pembrolizumab	NCT03765229	Completed
Radio-chemotherapy	RLR signaling (triggered by radio-chemotherapy)	I	Small cell lung cancer	Combination with nivolumab/ipilimumab	NCT03223155	Active, not recruiting
		II	Non-small cell lung cancer	Combination with pembrolizumab	NCT03631784	Completed
		II	Non-small cell lung cancer	Combination with pembrolizumab	NCT02492568	Completed
		II	Colorectal cancer, pancreatic cancer	Combination with nivolumab, ipilimumab	NCT03104439	Recruiting

Table 2 (*continued*)

Drug	Target	Phase	Disease	Treatment	NCT number	Status
		II	Squamous cell carcinoma of the head and neck	Combination with pembrolizumab/ cetuximab	NCT02707588	Completed
		III	Non-small cell lung cancer	Combination with MEDI4736	NCT02125461	Completed
		III	Prostate cancer	Monotherapy (+/− ipilimumab)	NCT00861614	Completed
		III	Brain cancer	Combination with nivolumab/temozolomide	NCT02617589	Completed
		III	Brain neoplasm	Combination with temozolomide	NCT02667587	Completed

RLR, retinoic acid-inducible gene 1-like receptor; HDAC, histone deacetylase.

systemic administration of RLR agonists could lead to varied results. Furthermore, a preliminary study reveals that merging systemic anti-PD1 treatment with the intratumoral delivery of CV8102, a cationic peptide-bound ssRNA stimulant of TLR7/8 and RIG-I, can trigger an antitumor immune reaction[488]. The completion of a Phase I trial assessing the effectiveness of intratumoral CV8102 administration, either singly or alongside systemic anti-PD1 treatment, in individuals with advanced melanoma, skin, HNSCC, or adenoid cystic carcinoma is anticipated shortly. Additionally, a Phase I/II trial involving CV8102 and HepaVac-101, a therapeutic vaccine for HCC, reveals the efficacy of this treatment in triggering immune reactions[489].

Despite the scarcity of clinical studies on the systemic delivery of 5′-pppRNA, numerous trials have been carried out to assess the safety and effectiveness of administering poly I:C, a synthetic dsRNA analogue aimed at TLR3 and MDA5. Insights can be gained from the practice of administering poly I:C either intravenously or intramuscularly. Three poly I:C-based compounds, Rintatolimod, Hiltonol, and BO-112, have progressed to the stage of clinical development[490]. Hiltonol and BO-112 have been safely administered intratumorally, subcutaneously, or intramuscularly to patients with cancer[491-494]. Significantly, research using mouse tumor models shows that Hiltonol's intratumoral administration was markedly less efficient than systemic delivery[235,495]. From a mechanistic standpoint, Hiltonol's enhanced effectiveness against tumors could stem from its ability to activate MDA5 in immune cells originating from bone marrow and tumor vascular endothelial cells. This stimulation results in the generation of type I IFN and chemokines that attract T cells, like CXCL9/CXCL10, thereby encouraging the infiltration of tumor T cells[235]. Future clinical trials might explore the widespread administration of RLR agonists.

3.4.2. Oncolytic viruses

Following the US FDA's 2015 approval of talimogene laherparepvec (T-VEC, IMLYGIC), the initial oncolytic virus medication, numerous oncolytic virotherapies have undergone clinical trials[495,496]. Considering dsRNA as the primary ligand for RLRs, this analysis will concentrate on oncolytic RNA viruses capable of triggering RLR signaling. Administering VSV, infused with IFN-β, intravenously to 15 individuals suffering from relapsed, resistant blood cancers presents no limiting toxic effects and demonstrates promising dose−responsive effectiveness in those with advanced, treatment-resistant T cell lymphoma[497]. In an effort to mitigate VSV's neurotropic effects, a modified VSV (VSV-GP) was engineered, replacing its neurotropic glycoprotein G with the non-neurotropic GP found in the LCMV, as documented in previous studies[498,499]. Early-stage research indicates that administering this recombinant VSV intratumorally and intravenously is efficient in preventing tumor expansion and metastasis[500]. The commencement of a Phase I clinical trial aims to assess the safety and initial effectiveness of administering VSV-GP either intratumorally or intravenously, either solely or alongside ezabenlimab[498]. Pexa-Vec (JX-594), a different recombinant VSV lacking the thymidine kinase gene, reduces VSV replication in cancerous tissues[501]. Administering Pexa-Vec intravenously before surgery could activate immunity against cancer and aid in the treatment of individuals with cancer metastasis[502]. Additional research is needed to ascertain whether deactivated VSV can work in conjunction with an ICI in treating patients with cancer.

Earlier clinical studies have shown the safe combination of the naturally existing reovirus type 3 Dearing and the unmodified serotype 3 reovirus pelareorep with standard chemotherapy in patients with advanced cancer[503-505]. Among 34 patients inexperienced with chemotherapy and suffering from severe pancreatic adenocarcinoma, administering pelareorep and gemcitabine intravenously induces a partial improvement in one individual and brings stability to the condition in 23 others[506]. In a Phase II, randomized trial, the combined use of pelareorep and paclitaxel in treating metastatic BC resulted in a notably extended OS rate, with no variance in PFS[507]. Nonetheless, Phase II randomized trials combining pelareorep and paclitaxel in individuals with preexisting, advanced, or metastatic NSCLC, or those with untreated metastatic pancreatic adenocarcinoma, failed to demonstrate enhanced PFS[505,508]. Concerning the use of pelareorep alongside an ICI, a Phase Ib study indicates that the pelareorep−pembrolizumab duo in treating patients with pancreatic adenocarcinoma is generally well-received and maintains extended effectiveness in certain cases[509]. An ongoing Phase II trial is being conducted, focusing on pelareorep and pembrolizumab as an alternative therapy for pancreatic adenocarcinoma. Given that earlier research suggests reovirus replication isn't essential for creating human antitumor immunity, additional investigations are needed to ascertain if intravenous administration of deactivated reovirus can prepare "immune-cold" tumors for reacting to ICIs.

3.4.3. Viral mimicry

Concerning the imitation of viruses, a randomized Phase III trial involving patients with AML reveals that the safety profile of oral azacitidine maintenance is largely positive[510]. Clinical trials have

thoroughly assessed the integration of viral imitation and ICIs. A Phase II clinical trial reveals that azacitidine, when used alongside nivolumab, is safe for tolerability, and adverse effects linked to the treatment encompass neutropenia, anemia, and immune-related complications like pneumonitis[511]. The combination of azacitidine and nivolumab seems to be a potent treatment for recurrent or resistant AML, particularly in patients who have recovered, have not previously used hypomethylating agents, or exhibited heightened pretherapy $CD3^+$ bone marrow infiltration[511].

A further Phase Ib/II investigation into the effects of azacitidine and PD-L1 antibody avelumab on relapsed/refractory AML indicates a lack of clinical advantage from this therapy, likely owing to the heightened expression of PD-L2 in these individuals and the small proportion of subjects new to hypomethylators[512]. An initial investigation into the effects of decitabine and pembrolizumab, a PD1 antibody, on adults with resistant or recurrent AML revealed that 6 out of 10 patients exhibited the most favorable outcomes for stable conditions or more favorable ones[513]. Yet, a Phase II clinical trial reveals that the combination of pembrolizumab and azacitidine offers limited effectiveness in managing mCRC resistant to chemotherapy[514]. Furthermore, a Phase II trial involving decitabine and PD1 antibody camrelizumab in treating relapsed or refractory classical Hodgkin lymphoma indicates that the decitabine−camrelizumab duo is more effective than using camrelizumab alone[515]. Despite the recurrence of classical Hodgkin lymphoma following previous camrelizumab monotherapy, a mix of decitabine and camrelizumab remained linked to elevated response rates and enhanced PFS[516]. Following these encouraging outcomes, the commencement of Phase III clinical trials for decitabine and camrelizumab or talacotuzumab in treating AML and Hodgkin lymphoma has been undertaken.

Additionally, the fusion of guadecitabine and ipilimumab, a cutting-edge hypomethylator, is deemed safe and bearable for individuals with inoperable melanoma, exhibiting encouraging immunomodulatory and antitumor effects[517]. A further Phase I study reveals that the combination of guadecitabine and pembrolizumab is bearable, exhibiting both immunomodulatory and anticancer effects in individuals with advanced solid tumors, myelodysplastic syndromes (MDS), or AML[518]. This research shows a reversal of prior resistance to ICIs[518]. Trials are underway to evaluate the efficacy of histone deacetylase (HDAC) inhibitors like vorinostat combined with pembrolizumab in treating BC. The pembrolizumab−vorinostat duo is generally well-received and exhibits initial anticancer effects, even after advancing in previous ICI treatments for advanced/metastatic NSCLC[519].

A different Phase II research suggests that merging entinostat, an HDAC inhibitor, with pembrolizumab results in lasting effects in certain individuals with metastatic uveal melanoma[520]. Furthermore, the combination of pembrolizumab and entinostat in treating patients with PD-L1 antibody-resistant/refractory NSCLC yields significant clinical advantages, evidenced by an ORR of 9% of patients[521]. Baseline concentrations of circulating classical monocytes could serve as a possible biomarker for the efficacy of this treatment[521]. There is an absence of a registered Phase III clinical trial for the combined use of HDAC inhibition and immune checkpoint blockade in cancer treatment.

3.4.4. Radio-chemotherapy

Given that DNA damage caused by radio-chemotherapy can trigger RLR signaling and provoke an immune reaction, the integration of radio-chemotherapy and ICIs has undergone assessment in clinical trials[522,523]. The PACIFIC randomized trial revealed that administering durvalumab, an anti-PD-L1 antibody, to patients with stage III NSCLC who show no disease advancement post-platinum-based chemoradiotherapy enhances their OS[524]. Results from the randomized PEMBRO-RT trial indicate that administering pembrolizumab to patients with NSCLC following three rounds of 8 Gy radiotherapy enhances both the response rates and median survival rate[525].

A different study involving patients with castration-resistant prostate cancer reveals that combining radiotherapy with ipilimumab immunotherapy markedly enhances survival rates, in contrast to those treated solely with ipilimumab[526]. Furthermore, initial findings from a Phase II study suggest that radiation treatment could improve the effectiveness of immune checkpoint blockade in cases of MSS colorectal and pancreatic adenocarcinoma[527]. The degree of involvement of RLRs in reacting to radioimmunotherapy is yet to be determined.

Although combining radiotherapy with immune checkpoint blockade might be beneficial in certain cancers, some clinical studies do not demonstrate that radiotherapy with immune checkpoint blockade outperforms the combination of radiotherapy with chemotherapy or molecular-targeted therapy[528-531]. Administering pembrolizumab along with radiotherapy to individuals suffering from locally advanced squamous cell carcinoma in the head and neck area does not enhance tumor management or survival rates when compared with the cetuximab (anti-EGFR monoclonal antibody)-radiotherapy group, and the toxicity appears to be reduced in the pembrolizumab−radiotherapy group[531].

In a Phase III randomized study involving patients with GB with an unmethylated methylguanine-DNA methyltransferase (MGMT) promoter, it was found that the median survival duration for those receiving conventional radiotherapy and nivolumab is less than those on radiotherapy and temozolomide[529]. A different Phase III randomized CheckMate 548 trial, focusing on patients recently identified with GB having a methylated or indeterminate MGMT promoter, revealed that the combination of nivolumab, radiotherapy, and temozolomide fails to enhance progression-free and OS rates compared to conventional treatments (radiotherapy plus temozolomide)[530]. Conversely, a Phase II, nonrandomized trial suggests that pembrolizumab, when used alongside chemoradiation therapy, shows potential in combating stage III NSCLC in patients who have not received prior treatment and are locally advanced[523]. Pinpointing predictive biomarkers and scheduling radiotherapy and immune checkpoint blockade could be crucial for enabling certain patients with cancer to benefit from combined therapy[532].

4. Targeting NLRs for cancer immunotherapy

4.1. NLRs

NLRs, a type of intracellular PRR, consist of three distinct segments: the central nucleotide-binding domain or NACHT domain (shortened as NLR family's apoptosis inhibitory protein (NAIP), class II MHC transactivator (CIITA), HETE, TP1), a shared element among NLRs crucial for NLRs' nucleic acid binding and oligomerization; the C-terminal leucine-rich repeats (LRRs) for ligand identification; and the N-terminal effector domain, a protein interaction area, such as the CARD or pyrin domain (PYD)[533-538].

To date, over 20 members have been documented in the NLR family. Based on the diversity and characteristics of the N-terminal domain, human NLRs are categorized into four distinct subgroups[534,539,540]: (1) NLRA subfamily consists of a single entity, the CIITA, which includes an N-terminal activation

domain[541]; (2) NLRB subfamily consists solely of the NAIP, which includes a Bir (baculovirus 'inhibitor of apoptosis' repeat) domain at its N-terminal end; (3) NLRC subfamily, comprising six members—NLRC1 (NOD1), NLRC2 (NOD2), NLRC3, NLRC4, NLRC5, and NLRX1—features either a CARD or a CARD-related X domain[536,542]; (4) and NLRP subfamily, distinguished by its PYD domain, comprises 14 distinct members—NLRP1, NLRP2, NLRP3, NLRP4, NLRP5, NLRP6, NLRP7, NLRP8, NLRP9, NLRP10, NLRP11, NLRP12, NLRP13, and NLRP14[542,543].

Typically, the LRR domain of an NLR molecule adopts a U-shaped conformation with its central NACHT domain, preventing its multimerization and rendering the NLRs inactive[544]. When PAMPs attach, either directly or indirectly, to the LRRs, the NLR molecule alters its structure, revealing the NACHT oligomerization area that initiates oligomerization, thereby activating the NLR molecule[545]. Concurrently, the N-terminal effector domain becomes exposed, and through homotypic interactions, subsequent adaptor molecules and similarly structured signaling proteins are engaged to initiate the related signal transduction[546]. Each of these NLRs functions as a crucial activator, either directly or indirectly involved in inflammatory caspases following the detection of infection or cellular harm[547].

4.2. *An overview of NLRs signaling*

It appears that the signaling process of NLRs is preserved across the entire family[548,549]. The recognition of a PAMP by the LRR domain of an NLR triggers structural alterations in the NLR, facilitating the swapping of ADP/GDP for ATP/GTP and its formation into complex scaffolds, thereby initiating subsequent signaling[550-552] (Fig. 4). CIITA's activation, a key controller of

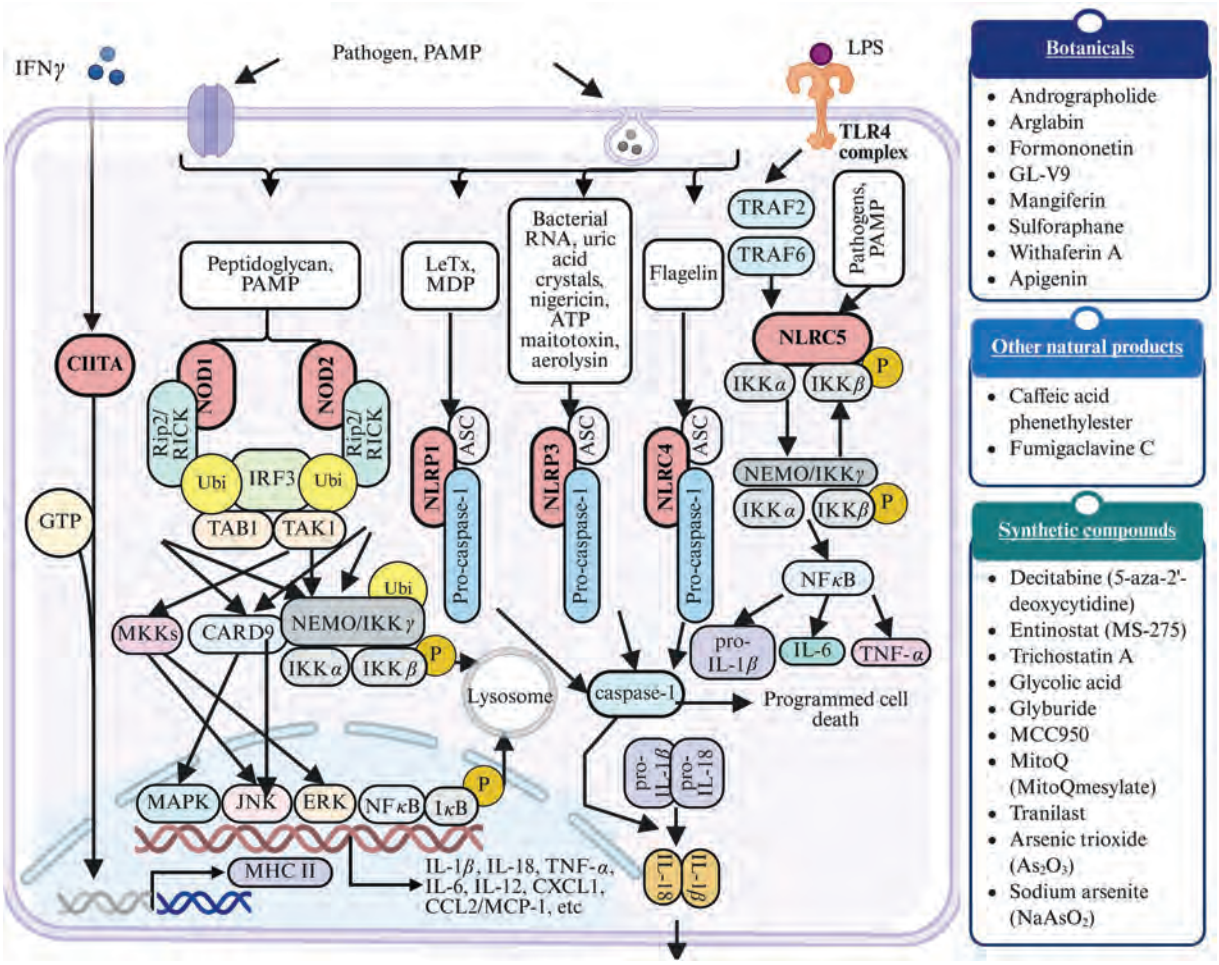

Figure 4 NLR signaling pathway. NLRs are activated by intracellular PAMPs and DAMPs, leading to the assembly of inflammasomes. The activated NLRs recruit ASC and caspase-1, resulting in the release of IL-1β and IL-18 and inducing pyroptosis, which contributes to the immune response. NOD, nucleotide-binding oligomerization domain; NLR, NOD-like receptors; PAMP, pathogen-associated molecular patterns; CIITA, class II major histocompatibility complex transactivator; CARD, caspase activation and recruitment domain; MAPK, mitogen-activated protein kinase; MKK, MAPK kinase; Ubi: ubiquitin; TAK1, TANK-binding kinase 1; TAB1, TAK1-binding protein 1; NEMO, NF-κB essential modulator; IκB, inhibitor of NF-κB; IKK, IκB kinase; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated protein kinase; NF-κB, nuclear factor-κB; MHC II, major histocompatibility complex II; IL, interleukin; TNF: tumor necrosis factor; CXCL, C−X−C motif chemokine ligand; CCL2, C−C motif chemokine ligand 2; MCP-1, monocyte chemotactic protein-1; NLRP, nucleotide-binding oligomerization domain-like receptor family pyrin domain; ASC, apoptosis-associated speck-like protein containing CARD; NLRC, nucleotide-binding oligomerization domain-like receptor family CARD domain; LPS, lipopolysaccharide; TLR, toll-like receptor; TRAF, tumor necrosis factor receptor-associated factor.

adaptive immune reactions, is actively controlled through post-translational modifications (PTMs), including acetylation, phosphorylation, and ubiquitination[553,554]. Following phosphorylation and ubiquitination by ERK1/2, there is an enhancement in the oligomerization and movement of CIITA into the nucleus[555-557]. The dynamic self-linked CIITA serves as a stage for engaging and interacting with an enhancedosome complex, triggering the transcription of MHC-II genes[553,558,559]. The MHC-II complexes play a key role in introducing extracellular antigens to CD4 T-cells[560,561]. A variety of cytokines linked to MHC-II, such as IFN-γ, TNF-α, TGF-β, and IL-1/4/10, to increase or decrease the levels of CIITA[558].

The proteins NOD1 and NOD2 play a crucial role in identifying segments of bacterial peptidoglycan, essential for the structure of bacterial cell walls. The activation of NOD1 and NOD2 multimers triggers the autophosphorylation of RIP2 kinase (alternatively RICK, CARDIAK, CCK, or RIPK2), leading to the stimulation of the NF-κB and MAPK pathways[562-565]. The NLRC4 and NAIP enzymes detect flagellin, a key element of the bacterial flagellum[566,567]. NLRP1 is reactive to the deadly anthrax toxin and muramyl dipeptide[568-570]. The activation of NLRP3 occurs through the binding of bacterial RNA, uric acid crystals, ATP, nigericin, maitotoxin, aerolysin, and various other molecules[571-574]. The involvement of NLRC4, NAIP, NLRP1, and NLRP3 multimers in attracting apoptosis-associated speck-like protein containing CARD (ASC) triggers the activation of caspase-1 (CASP1), leading to the release of IL-1β and subsequent apoptosis[550,575,576]. Conversely, the activation of NLRC5 through bacterial LPS-triggered TRAF2/6 pathways leads to the suppression of NF-κB signaling routes, culminating in reduced quantities of diverse cytokines[577,578].

4.3. NLR expression in different types of cells of TME

4.3.1. NLR expression and function in immune cells

The NLR family members play a dual role in cancer related to the immune system[20]. Within the BC linked to obesity, the NLRC4 inflammasome−IL-1β route in macrophages stimulates fat cells to release VEGF, thus aiding in blood vessel formation[579]. In the context of colorectal cancer, NOD1 triggers the activation of ATG16L1, a protein involved in autophagy, which in turn elevates arginase 1 levels in myeloid cells, thus amplifying the immunosuppression of T cells[580]. In the context of lung cancer, the interplay between NLRP3 inflammasome and IL-1β in immune cells escalates inflammation, boosts the production of chemokines, and suppresses NK cells[581,582].

In an implanted thymoma tumor model, chemotherapy medications activate the cathepsin B−NLRP3−IL-1β route in MDSCs, leading to IL-17 production by CD4 T cells and diminishing the cancer-fighting effects of chemotherapy[583]. Regarding colorectal cancer, inflammation is triggered by immune cells' TLR2, NOD1, and NLRP3, which amplify their anticancer effects. Other receptors akin to NODs (NLRP6, NLRP12, and NLRC3), in conjunction with cGAS, hinder processes that encourage tumor development[584].

4.3.2. NLR expression and function in tumor cells

Colorectal cancer stands as a prime illustration of cancers linked to inflammation. Patients suffering from ulcerative colitis face a significant likelihood of developing colorectal cancer, a common type of idiopathic inflammatory bowel disease[585]. Researchers, encompassing 3117 patients with ulcerative colitis, approximated the incidence of colorectal cancer in these individuals to be 5.7 times greater than in the control group[585]. In evaluating NLR signaling's role in CAC, Chen and team analyzed a well-known mouse model lacking NOD1 for CAC, revealing a link between CAC and colon tumors associated with APC[586]. The presence of NOD1 in T-cells curtails the development of intestinal tumors linked to colitis through the modulation of IFN-γ signaling pathways[587]. Studies involving NOD2-deficient mice and patients with colitis also revealed a predisposing impact of NOD2 in CAC[588,589]. NOD2 hinders the development of CAC tumors by reducing NF-κB and MAPK pathways, leading to the activation of IRF4[590]. Allen and his team observed a rise in both acute and recurrent CAC in *Nlrp3*$^{-/-}$ mice relative to normal mice, indicating NLRP3's role in reducing CAC carcinogenesis[591]. Within colorectal cancer cells SW620, HCT116, and HT29, TNF-α and TGF-β1 triggered an increase in NLRP3 levels and facilitated the transition from epithelial to mesenchymal cells, independent of inflammasomes[592].

Different research indicated that the NLRP3 inflammasome hindered the spread of colorectal cancer in the liver through the disruption of IL-18 signaling and the enhancement of tumor-killing functions in liver NK cells[593]. Nonetheless, the precise functions of NLRP3 in colorectal cancer are still a matter of debate. Recent research has revealed that colorectal cancer cells, heavily encircled by macrophages, exhibit elevated NLRP3 levels and accelerated movement towards the liver. Inhibiting NLRP3 signals led to a decrease in the movement of colorectal cancer cells *in vitro* and their metastasis *in vivo*[593]. Additional NLRs play a role in the development of CAC tumors. Studies involving *Nlrp1*$^{-/-}$ mice have demonstrated NLRP1's role as a defensive controller in CAC[594]. Allam and colleagues noted that mice lacking NAIP showed heightened STAT3 activity and hindered P53 activation, leading to more tumor development, indicating NAIP's defensive function in CAC[595]. NLRC3 fosters cell death to reduce the development of colorectal tumors. From a mechanistic standpoint, NLRC3 inhibits MYC, FOXO3AF, and FOXO1 by blocking PI3K, TRAF6, and mTOR in the colon of *Nlrc3*$^{-/-}$ mice[596]. Additionally, the increased expression of BMI1 and OLFM4 stem cell markers in the colon of *Nlrc3*$^{-/-}$ mice indicates NLRC3's involvement in cell stemness[596].

NLRP6 inhibits the development of colorectal tumors through the control of epithelial self-renewal processes[597]. Inflammasomes of NLRP6 detect damage to intestinal tissues and trigger a decrease in IL-22BP dependent on IL-18, resulting in the growth of tumors due to excessively activated IL-22[598]. NLRP12 reduces atypical NF-κB signaling through TRAF3, AKT, and ERK routes, resulting in the inhibition of colorectal cancer development[599]. A decrease in the levels of NLRC4 or NLRC5 is associated with an increased likelihood of developing colorectal cancer[600,601].

It has been demonstrated that CD, a common type of idiopathic inflammatory bowel disease, is intimately linked with colorectal cancer. A variety of alterations in the NOD2 gene have been pinpointed in CD cases[602,603]. A recent study employing quantitative reverse transcription PCR revealed activation of NLRP3 inflammasome in 60.0% of patients with CD, in contrast to 28.6% in control subjects ($P = 0.042$) [604].

The bacterium *H. pylori*, characterized by its gram-negative, curved rod structure, is commonly located under the mucus covering the gastric epithelium[605]. Statistical research indicates a significant link between *H. pylori* infection and a heightened risk of GC, leading to the belief that the infection plays a role in the development of GC. Similarly, individuals testing positive

serologically for *H. pylori* are at an increased risk of contracting mucosa-associated lymphoid tissue (MALT) lymphoma[606].

It was discovered that NOD1 and NOD2 have a connection to *H. pylori* infection and stomach lesions[607,608]. Research into the mechanisms showed that the activation of TRAF3 by NOD1 suppresses the CDX2 expression triggered by *H. pylori* infection in AGS and GSM06 GC cells[607]. A lack of NLRP6 showed notable correlations with the spread to lymph nodes and reduced survival rates in GC cases[609]. NLRP6 hindered the development of tumors through the P14ARF-MDM2−P53-dependent aging process in stomach cancer cells[609]. Research on human monocytic cells and neutrophils has shown that infection by *H. pylori* triggers the production of IL-1β and IL-18 by activating NLRP3 inflammasomes[610,611]. Furthermore, despite the significant increase in NLRP3 expression in GC, recent findings indicate that inhibiting NLRP3 with MIR22 microRNA can reduce the carcinogenesis caused by *H. pylori*[612]. Additionally, it has been documented that NLRP3 stimulates the production of cyclin-D1 in gastric epithelial cells, leading to increased cell growth and cancer development[612].

Growing evidence indicates a link between infections of the HBV and HCV and the emergence of HCC[613]. Compared to non-carriers, carriers of HBV surface antigen and anti-HCV antibodies or HCV RNA face a 13.7- and 11.5-fold increased risk of HCC, respectively[614]. Elevated NLRP3 levels in patients with chronic HBV infection indicate NLRP3's critical role as an internal detector of HBV infection, crucial for triggering inflammatory reactions through the stimulation of IL-1β and IL-18 secretion[615]. Systems biology studies suggest that NLRP3 signaling is responsible for increasing IL-1β levels in the serum of individuals with long-term HCV infection[616].

In a clinical trial involving patients with HCC, Wei and team evaluated NLRP3 levels across various hepatocarcinogenesis phases, uncovering a notable link between NLRP3 expression and HCC[617]. Significantly, there was an upsurge in NLRP3 expression during hepatitis and cirrhosis, yet it diminished as HCC emerged[617]. The enhancement of NLRP3 inflammasome by estrogen through the estrogen receptor-β signaling route has been demonstrated to inhibit HCC[618]. Furthermore, additional research has shown that the increase in NLRP3 due to stanniocalcin-1 diminishes the size of HCC tumors[619]. Approaches that activate NLRP3 might thus provide therapeutic advantages in HCC linked to HBV and HCV. In contrast, it has been demonstrated that NLRC5 levels increase in HCC. Eliminating NLRC5 has been demonstrated to suppress the growth, movement, and penetration of HCC cells by focusing on the Wnt/β-catenin signaling route[620]. Furthermore, recent studies have shown that the mRNA quantities of NOD2 and NLRX correlate with separate prognoses[621]. Consequently, it is proposed that NOD2 and NLRX might serve as valuable predictive indicators in HCC post-hepatectomy.

Patients suffering from *Schistosoma haematobium* face a roughly fivefold heightened risk of bladder cancer. Consequently, the International Agency for Research on Cancer regards *S. haematobium* as a clear contributor to bladder cancer. Latest research has shown significant activation of NLRP1 and NLRP3 inflammasomes in cases of schistosomiasis (*Schistosoma japonicum*) infections[622]. Subsequent research indicated that the activation of NLRP3 inflammasomes hinges on the stimulation of spleen tyrosine kinase (Syk), a process intimately linked to cancer related to schistosomiasis[622]. Furthermore, the activation of NLRP3 inflammasomes in *S. japonicum* infections is influenced by Dectin-1 and JNK signaling pathways[622]. Activation of the NLRP3 inflammasome triggers the production of IL-1β, activation of CASP1, and ASC signaling, hinting at NLRP3's potential involvement in bladder cancer linked to schistosomiasis[622].

4.3.3. NLR expression and function in epithelial cells

NOD1 and NOD2 hold the distinction of being the initial NLRs identified as intracellular microbial detectors[623]. Activating NOD1 or NOD2 leads to the release of proinflammatory cytokines and chemokines, along with the generation of antimicrobial peptides by IECs. Mice devoid of either NOD1, NOD2, or a combination of both exhibit a high vulnerability to colitis caused by DSS and exhibit dysbiosis. Alterations in the NOD2 gene are associated with a heightened risk of developing CD[602,624]. Beyond the NOD proteins, NLRP6 is essential for sustaining gut balance and a robust intestinal microbiome[625]. The primary expression of NLRP6 is in IECs[626]. Mice lacking NLRP6 are more prone to intestinal inflammation and chemically triggered colitis, owing to NLRP6's crucial function in mucosal regeneration and growth[597,627]. Additionally, NLRP6 plays a role in maintaining the equilibrium of the intestinal microbiome, in part by the fundamental release of IL-18[625]. NLRP6 is linked with the inflammasome adapter, a speck-like protein associated with ASC, triggering the CASP1-dependent conversion of pro-IL-18 and pro-IL-1β into active IL-18 and IL-1β[628]. IL-18 regulates the production of antimicrobial peptides and the mucus excretion by IECs[629]. Beyond its vital function in sustaining the gut microbiome and controlling antibacterial responses, recent findings indicate NLRP6's involvement in managing enteric virus infections[630]. Mice devoid of NLRP6 were unable to regulate the multiplication of EMCV or MNV within their gastrointestinal system[630]. Fascinatingly, the activation of NLRP6 occurred due to viral RNA attaching to the RNA helicase DHX15 and engaging with the RLR signaling adapter MAVS, triggering both type I and type III IFNs, along with ISGs.

Recent research has shown that NLRP9B, uniquely present in IECs, impedes rotavirus replication[631]. NLRP9B identifies short dsRNAs originating from rotavirus (unlike long dsRNAs like those from EMCV) through the RNA helicase DHX9, subsequently creating the inflammasome, which triggers the release of active IL-18 and the pyroptosis of the infected cell. Consequently, IECs infected by rotavirus are ejected early from the epithelial layer to limit the virus's replication and dissemination[631]. NLRC4, known for detecting flagellin and bacterial secretions, exhibits a comparable process of ejecting infected or cancerous cells from the intestinal lining[629,632]. The expulsion of infected cells might also play a significant role in managing MNV or other viruses that infect IECs. Latest findings regarding NLRP6 and NLRP9B reveal that NLRPs, surprisingly, are crucial in combating enteric RNA viruses due to their interaction with RNA helicases containing the DHX[630,631].

4.4. NLRs pathway for cancer immunotherapy

Given the evident significance of NLRs in cancer development, the emergence of drugs targeting NLRs shows potential. Various plants and artificial compounds known to block NLR signaling are documented to diminish the growth, penetration, and blood vessel formation of tumor cells. In this review, we will summarize the drugs that target NLRs from three aspects (Table 3).

4.4.1. Synthetic compounds

Reinstating CIITA expression in cancer cells using inhibitors of histone deacetylases and DNA methyltransferase, due to the

Table 3 The drugs targeting NLRs.

Drug	Target	Phase	Disease	Treatment	NCT number	Status
Trichostatin A	CIITA	I	Hematologic malignancy	Monotherapy	NCT03838926	Unknown status
Andrographolide	NLRP3	II	Colorectal neoplasm	Combination with capecitabine	NCT01993472	Terminated
		III	Esophageal cancer	Monotherapy	NCT04196075	Completed
MitoQ	NLRP3	Not applicable	Breast cancer	Monotherapy	NCT05146843	Unknown status
Sulforaphane	NLRP3/NLRC4	I/II	Breast cancer	Monotherapy	NCT03934905	Recruiting
		Not applicable	Prostate cancer	Monotherapy	NCT03665922	Active, not recruiting
		Not applicable	Prostate cancer	Combination with allin	NCT04046653	Completed
Withaferin A	NLRP3	I/II	Ovarian cancer	Combination with iposomal doxorubicin	NCT05610735	Recruiting
Tranilast	NLRP3	II	Nasopharyngeal carcinoma	Monotherapy	NCT05626829	Recruiting
Arsenic trioxide	NLRP1/NLRP3, NAIP5/NLRC4	I/II	Ovarian cancer	Combination with fuzuloparib	NCT04518501	Recruiting
		I/II	Leukemia	Combination with all-*trans* retinoic acid	NCT05497310	Recruiting
		I/II	Colorectal cancer	Combination with D-isoascorbic acid (D-VC)	NCT05721872	Recruiting
		II	Pediatric cancer	Monotherapy	NCT06088030	Recruiting
		II	Neuroblastoma	Monotherapy	NCT03503864	Recruiting
		II	Leukemia	Monotherapy	NCT04687176	Recruiting
		II	Leukemia	Combination with all-*trans* retinoic acid	NCT04793919	Recruiting
Sodium arsenite	NLRP1/NLRP3, NAIP5/NLRC4	I	Small cell lung cancer	Combination with cisplatin	NCT01110226	Terminated

CIITA, class II major histocompatibility complex transactivator; NLRP, nucleotide-binding oligomerization domain-like receptor family pyrin domain; NLRC, nucleotide-binding oligomerization domain-like receptor family CARD domain; NAIP, nucleotide-binding oligomerization domain-like receptor family's apoptosis inhibitory protein.

transcriptional reduction of CIITA triggered by histone deacetylation, is linked to a notable drop in the survival rates of patients with cancer[633,634]. Reports indicate that treating rhabdomyosarcomas with histone deacetylase inhibitors (HDACi), such as trichostatin A, or a DNA methyltransferase inhibitor, 5-aza-2′-deoxycytidine[634], reinstates CIITA expression. Administering an HDACi, MS-275 (entinostat), led to a slight increase in CIITA and MHC-II levels in diffuse large B-cell lymphoma cells[633]. The combined anticancer properties of MS-275 and IL-2 were additionally noted in a mouse model of renal cell carcinoma with a healthy immune system[635]. Additional epigenetic alterations, such as the methylation of H3-K4 or the dimethylation of H3-K9, play a role in the transcriptional control of CIITA. Conducting a thorough and detailed examination of these epigenetic modification regulators will aid in the discovery of new anticancer agents.

The anti-allergic medication Tranilast attaches to NLRP3, inhibiting the formation of NLRP3 inflammasomes in macrophages. Furthermore, Tranilast has demonstrated significant preventative or healing impacts in mouse models of diseases linked to NLRP3[636]. Inhibiting NLRP3 using glyburide led to the prevention of bladder issues in a rat cystitis model, where NLRP3 was identified as a potential therapeutic target[637]. Furthermore, the presence of arsenic trioxide and sodium arsenite hindered the activity of NLRP1, NLRP3, and NAIP5/NLRC4 inflammasomes, thereby obstructing the autoproteolytic stimulation of CASP1 and IL-1β release from macrophages[638]. Additionally, MCC950 distinctly inhibited NLRP3 activation, resulting in a decrease of IL-1β production in a human multiple sclerosis mouse model[639].

It is also believed that CSCs play a pivotal role in the onset and advancement of cancer[640,641]. Recent research has uncovered a distinct positive link between the activation of NLRP3 inflammasomes and CSC indicators BMI1, ALDH1, and CD44 in tumor specimens from human HNSCC[642]. The underlying cause likely involves cytokines regulated by NLR during inflammation, such as IL-1α, IL-1β, IL-6, TNF-α, and TGF-β, which all aid in the advancement of stem cells in tumor development[643]. Research indicates that MCC950, which inhibits NLRP3, diminishes the *in vitro* levels of BMI1, ALDH1, and CD44, thereby slowing down tumor development in mice afflicted with HNSCC[639,642]. Another inhibitor of NLRP3 signaling is the mitochondria-focused antioxidant MitoQ, which has demonstrated efficacy in managing colitis[644]. Moreover, the hypermethylation of the NLRC4 promoter, triggered by glycolic acid, impedes the formation of the inflammasome in skin HaCaT cells[645].

4.4.2. Botanicals

The natural diterpenoid Andrographolide, derived from *Andrographis paniculata*, reduces the progression and tumor load of colon cancer caused by azoxymethane/dextran sulfate sodium in mice by inhibiting the NLRP3 inflammasome through

mitophagy[646]. Similarly, GL-V9, an activator of the small-molecule AMPK, triggers the breakdown of the NLRP3 inflammasome through autophagy, thus safeguarding against colitis and cancer development in CAC[647]. Formononetin, a mitochondria-focused antioxidant that can inhibit NLRP3, has demonstrated efficacy in managing colitis[648].

Additional plant-based substances could potentially prevent or treat cancers driven by NLRP. The organosulfur compound Sulforaphane, derived from cruciferous vegetables, reduced the activation of NLRP3 inflammasomes and the release of IL-1β in mice suffering from peritonitis[649]. Originating from *Acnistus arborescens* or *Withania somnifera*, Withaferin A effectively inhibited NLRP3 inflammasome activation and NF-κB signaling in mice with chronic pancreatitis[650]. Qu et al.[651] documented that mangiferin inhibited mastitis caused by LPS through the suppression of NF-κB and NLRP3 signaling pathways. The sesquiterpene lactone Arglabin, derived from *Artemisia glabella*, was found to suppress the NLRP3 inflammasome in ApoE2.Ki mice on a high-fat diet[652].

A significant number of small molecules aimed at NLRC have been discovered, showing potential in their anticancer properties. Sulforaphane, derived naturally from cruciferous vegetables, reduced the activation of NLRC4 and the release of IL-1β in mice suffering from peritonitis[649]. By altering NLRP6 signaling, independent of inflammasomes, Apigenin shielded mice from colitis[653].

4.4.3. Other natural products

It was shown that caffeic acid phenethyl ester inhibits CASP1 activation and IL-1β synthesis by reducing NLRP3 in primary mouse macrophages and mouse models of gouty arthritis[649]. An additional inhibitor of NLRP3 signaling, fumigaclavine C, has demonstrated efficacy in managing colitis[654]. Yet, the effectiveness of their treatment in CAC remains unclear.

5. Targeting cytosolic DNA sensors for cancer immunotherapy

5.1. Cytosolic DNA sensors

Given that cancer cells are characterized by an increased presence of cytosolic dsDNA originating from nuclear, mitochondrial, or microbial sources, cytosolic DNA sensors have recently become intriguing for their role in cancer[17]. Absent in melanoma 2 (AIM2)-like receptors (ALRs) represent a novel category of PRRs capable of identifying intracellular DNA[655]. At the C-terminus lies the DNA-binding domain HIN-200, while the N-terminus is the PYD[656-658]. The HIN-200 domain is responsible for recognizing and attaching to dsDNA. The N-terminal PYD attaches to the PYD in the ASC[659], facilitating inflammasome creation and the development and discharge of IL-1β and IL-18[660]. The binding affinity of AIM2 to DNA and its inflammasome's function rely on dsDNA, which is capable of forming filamentous formations along dsDNA. Nonetheless, in the absence of dsDNA, it is possible to create filaments at elevated protein levels[661-663]. ALRs are capable of both contributing to the innate immune reaction and controlling apoptosis, a process linked to tumor emergence and growth[664].

Another extensively researched cytosolic dsDNA sensor, namely cGAS and the endoplasmic reticulum (ER)-resident protein STING (also known as mediator of IRF3 activation, ER IFN stimulator, N-terminal methionine−proline−tyrosine−serine plasma membrane tetraspanner, and transmembrane protein), often shows increased expression in various human cancers (such as lung, brain, and kidney cancer)[16,665-667]. The MB21D1 gene encodes cGAS, also known as C6orf150, a 500-amino-acid protein, which identifies dsDNA in the cytosol[668]. cGAS's identification of dsDNA leads to a structural alteration in its activation loop, as it reorganizes the catalytic site. This rearrangement facilitates the integration of ATP and GTP into the catalytic pocket, resulting in the synthesis of cGAMP, a secondary messenger that effectively activates STING−TBK1 or T2K or NAK axis activation of the transcription factor IRF-3[669-672].

STING comprises five putative transmembrane regions that primarily reside in the ER and partly in mitochondria and mitochondria-associated membranes *via* its N-terminal transmembrane domains[673]. Comprising the initial 130 amino acids, STING's amino-terminal domain includes four transmembrane domains, while its final 250 amino acids create a spherical carboxy-terminal domain[57]. Situated in the cytosol, STING's carboxy-terminal domain guides TBK1 in phosphorylating and activating IRF-3, thereby activating type 1 IFNs[674]. cGAMP's attachment to the STING dimer's central crevice happens through hydrogen bond creation and hydrophobic interaction[675,676]. Consequently, cGAMP's attachment to STING triggers a structural alteration, leading to the release of the carboxy-terminal tail (CTT), which in turn attracts and stimulates TBK1 to phosphorylate the CTT[677,678]. This process of releasing and phosphorylating CTT plays a pivotal role in activating IRF3, making STING an essential part of the innate immune system and facilitating the activation of IRF3, type 1 IFNs (IFN-α and IFN-β), along with the release of various pro-inflammatory cytokines[677,678].

Given that dsDNA from both host and microbial sources activates innate immunity, leading to various molecular and cellular mechanisms linked to cancer development, including inflammation, antitumor immunity, DNA damage response, aging, and cell death[666], more clinical research is expected to uncover connections between dsDNA sensors and different human cancers.

5.2. An overview of cytosolic DNA sensors signaling

Research conducted in living organisms on the signaling of cytosolic DNA detectors in cancer predominantly focuses on the AIM2 and cGAS−STING axes of DNA detection. AIM2 oligomerizes on cytosolic dsDNA, initiating the nucleation of ASC adaptor filaments and inducing pro-CASP1 filament polymerization, thereby activating CASP1 through autoprocessing. This activation results in the production of pro-inflammatory cytokines (IL-1β and IL-18) and pyroptosis[658,662,679-681]. During cGAS−STING signaling, various pro-inflammatory cytokines and molecules are emitted when NF-κB is activated by the IKKβ[16,682-685]. The activation of IKKβ is facilitated by TRIM32 and TRIM56, which are cytosolic DNA synthesizers of ubiquitin chains attached to the NF-κB essential modulator or IKKγ53. Additionally, IKKβ plays a role in activating TBK1 and NF-κB. Consequently, TBK1−IKKβ creates a necessary positive feedback cycle for the robust production of cytokines and chemokines (IL-6, CCL2, CCL5, and CXCL10) in the cGAS−STING signaling pathway[686,687]. Beyond the release of pro-inflammatory cytokines *via* type 1 IFNs and NF-κB, the activation of STING also triggers the release of ADAM metallopeptidase domain 17 or TNF-α converting enzyme from the membrane-bound immune semaphorin SEMA4D/CD100 into a soluble state, leading to actions similar to pro-inflammatory cytokines[688]. The cGAS identifies cytosolic dsDNA for the synthesis

of cGAMP. The identification of cGAMP by STING triggers its dimerization, moving into the perinuclear area to attach to TBK1[673]. This interaction between STING and TBK1 is vital for initiating the subsequent signaling route[673,689,690]. STING is capable of identifying cyclic dinucleotides (CDNs) and cyclic nucleic acids, either directly or attached to cytosolic DNA detectors like cGAS, nuclear resident IFN-inducible protein 16 (IFI16), and helicase DDX41[691-693] (Fig. 5).

5.3. Cytosolic DNA sensors expression in different types of cells of TME

5.3.1. Cytosolic DNA sensors expression and function in immune cells

Investigations into the immune roles of TLRs, NLRs, ALRs, and assorted cytosolic DNA sensors in cancer mainly concentrate on the AIM2 and cGAS−STING pathways for DNA identification. Research on HCC has shown that the activation of AIM2 inflammasomes in Kupffer cells, the resident liver macrophages, initiates tumors associated with liver inflammation[694]. In mouse models of sarcoma, recognized for their immune response, and melanoma, infamous for its weak immune response, cGAS detects tumor DNA, triggering STING-dependent type I IFNs and inflammatory cytokine generation in DCs, which in turn activate NK cells and foster adaptive antitumor immunity. Furthermore, STING detects 2′3′-cGAMP, an outcome of cGAS in cancerous cells. During cell death, ATP is released by cells to activate the P2X7R−NLRP3 inflammasome-IL-1β pathway, which in turn promotes adaptive immune responses to tumors[695,696]. Bone marrow-derived immune cells exhibiting STING have the potential to accelerate skin cancer progression associated with inflammation from 7,12-dimethylbenz[*a*]anthracene (DMBA) by boosting the release of pro-inflammatory cytokines and chemokines[697].

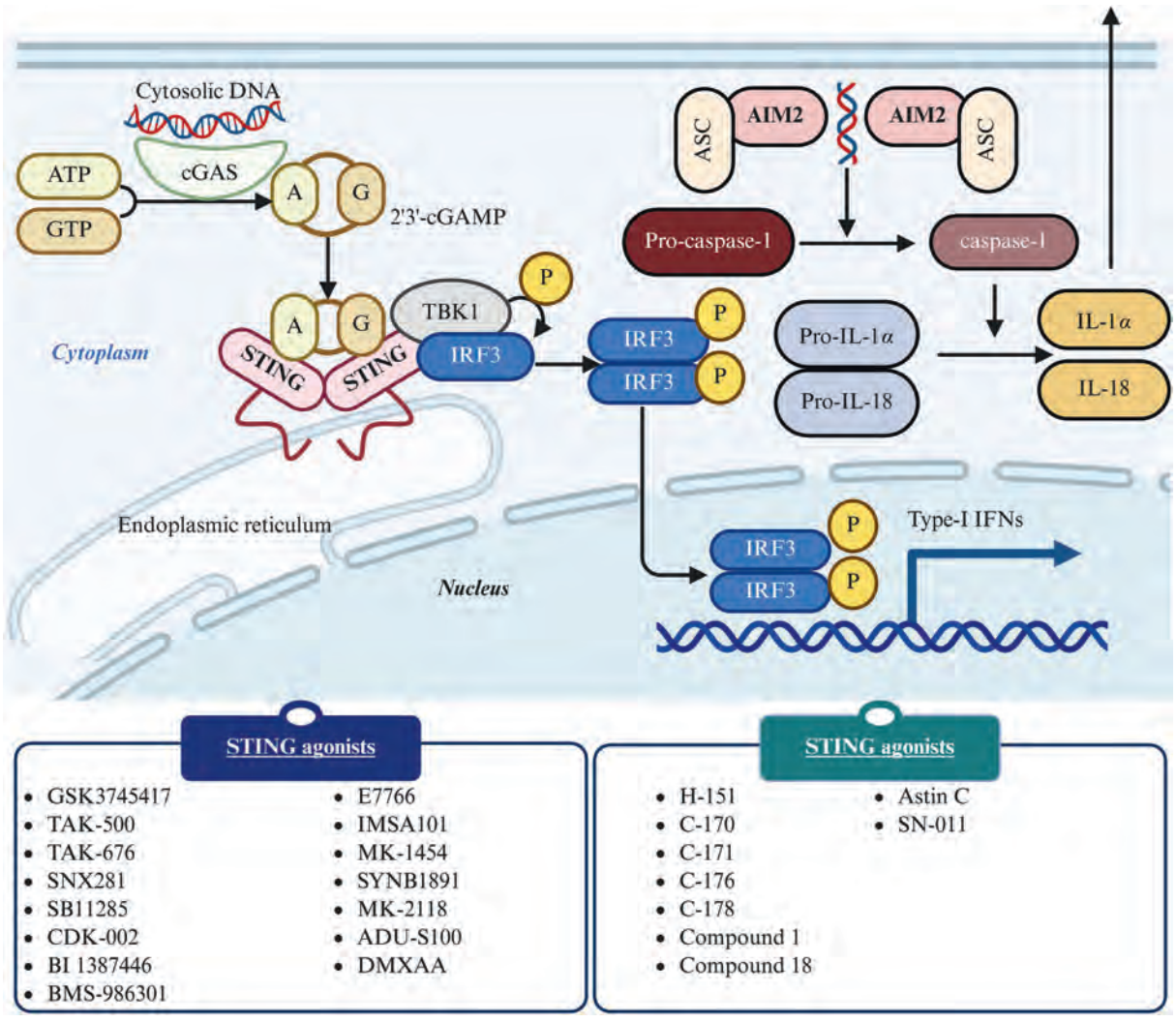

Figure 5 Cytosolic DNA sensors signaling pathway. Cytosolic DNA sensors, particularly through the cGAS−STING pathway, detect cytosolic double-stranded DNA (dsDNA), leading to the production of cGAMP, which activates STING on the endoplasmic reticulum. STING translocation to the perinuclear region recruits TBK1, resulting in IRF3 phosphorylation and type I interferon production. This pathway also activates NF-κB, inducing pro-inflammatory cytokines and contributing to immune responses against tumors. GTP, guanosine triphosphate; ATP, adenosine triphosphate; cGAS, cyclic GMP−AMP (cGAMP) synthase; IFN, interferon; STING, stimulator of IFN gene; TBK1, TANK-binding kinase 1; IRF, IFN regulatory factor; AIM2, Absent in melanoma 2; ASC, apoptosis-associated speck-like protein containing caspase activation and recruitment domain (CARD); IL, interleukin.

5.3.2. Cytosolic DNA sensors expression and function in tumor cells

The inherent instability of a tumor cell's genome enables it to trigger the cGAS−STING pathway[698]. When the micronuclear envelope breaks, it reveals the genomic DNA to the cytosol, and occasionally, mitochondrial malfunction leads to the emission of mitochondrial dsDNA[699]. This cGAMP, originating from tumor cells, can be introduced into immune cells *via* cell openings. A variety of recently discovered pathways facilitate the movement of 2′3′-cGAMP in the tumor setting, such as connexins[700], connexin 43-PCDH7 gap junctions[701], SLC19A1[702,703], SLC46A2[704], and volume-regulated anion channels LRRC8[704-706]. The redistribution of extracellular cGAMP through the control of these transporters could be utilized in treating cancers with reduced immunogenicity[707].

Occasionally, STING may also contribute to tumor growth amidst persistent inflammation. Unlike the suppression of tumors facilitated by the immediate activation of STING, persistent inflammation within the tumor milieu creates a conducive environment for cancer development. Take, for instance, the role of mutagenic DMBA, cisplatin, and etoposide in fostering skin cancer development through the generation of STING-dependent inflammatory cytokines and phagocytic invasion[697]. In a similar vein, tumor cells with chromosomal instability adopt prolonged activation of atypical NF-κB signaling following STING, leading to cell invasion and metastasis[699]. These brain metastatic cancer cells can commandeer the astrocyte STING pathway, thereby sustaining IFNα and TNFα production, which subsequently activate STAT1 and NF-κB pathways in brain tumor cells, fostering tumor growth and resistance[701]. Moreover, activating STING can lead to immunosuppression by attracting MDSCs *via* the CCR2 pathway[697] or by enhancing immunosuppressive proteins like PD-L1 and IDO1[708]. The fundamental process behind these divergent effects of STING activation in tumors warrants more research.

5.3.3. Cytosolic DNA sensors expression and function in epithelial cells

cGAS, the primary sensor of cytosolic DNA, produces cGAMP that attaches to the STING protein, STING, activating TBK1 to phosphorylate IRF3 and trigger type I IFNs, contributing to the preservation of the gut epithelial barrier post-irradiation and hematopoietic stem cell transplantation[467,709]. Additional research has shown an increased vulnerability of STING knockout mice to colitis caused by DSS or T cell transfer, linked to impaired mucus and IgA production, alterations in innate lymphoid cells (ILCs), and weakened Tregs, underscoring the significance of this route in preserving intestinal barrier functionality and averting inflammation[710]. Additionally, mice lacking STING exhibit a heightened vulnerability to cancer linked to colitis, attributed to the unregulated NF-κB and STAT3 signaling pathways[711].

The cGAS/STING pathway might be activated by DNA originating from commensal bacteria, internal DNA, or enteric virus DNA. Reports indicate that the cytosolic DNA detectors AIM2 and IFI16 are instrumental in identifying DNA viruses, notably herpesviruses[712-714], with increased concentrations of AIM2 and IFI16 noted in the mucosal layers of individuals with active inflammatory bowel diseases (IBD)[715]. Activated by bacterial or viral cytosolic DNA and irradiated nuclear DNA, the AIM2 inflammasome shields mice from colitis by stimulating IL-18-induced production of antimicrobial peptides and Akt-induced production of tight junction proteins[716-718].

5.4. Cytosolic DNA sensors for cancer immunotherapy

STING stands as an exceptionally appealing and hopeful focus for cancer immunotherapy. The immediate stimulation of STING within the tumor's microenvironment results in significant and widespread tumor shrinkage and immune response[719]. However, there are instances where STING may contribute to tumor growth amidst ongoing inflammation. Therefore, utilizing drugs to combat STING presents significant challenges. The research on drugs targeting STING can be divided into two aspects: agonists and inhibitors (Table 4). Due to a paucity of preclinical *in vivo* studies using bona fide spontaneous or experimentally induced cancer models coupled to genetic modulation of endogenous AIM2, and as many of the tumor-related activities of AIM2 reported thus far have been independent of inflammasomes, the role of AIM2 in its native cellular and tissue contexts in cancer is ill-defined. We look forward to more studies on AIM2-related treatment in the future.

5.4.1. STING agonists

A variety of clinical studies involving STING agonists are currently underway. During the preliminary phases, multiple approaches are concurrently functioning to create modulators aimed at STING. The simplest approach involves creating nucleotide cGAMP analogs, suitable for treating solid tumors that can be delivered intratumorally. The latest advancements have led to the creation of synthetic biosynthetic routes employing a modified kinase—cGAS sequence to synthesize a nucleotide cGAMP analogue, boasting a significant production capability[720].

Nonetheless, the protein kinases (PKs) of these nucleotide mimetics are suboptimal due to metabolic instability and the impermeability of membranes. The enzyme ectonucleotide pyrophosphatase/phosphodiesterase 1 has been recognized for its capacity to break down cGAMP outside cells, leading to the production of substances like the immune inhibitor adenosine, which in turn weakens anticancer defenses and fosters the spread of tumors[721]. Addressing this obstacle, immunomodulatory nanosystems offer a potent method for administering STING agonists[722], and the use of stimuli-responsive nanoparticles aids in the precise and regulated release of drugs, tailored to the tumor environment's nature, while preventing adverse effects[723]. Studies have shown that endosomolytic polymersomes containing cGAMP can significantly boost cGAMP activity through both intravenous and intratumoral methods[724]. Additionally, it boosts the activation of STING in both the tumor and sentinel lymph nodes, setting the stage for a more synergistic effect with ICIs[724].

Concurrently, the creation of the non-nucleotide STING agonist aimed to rectify the nucleotide's suboptimal PK properties, encompassing ABZI, MSA-2, and SR-717[725-728]. Within this group, MSA-2 and SR-717 are suitable for oral consumption, chosen for their ease of delivery and affordability. Compounds in nature that selectively control STING could be crucial for screening purposes. A variety of natural substances capable of altering STING activation were identified[729], potentially positioning them as prime candidates for additional alteration. Considering the process of the selective human STING agonist C53 and the idea of an additional pocket, creating new modulators aimed at the second pocket of STING would be intriguing.

A different approach to trigger STING activation is derived from a study on a versatile STING agonist—a man-made polymer featuring a cyclic seven-membered ring (PC7A)—that attaches to a non-competitive STING surface, separate from the cGAMP binding site, leading to the phase condensation of STING[730,731].

Table 4 The drugs targeting STING: agonists and inhibitors.

Drug	Target	Phase	Disease	Treatment	NCT number	Status
MK-1454	STING	I	Solid tumors or lymphoma	Combination with pembrolizumab	NCT03010176	Completed
		II	Head and neck squamous cell carcinoma	Combination with IV pembrolizumab	NCT04220866	Completed
SR-8541A	ENPP1	I	Solid tumor	Monotherapy	NCT06063681	Recruiting
TXN10128	ENPP1	I	Solid tumor	Monotherapy	NCT05978492	Recruiting
RBS2418	ENPP1	I	Advanced cancer	Monotherapy	NCT05270213	Recruiting
ADU-S100	STING	I	Solid tumor or lymphoma	Monotherapy (+/− ipilimumab)	NCT02675439	Terminated
		I	Solid tumor or lymphoma	Combination with PDR001	NCT03172936	Terminated
		II	Head and neck cancer	Monotherapy	NCT03937141	Terminated
DMXAA (ASA404)	STING	I	Metastatic cancer	Combination with paclitaxel +, carboplatin, or docetaxel	NCT01240642	Terminated
		I	Solid tumor	Monotherapy	NCT01278849	Terminated
		I	Metastatic cancer	Monotherapy	NCT01278758	Terminated
		I	Solid tumor	Monotherapy (+/− taxane-based chemotherapy)	NCT01290380	Terminated
E7766	STING	I	Solid tumor or lymphoma	Monotherapy	NCT04144140	Terminated
KL340399	STING	I	Solid tumor	Monotherapy	NCT05549804	Recruiting
		I	Solid tumor	Monotherapy	NCT05387928	Recruiting
GSK3745417	STING	I	Solid tumor	Monotherapy (+/− dostarlimab)	NCT03843359	Active, not recruiting
		I	Acute myeloid leukemia and high-risk myelodysplastic syndrome	Monotherapy	NCT05424380	Active, not recruiting
CRD3874-SI	STING	I	Sarcoma and Merkel cell carcinoma	Monotherapy	NCT06021626	Recruiting
IMSA101	STING	I	Solid tumor	Combination with an immune checkpoint inhibitor	NCT06026254	Active, not recruiting
		I/II	Solid tumor	Monotherapy (+/− immune checkpoint inhibitor)	NCT04020185	Completed
		II	Solid tumor	Combination with PULSAR-integrated radiotherapy + pembrolizumab or nivolumab	NCT05846659	Recruiting
		II	Oligometastatic disease	Combination with PULSAR-ICI	NCT05846646	Recruiting
SNX281	STING	I	Solid tumor and lymphoma	Monotherapy (+/− pembrolizumab)	NCT04609579	Terminated
TAK-500	STING	I/II	Solid tumor	Monotherapy (+/− pembrolizumab)	NCT05070247	Recruiting
ONM-501	STING	I	Solid tumor and lymphoma	Monotherapy (+/− anti-PD1)	NCT06022029	Recruiting
PF-07820435	STING	I	Solid tumor	Monotherapy (+/− sasanlimab)	NCT06285097	Recruiting
BMS-986301	STING	I	Solid tumor	Monotherapy (+/− nivolumab and ipilimumab)	NCT03956680	Completed
SB-11285	STING	I	Solid tumor	Monotherapy (+/− atezolizumab)	NCT04096638	Active, not recruiting

STING, stimulator of IFN gene; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1.

Consequently, this can also successfully trigger the activation of cGAMP-resistant STING variants, like the inherent R232H STING variant. Furthermore, these polymer-facilitated STING biomolecular condensates exhibit greater resistance to breakdown, resulting in a prolonged and robust STING activation pattern. Considering distinct activation processes, the polymer collaborates with cGAMP to produce the most effective STING activity profile, ensuring a swift and lasting reaction[730].

The clinical trials of STING-based treatments reveal a clear pattern: the use of ICIs in combination, such as the PD-1/PD-L1 blocking antibody and the CTLA4 blocking antibody. The latest studies have additionally confirmed the effectiveness of certain

innovative combination methods. For example, using a mix of STING agonist and CXCR3 antagonist has been shown to counteract resistance to anti-PD-L1 in lung adenocarcinoma when subjected to oxidative stress[732]. Utilizing both the oral STING agonist MSA-2 and the anti-TGF-β/PD-L1 bispecific antibody YM101, it is possible to successfully counteract resistance to immunotherapy in both immune-excluded and immune-desert models[733]. Furthermore, a methoxy PEG masked CD44 $\times$ PD-L1/CD3 trispecific T-cell nanoengager, infused with the STING agonist, to convert a cold tumor into a hot one and eliminate the well-known large TNBC[734].

Mn, serving as a complement to cGAS−STING activation, plays a crucial role in the anticancer impact, evidenced by mice lacking Mn showing markedly increased tumor expansion and metastasis, and a substantial decrease in tumor-penetrating $CD8^+$ T cells[735,736]. In conjunction with the anti-PD-1 antibody, Mn significantly enhanced the effectiveness against tumors and decreased the necessary dosage of the anti-PD-1 antibody[736]. Different research developed a cGAMP nanovaccine with thiolation and Mn^{2+} coordination, enhancing the management of both primary and distal tumors[737]. Finally, in the case of KRAS-LKB1 mutant lung cancers where STING is inactivated in epigenetic studies, it is noted that one treatment with an MPS1 inhibitor can effectively reactivate STING activation and reinstate T cell penetration by epigenetically de-repressing STING[738]. There is a significant need for future research to advance this amalgamation into sophisticated clinical testing phases.

5.4.2. STING inhibitors

Beyond the advancements in STING agonists, the potential of STING inhibitors in treating inflammatory conditions is significant, necessitating additional exploration in clinical studies. It is noted that STING undergoes significant PTMs, with palmitoylation at cysteine residue 91 being crucial for its activation. Ablasser and his team identified various substances as covalent suppressors of STING, such as H-151, and C-170, C-171, C-176, and C-178, which irreversibly attach to Cys91[739]. Additional STING inhibitors, such as Compound 1, Compound 18, Astin C, and SN-011, have been identified to target the ligand-binding site of STING through cell-based phenotypic chemical screening or *in silico* docking screening[740-742]. These inhibitors have been shown to reduce STING-related autoinflammatory conditions in mice, demonstrating the effectiveness of STING antagonists in managing autoinflammatory diseases[725,741,742].

5.4.3. AIM2 for cancer immunotherapy

Regarding their function in natural immune reactions, AIM2 may serve as a potential target for treatment in autoimmune diseases, inflammatory conditions, and various cancers. Synthetic ODNs A151, for instance, to suppress immune reactions by attaching to AIM2, potentially offering treatments for infectious and autoimmune disorders[743]. The human cathelicidin antimicrobial peptide, LL-37, can bind with DNA, inhibiting the activation of AIM2 inflammasomes, providing a potential treatment for chronic skin conditions[744].

6. Targeting CLRs for cancer immunotherapy

6.1. CLRs

CLRs, part of the phagocytic PRRs category, are a widely studied receptor type[745]. The role of the phagocytic receptor differs from that of the receptor that stimulates cells through signal transduction. This mechanism identifies and attaches to PAMPs *via* PRRs, positioning pathogens within cytoplasmic vesicles for immediate digestion and eradication to manage infection[746].

CLRs represent a category of receptors that identify carbohydrates present on the surfaces of pathogenic microorganisms and involve calcium[747]. They are found on macrophages, DCs, and specific types of tissue cells. The carbohydrate recognition domain (CRD) facilitates the capacity of CLRs to identify carbohydrates present in both self and external structures[748]. The CRD of CLRs manifests as a dense spherical formation, known as the C-type lectin-like domain (CTLD) [749,750].

CLRs are categorized into transmembrane and secretory receptors based on the protein's position on the cell membrane[748,751,752]. The collagen lectin family, known as "extracellular pattern recognition molecules", represents the secretory receptors[753]. Based on their structural design, transmembrane receptors are categorized into type I and type II[754,755]. Type I receptors have an N-terminal that extends to the extracellular area, housing several CRDs, in contrast to type II receptors, whose N-terminal extends to the intracellular region and has a single CRD[756,757].

Research indicates that most CLRs play a role in displaying antigens as active receptors associated with membranes, predominantly found on APCs such as DCs and macrophages[747]. CLRs consist of circular formations linked by a pair of disulfide bonds[758]. They possess at least one CTLD external to the cell, with a distinct intracellular domain.

Mannose receptors (MRs) are part of membrane CLRs, characterized as single-chain transmembrane entities[759-761]. The extracellular portion of MRs is bifurcated: the proximal end of the membrane, housing eight successive CTLDs, facilitates ligand endocytosis and transport, while the distal end of the cysteine-abundant lectin domain identifies carbohydrate conjugate sulfation[762]. MR's inherent ligands include lysosomal hydrolase and myeloperoxidase, along with the mannan-abundant structure produced by pathogens[763,764].

6.2. An overview of CLRs signaling

When ligands are identified, Dectin-1 forms homodimers and conveys signals inside cells directly *via* hem-ITAM in its cytoplasmic ends, whereas Dectin-2 and Mincle form heterodimers with Dectin-3 and convey signals inside the cell through FcR-γ that includes ITAM[765]. The activation of CLR triggers Src family kinases to phosphorylate tyrosine in hem-immunoreceptor tyrosine-based activation motif (ITAM) or ITAM, resulting in the engagement and activation of Syk. Following this, the kinase triggers the activation of caspase, enlists the complex of domain-containing protein 9 (CARD9)/B-cell lymphoma/leukemia 10/MALT1, stimulates the standard NF-κB signaling route, and prompts the production of proinflammatory molecules[766].

The activation of Dectin-1 triggers the production of inflammatory cytokines *via* five distinct routes: (i) The signaling of Dectin-1-Syk kinase starts the creation of reactive oxygen species (ROS), influencing the development and maturation of IL-1β and IL-18 in NLRP3 inflammasomes[767]; (ii) The signaling of Dectin-1-Syk kinase also plays a role in the development of the noncanonical caspase-8 inflammasome, essential for the active production of IL-1β[768]; (iii) The signaling of Dectin-1-Syk kinase initiates the activation of the nuclear factor of activated T cells (NFAT) in a manner reliant on calcineurin, integrating with NF-κB

signaling[767]; (IV) The signaling pathway of Dectin-1 activates RAS-GRF1 in a SYK-dependent manner, attracting H-RAS through CARD9, culminating in the activation of extracellular signal-regulated protein kinase[767,769]; (V) Dectin-1 conveys signals *via* the RAF-1-dependent pathway, similar to DC-SIGN. Dectin-1 and DC-SIGN stimulate RAS, trigger RAF-1-induced atypical NF-κB signaling activation, and then encourage the expression of proinflammatory molecules[751] (Fig. 6).

6.3. *CLRs expression in different types of cells in the TME*

6.3.1. *CLRs expression and function in immune cells*

Tumor cells can orient macrophages towards a phenotype that promotes metastasis[770]. Macrophages, specifically M1 and M2, are assimilated by macrophages that are influenced by IL-10 and IL-4/IL-13, respectively[771]. It has been discovered that DC-SIGN is present on tumor immunosuppressive M2 macrophages, and its expression can be triggered by IL-4 and macrophage colony-stimulating factor, suggesting DC-SIGN as an indicator of M2 macrophages[771]. Consequently, this suggests that DC-SIGN plays a role in creating an immunosuppressive microenvironment. For example, Lewis lung cancer cells can emit IL-4, leading macrophages to adopt M2 phenotypes that exhibit DC-SIGN, thereby aiding in immune evasion[770].

TAMs make up 5%−40% of tumor tissues, with their prevalence linked to unfavorable outcomes[772,773]. CD206, a different CLR member, to alter the tumor's surroundings[774]. This is absent in traditional M1 macrophages and is exclusively present in M2 macrophages, known for their cytokine secretion of IL-4, IL-13, and IL-10[775]. Enninga and colleagues discovered that the carbohydrate-binding protein galectin-9 attaches to CD206 on macrophages, leading to the development of tumors[774]. Haque and colleagues discovered the existence of $CD206^+$ tumor-related macrophages in cases of OSCC[776]. The joint cultivation of OSCC cells and $CD206^+$ cells enhances their growth and invasive capabilities, attributed to the epidermal growth factor generated by $CD206^+$ TAMs[776].

Skin lesions in cutaneous T-cell lymphoma are characterized by the expression of CLRs such as CD206, CD207, and CD209[777].

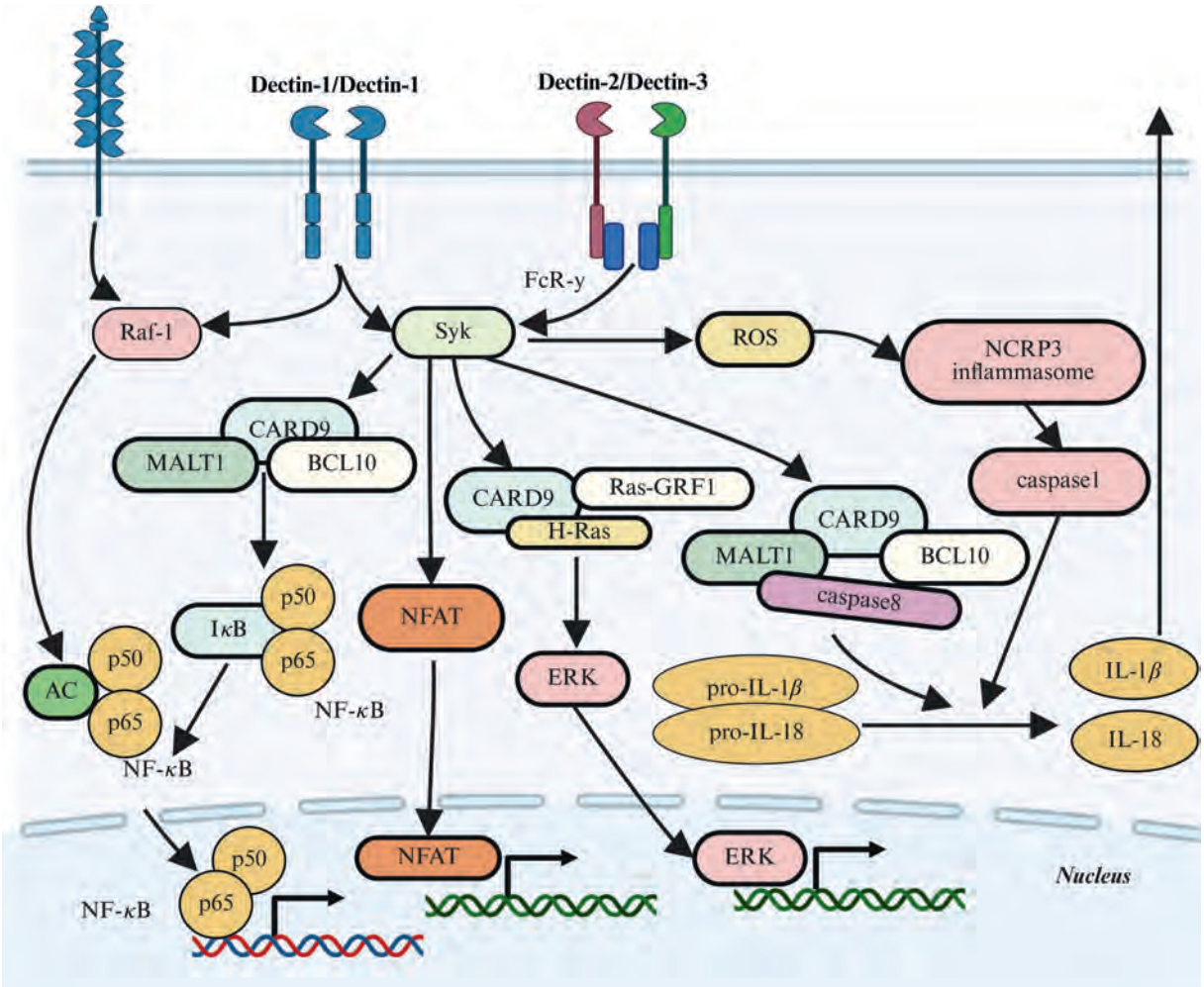

Figure 6 CLR signaling pathway. CLRs like Dectin-1, Dectin-2, and Mincle recognize carbohydrate ligands and activate Syk kinase through hem-ITAM or FcR-γ. This triggers NF-κB and MAPK signaling, leading to cytokine production. The recruitment of MALT1 and CARD9 further amplifies immune responses by enhancing cytokine signaling and immune cell activation. CLR, C-type lectin receptor; Syk, spleen tyrosine kinase; ROS, reactive oxygen species; CARD9, caspase recruitment domain protein 9; MALT1, mucosa-associated lymphoid tissue 1; BCL10, B-cell lymphoma 10; IKK, IκB kinase; NF-κB, nuclear factor-κB; NFAT, nuclear factor of activated T cell; Ras-GRF1, ras-specific guanine nucleotide-releasing factor 1; ERK, extracellular signal-regulated protein kinase; IL, interleukin.

The presence of CD209 is associated with unfavorable outcomes in cases of acute lymphoblastic leukemia[778]. TAMs that express DC-SIGN are linked to a tumor setting that suppresses immune responses[779]. Utilizing a neutralizing antibody to suppress DC-SIGN-expressing TAMs has the potential to reawaken antitumor immunity and enhance immunotherapy for bladder cancer[779].

DCs and macrophages play a crucial role in triggering immune responses against tumors[780]. Van Kooyk observed that DC-SIGN on immature DCs, as opposed to mature DCs, is capable of identifying the glycosylated carcinoembryonic antigen (CEA) in colorectal cancer cells[780]. The interaction occurs through the attachment of CEA-bearing LewisX/Y to colorectal cancer cells and DC-SIGN on DCs[780]. Such an interaction is absent between DC-SIGN and CEA when Lewis antigen levels are low in normal colon cells. This could lead to colon cancer cells evading immune monitoring[780].

6.3.2. CLRs expression and function in tumor cells

CD205 is present in thymic cortical epithelial cells, notably in thymic epithelial tumors, serving as a potential diagnostic indicator[781]. Additionally, CD205 is found in NSCLC, HNSCCs, and ESCCs. OC exhibited CD205 expression, which plays a role in altering metastasis[782]. However, the operational significance of CD205 expression in certain tumors, such as HNSCC and NSCLC, warrants additional research. $CD205^{+}$ polymorphonuclear MDSCs are capable of enhancing tumor suppression[783].

Additionally, CD206 shows increased expression in cases of AML and the alveolar lavage fluid of individuals with small cell lung cancer[784,785]. Identified in HCC, its presence is associated with reduced overall and DFS rates[786]. *In situ* expression of C-type lectin is possible in human cancer cells[786-788]. Reports indicate the presence of DC-SIGN in various tumor cells, such as those in colon cancer[789,790], GC cells, and in the control of tumor cell growth, movement, and spread[791,792]. The attachment of DC-SIGN to the glycosylated antigen of colorectal cancer cells stimulates the release of IL-6 and IL-10, leading to the creation of an immune-tolerant environment.

A variety of cancerous cells, such as those in the colon and Lewis lung, are capable of expressing human DC-SIGN[770,791,793]. The expression is notably elevated in mCRC cell lines. When DC-SIGN binds to DC and undergoes tumor-specific glycosylation, it can inhibit DC activities and facilitate the immunosurveillance of tumor cells[780,793-795]. It was documented that C-type lectin domain family (CLEC) fosters the development of GB through the modulation of PI3K/V-akt mouse thymoma viral oncogene homolog (AKT) signaling pathways[796]. The colorectal mucosal surfaces showed expression of CLEC[787]. Jiang and colleagues documented the presence of DC-SIGN and DC-SIGNR in cancer tissue immunohistochemical tests, yet noted minimal expression in healthy tissues[790]. Conversely, the serum concentrations of DC-SIGN exceeded those found in healthy individuals. Elevated counts of DC-SIGN + DCs were also detected in the lesions associated with cutaneous T-cell lymphoma[777].

Variations in single nucleotides within the DC-SIGN gene-encoding area have been linked to an increased risk of various cancers, such as nasopharyngeal carcinoma and colorectal cancer. Lu and colleagues[797] discovered a correlation between colorectal cancer risk and single-nucleotide polymorphisms in the C-type lectin genes CD209, MBL2, and REG4. The findings suggest that DC-SIGN may serve as an indicator for the initial detection of cancer and foresee the clinical progression of cancerous conditions[794].

BC cells exhibit clusterin expression, experiencing unusual fucosylation and engaging with DC-SIGN[798]. Glycan present in cancer cells to attach to CLEC, leading to the spread of cancer. Colon cancer cells attach to DC-SIGNR on liver sinusoidal endothelial cells, facilitating the movement of these cells to the liver. DC-SIGNR controls the expression of metallothioneins and MMP-9, enzymes capable of breaking down the extracellular matrix in colon cancer cells[789].

Within follicular lymphoma, alterations in glycosyltransferase levels, crucial for producing *N*- and *O*-linked oligosaccharides, resulted in irregular glycosylation in tumor cells, aiding their movement and metastasis[799]. Additionally, the manifestation of DC-SIGN in lymphatic endothelial cells may play a role in facilitating the spread of follicular lymphoma[799,800]. L-SIGN, produced by lymphatic endothelial cells, to attach to high-mannose glycans in malignant follicular lymphoma B cells, facilitating the spread of follicular lymphoma[800].

Through the PI3K/AKT/β-catenin signaling route, DC-SIGN may facilitate the spread of colorectal cancer and enhance the expression levels of MMP-9 and VEGF[791]. The spread of colorectal cancer is aided by DC-SIGN both in laboratory conditions and in living organisms. The activation of tyrosine-dependent signaling is triggered by DC-SIGN, leading to the activation of PI3K/AKT/β-catenin signaling, which in turn promotes tumor growth[791].

The platelet-activating CLEC-2 is known to enhance the spread of blood-borne tumors and aid in their advancement[801]. The spread to the bloodstream is intensified by the clumping of platelets caused by tumor cells, a process facilitated by the interaction between CLEC-2 and podoplanin.

6.3.3. CLRs expression and function in epithelial cells

Dectin-1 serves multiple roles in the intestinal mucosal defense against pathogen invasion. IECs offer the largest interaction zone with luminal plentiful antigens and are known to produce the β-glucan receptor Dectin-1 along with other subsequent adaptor proteins[802]. Following the Dectin-1-reliant route, human IECs react to intestinal β-glucan activation by secreting various cytokines and chemokines (like IL-8 and CCL2), highlighting the importance of CLRs in the interplay between fungi and non-myeloid cells like IEC[802]. Beyond its mechanical protective role, the intestinal lining is composed of various biologically active elements, including mucin glycoproteins and secreted immunoglobulins (secretory IgA (sIgA) and IgG), both of which have a connection to Dectin-1. The thick mucus layer not only acts as a physical divider between intestinal epithelia and luminal microbiota or food, safeguarding the mucosa barrier, but it also hinders intestinal inflammation and improves oral tolerance through the mucin MUC2-driven creation of the Gal-3-Dectin-1-FcγRIIB complex. This complex suppresses NF-κB activation, reduces pro-inflammatory cytokines, and marks DCs with anti-inflammatory traits[803]. At the molecular level, Gal-3 can identify the *N*-glycan configurations of both Dectin-1 and SIGN-R1[804]. A different study discovered that Dectin-1, present on microfold cells, also facilitates the intestinal reverse transcytosis of sIgA, moving from the lumen to the gut-associated lymphoid tissue[805].

Only recently has the function of Mincle in the functioning of the mucosal barrier and the immune system of the intestines been uncovered. Murine Mincle plays a key role in the intestinal Peyer's patches' commensal-detecting capability, controlling the

production of IL-17 and IL-22 from ILCs and T cells, ensuring the synthesis of various intestinal antimicrobial proteins (like RegIIIγ and IgA), and ultimately limiting irregular microbial movement[806]. Additionally, another study highlighted the significance of CX3CR1$^+$ gut-resident mononuclear phagocytes (MNPs), a group of phagocytes characterized by elevated gene expression linked to fungal identification (such as Mincle, Dectin-1, and Dectin-2). This entity acts as a new intermediary in the dynamics between the fungal population and the host's gut immune response, dependent on SYK[807]. Aligning with findings that mice lacking CX3CR1$^+$ MNPs show changes in GM and intense colitis caused by DSS, a missense mutation in the CX3CR1 gene in patients with CD correlates with weakened antifungal antibody reactions[807]. Additionally, compared to patients in CD remission and healthy individuals, patients with active CD show a notable rise in SAP130 serum levels, a typical DAMP of Mincle. Concurrently, the levels of SAP130 and Mincle in the colon tissues of patients with active CD have increased, offering a hopeful biomarker for assessing CD severity and treatment effectiveness[808].

Studies showed Dectin-3's importance in maintaining intestinal balance by interacting with commensal fungi. A lack of Dectin-3 significantly heightens the risk of mouse colitis caused by DSS, resulting in a noticeable rise in specific fungal load and microbial movement, particularly a widespread commensal *Candida tropicalis*[809]. The use of *C. tropicalis* exacerbates colitis exclusively in mice lacking Dectin-3, as this deficiency hinders NF-κB activation, resulting in impaired cytokine production, epithelial recovery, and macrophage phagocytosis amid fungal invasion[809]. Antifungal treatment can successfully reduce intestinal inflammation in mice lacking Dectin-3. Additionally, recent research indicates that E3 ubiquitin ligase c-Cbl, a regulatory element following the Dectin-2 and Dectin-3 signaling routes, plays a role in controlling inflammation caused by intestinal fungi[810]. Mn derived from commensal fungi is capable of aiding in the transcription of the il10 gene and the production of anti-inflammatory cytokines, thereby reducing colitis risk in normal mice by stimulating Dectin-2, Dectin-3, and their subsequent c-Cbl in DCs[810]. Nonetheless, a lack of c-Cbl triggers the activation of the atypical NF-κB subunit RelB during mannan stimulation, thereby inhibiting the standard NF-κB subunit p65-driven il10 transcription and ultimately worsening DSS-triggered mouse colitis[810].

Numerous animal studies have revealed the involvement of mouse equivalents of human DC-SIGN in the onset of colitis. Post-DSS treatment, mice lacking SIGN-R1 demonstrate increased colitis resistance, milder intestinal damage, and reduced levels of pro-inflammatory cytokines compared to normal mice, linked to impaired macrophage reaction to commensal LPS stimulation[811]. Additionally, SIGN-R1 and TLR4 collaboratively work to control intestinal inflammation[811]. Conversely, another murine counterpart, SIGN-R3, is capable of identifying carbohydrate ligands on commensal fungi, and mice lacking SIGN-R3 show more intense colitis symptoms like weight loss and diarrhea, along with heightened TNF-α production in the colon in comparison to normal mice[812]. Additionally, it's important to recognize that SIGN-R3 possesses a hemITAM signal motif, in contrast to DC-SIGN and SIGN-R1, which are part of CLRs independent of ITAM-ITIM[751]. Fascinatingly, recent research indicates that the interplay of the surface layer protein A in the probiotics *Lactobacillus acidophilus* and murine SIGN-R3 safeguards the intestinal mucosal barrier, averts dysbiosis, enhances colonic control signaling, and aids in reducing experimental colitis—a protective function absent in mice lacking SIGN-R3[813].

6.4. *CLRs for cancer immunotherapy*

The focus on the CLR family is increasingly recognized for its promise in cancer treatment, attributed to the tumor-inhibiting characteristics of certain family members, exemplified by Dectin-1 and its fungal ligand, β-glucan. In tumor models such as mouse syngeneic (*e.g.*, lung cancer and lymphoma) and human xenograft (*e.g.*, BC), β-glucan influences the TME through its immunostimulatory effects on both innate and adaptive immune cells. This highlights its potential clinical efficacy and low toxicity as either a single-agent therapy or an immune enhancer in various advanced-stage cancers[814] (Table 5). Recently, β-glucans have garnered attention as polysaccharide-based nanocarriers for precise drug delivery to immune cells[815]. Although still in early preclinical trials, β-glucans show promise due to their advantageous biochemical properties and adaptable, porous architecture, which can be modified for drug incorporation. Their recognition by immune cells, particularly TAMs, makes them a promising target for cancer immunotherapy treatments[815]. Additionally, laminarin, a Dectin-1 inhibitor, has been shown to hinder the onset of DSS-induced colitis by stimulating Treg cells in mice[816].

SYK plays a crucial role as a signal transduction molecule in the CLR signaling pathway. Reports indicate that R788, a targeted SYK inhibitor, delays the onset of spontaneous diabetes in non-obese diabetic mice and mitigates skin damage in lupus-prone MRL/lpr mice[817,818]. Furthermore, a Phase II randomized clinical trial demonstrated R788's efficacy in treating patients with rheumatoid arthritis. However, its clinical application has been limited due to significant side effects[819,820].

7. Novel pathogens as PRR agonists

Most PRR agonists currently in use are synthetic. While numerous preclinical studies and clinical trials have demonstrated their ability to induce tumor regression and activate immune responses,

Table 5 β-Glucans targeting CLRs.

Drug	Target	Phase	Disease	Treatment	NCT number	Status
β-Glucan	CLR	II	Neuroblastoma	Combination with a bivalent vaccine, and GM-CSF	NCT04936529	Recruiting
		II	Neuroblastoma	Combination with a bivalent vaccine, and OPT-821	NCT06057948	Recruiting
		Not applicable	Oral squamous cell carcinoma (OSCC)	Monotherapy	NCT04387682	Unknown status

CLR, C-type lectin receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor.

their clinical application remains limited due to unclear safety profiles, administration methods, and specific mechanisms of action. In contrast, conventional pathogen vaccines and agonists may serve as promising natural sources of PRR agonists[821]. Since little is known about using pathogen vaccines to activate the immune microenvironment and immune response in cancer therapy—except for BCG, which has been fully evaluated and widely used in oncologic clinical trials—this section will discuss and summarize several novel pathogens or pathogen-derived products as potent natural sources of PRR agonists.

Capsular bacterial polysaccharides, such as *Haemophilus influenzae* type b, become immunogenic in humans when bound to carrier proteins. This binding activates $CD4^+$ T cells to produce cytokines and stimulate the cloning and expansion of bacterial polysaccharide-specific B cells, which then differentiate into memory cells[822]. The PedvaxHIB vaccine, which links *H. influenzae* polysaccharides with *Neisseria meningitidis* porins that may bind to TLR2, enhances the expression of the B cell co-stimulatory molecule B7-2 and boosts the immunogenicity of bacterial polysaccharides. Its immune effects are associated with the induction of IL-6 and TNF-α[823,824]. DCs are activated by TLR2 to release TNF-α in a MYD88-dependent manner[824].

Li et al.[825] demonstrated that attenuated rabies virus (RABV) strains are essential for inducing long-term humoral immunity, a process mediated by TLR7. Luo et al.[826] showed that TLR7 deficiency significantly reduces CXCL13 levels following RABV immunization, leading to impaired germinal center formation and B cell recruitment. Transcriptomic analysis revealed that differentially expressed genes were primarily cytokines and immunoglobulins. Additionally, TLR7 expression was correlated with several Th1-related cytokines and chemokines, including IL-6, IFN-γ, IL-27, and CXCL10, all of which were upregulated following RABV immunization[826].

Recently, Aleynick et al.[827] conducted an extensive immunoassay of 19 commonly used pathogen vaccines, including those mentioned above, and combined non-synthetic PRR agonists with different mechanisms of action to create a novel triplet combination. This approach involves mobilizing DCs using Flt3L, promoting TA release through radiation therapy, and administering natural PRR agonists intratumorally to activate antigen-loaded DCs *via* their respective receptors[237]. The researchers found that the triplet vaccine was more effective than synthetic PRR agonists in expanding DC populations in both humans and mice, promoting the cross-priming of TA-specific effector T cells[827]. This method led to stronger $CD8^+$ T cell-mediated killing and induced significant tumor regression.

The recombinant poliovirus/rhinovirus chimera (PVSRIPO) has demonstrated, in preclinical studies, the ability to stimulate a typical innate anti-pathogen inflammatory response in the TME. This response ultimately leads to DC infiltration and TA-specific CTL responses, showcasing significant potential for future cancer immunotherapy[828]. Brown et al. reported that recombinant poliovirus mediates antitumor immunity by directly infecting non-malignant cells within the TME. This process activates MDA5−TBK1−IRF3 signaling, thereby promoting the proliferation and differentiation of effective antitumor T cells. Of particular importance, type I IFN associated with the TLR signaling pathway plays a crucial role in the localization and function of T cells within tumors[829].

Other bacterial-derived ligands can be rapidly recognized by PRRs and activate immune responses aimed at pathogen elimination. LPS, found in the outer membrane of Gram-negative bacteria, can be hydrolyzed to produce a nontoxic derivative, monophosphoryl lipid A (MPL)[830]. This derivative retains the immunogenicity of LPS while significantly reducing its toxicity. By binding to TLR4, the Th1 adjuvant MPL stimulates the production of cytokines and chemokines, promotes the migration and maturation of APCs, and shows significant potential for enhancing immune responses and antitumor therapies[831]. Another bacterial preparation, OK-432, is derived from a low-virulence strain of *Streptococcus pyogenes* that has been incubated with penicillin and lyophilized. OK-432 is used in cancer treatments due to its ability to induce Th1-type cytokines[832]. Its efficacy in antitumor immunity has been increasingly demonstrated in cancer therapy, including in pediatric solid tumors and advanced OC[833].

Table 6 The advantages and disadvantages of PRRs.

Target	Advantages	Disadvantages
TLR	Critical in activating innate immunity, show promise in cancer immunotherapy by enhancing antigen presentation and tumor-specific T cell activation.	TLR activation can cause systemic inflammation, leading to toxicity and immune-related side effects, and some TLR agonists have demonstrated limited efficacy in clinical trials due to immune suppression or resistance.
RLR	Important for recognizing viral RNA and initiating type I interferon responses, with potential in cancer immunotherapy by activating innate immunity and enhancing antitumor responses.	Not always yield sustained or targeted immune responses, and can cause tissue damage or autoimmunity if uncontrolled.
NLR	Involved in inflammation and innate immunity, playing a role in activating the inflammasome to boost immune responses and antitumor immunity.	Excessive activation of NLRs can lead to chronic inflammation, contributing to diseases or tumor progression, making careful regulation essential.
Cytosolic DNA sensor	Key in recognizing foreign DNA and activating type I interferon responses, essential for detecting infections and promoting antitumor immunity	Activation can lead to chronic inflammation and autoimmune diseases, and tumors may evolve mechanisms to evade detection, reducing the effectiveness of DNA sensor-targeted therapies.
CLR	Involved in recognizing carbohydrate patterns on pathogens and tumor cells, promoting dendritic cell activation and T cell responses, with certain CLRs like Dectin-1 being explored for cancer therapies.	The specificity of CLRs for certain antigens limits their application, as not all tumors express the required glycan patterns, and CLR-targeted therapies may induce immune suppression in the tumor microenvironment.

TLR, toll-like receptor; RLR, retinoic acid-inducible gene 1-like receptor; NLR, nucleotide-binding oligomerization domain-like receptor; CLR, C-type lectin receptor.

8. Overcoming immune checkpoint blockade resistance with PRR agonists

Immune checkpoint blockade is currently one of the most prominent approaches in cancer immunotherapy. Many antibodies targeting CTLA4 and the PD-1/PD-L1 axis—such as ipilimumab, nivolumab, pembrolizumab, and atezolizumab—have been approved for use in various cancers. However, potential mechanisms of resistance to ICIs may include insufficient antigenicity of the tumor itself, disruption of tumor cell response to IFNγ signaling, downregulation or loss of MHC in tumors, and the effects of oncogenic signaling pathways such as the WNT−β-catenin pathway, CDK4−CDK6 and cell cycle, MAPK signaling, loss of the tumor suppressor PTEN, tumor dedifferentiation, and stem cell characteristics[834].

The combination of PRR agonists and ICIs be effective in patients with refractory or ICI-resistant tumors, as evidenced by numerous clinical trials and their outcomes. One strategy for overcoming ICI resistance is converting “cold” tumors into "hot" tumors. In patients resistant to anti-PD-1/PD-L1 therapy, the initial antitumor immune response may be inadequate; however, enhancing TA exposure could lead to stronger activation of antitumor T cells[835]. Immune “cold” tumors exhibit a weak response to ICIs due to the low infiltration of immune cells within their microenvironment. Various PRR agonists have been shown to activate APCs, thereby promoting the action of tumor-killing cells.

Since antigen presentation defects underlie many tumors’ resistance to ICIs, potent activators of innate immunity can modulate tumor homeostasis, transforming the TME from immunosuppressive to immunoactive[264]. Moreover, when combined with traditional therapies such as radiotherapy, PRR agonists can enhance antigen presentation, innate immune function, and T-cell activation. Radiotherapy complements PRR agonists by killing tumor cells and releasing TAs and DAMPs, further activating T cell and tumor interactions[836]. These activated T cells can subsequently be transported systemically to the target and exert effects on distant tumors.

Additionally, reversing ICI resistance can be achieved by restoring IFN-γ signaling. Tumors lacking IFN-γ signaling are unable to adaptively express immune checkpoints such as PD-L1. In certain tumors, the expression of MHC-I antigens primarily relies on IFN-γ signaling, and the loss of this signaling is tantamount to the loss of antigen presentation[834]. PRR agonists, including TLR agonists, have the potential to trigger IFN-mediated tumor inflammation. This leads to an increase in $CD8^+$ T cells, elevated PD-L1 expression, and an enhanced IFN-γ gene signature[837].

In summary, intra-tumoral injection of PRR agonists can stimulate localized inflammation, attracting infiltrating myeloid cells into the TME through chemokine signaling[838]. Furthermore, these agonists can activate myeloid cells, enhancing their ability to present TAs to T cells and thereby improving the antitumor immune response.

9. Conclusions and perspectives

Drugs targeting PRRs, owing to their capability to stimulate the immune system, play a pivotal role in eliciting both systemic and enduring adaptive immune responses against tumors. Additionally, they can directly trigger immunogenic cell death, a specific form of apoptosis that activates immune reactions targeting antigens associated with dead cells[839]. Administering cancer vaccines or intra-tumoral treatments with PRR-targeting drugs may result in robust immune responses against tumors. However, these therapies may fall short in mitigating TA tolerance within the tumor’s microenvironment and adjacent lymph nodes. Combining PRR agonist treatments with antibodies targeting immune checkpoints, such as anti-CTLA4 or anti-PD-1, may address this limitation and overcome resistance to single-agent immune checkpoint blockade therapy.

As noted, drugs targeting PRRs also have inherent limitations and face ongoing challenges. The advantages and disadvantages of each PRR type are summarized in Table 6. Activation of PRRs can lead to the expression of numerous cytokines, creating an inflammatory microenvironment that may cause tissue damage, such as hematotoxicity, infection, influenza-like symptoms, and gastrointestinal reactions. Nevertheless, these side effects are generally manageable, and the drugs are well-tolerated[254,259,840]. However, PRR activation can also drive chronic inflammation, contributing to an immunosuppressive microenvironment characterized by the accumulation of M2 macrophages, MDSCs, Tregs, and immunosuppressive cytokines, which collectively enhance tumor activity[17,75]. Gaining a deeper understanding of the mechanisms by which PRRs influence the cancer microenvironment is crucial to prevent unintended amplification of pro-tumor immunosuppression or chronic inflammation when using agonists or antagonists.

Moreover, the cellular composition and functional states of the TME vary depending on the tumor’s organ of origin, the intrinsic characteristics of cancer cells, tumor stage, and patient-specific factors, reflecting its heterogeneity. Addressing the therapeutic challenges posed by tumor heterogeneity and its impact on treatment efficacy remains a significant obstacle.

The future of PRR-based therapeutics lies in integrating innovative approaches to overcome current limitations and enhance efficacy. Combining PRR-targeted strategies with emerging modalities, such as ICIs or CAR-T therapies, holds promise for synergistically enhancing antitumor immunity. Leveraging multi-omics technologies, including genomics, transcriptomics, and proteomics, allows for the identification of patient-specific biomarkers and the development of personalized treatment regimens[841]. These advancements have the potential to improve therapeutic outcomes, minimize off-target effects, and position PRR-based strategies as a cornerstone of precision oncology.

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Author contributions

Dengjie Ouyang: Writing-Original Draft, Review, and Supervision. Ruixian Chen: Writing-Original Draft, Review, and Editing. Haitong Xie: Validation. Xiwen Yang: Conceptualization. Qintong Li: Methodology and Validation. Jie Chen: Conceptualization, Methodology, Resources, and Funding acquisition.

Conflicts of interest

The authors declare no conflicts of interest.

Declaration of generative AI in scientific writing

During the preparation of this work, the authors used ChatGPT and DeepSeek exclusively for language editing and grammar checking of non-technical content. After using these tools, the authors reviewed and edited the content critically. The authors take full responsibility for the scientific accuracy, integrity, and originality of the work.

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