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REVIEW ARTICLE

A new emerging target in cancer immunotherapy: Galectin-9 (LGALS9)

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Abstract Over the past few decades, advances in immunological knowledge have led to the identification of novel immune checkpoints, reinvigorating cancer immunotherapy. Immunotherapy, represented by immune checkpoint inhibitors, has become the leader in the precision treatment of cancer, bringing a new dawn to the treatment of most cancer patients. Galectin-9 (LGALS9), a member of the galectin family, is a widely expressed protein involved in immune regulation and tumor pathogenesis, and affects the prognosis of various types of cancer. Galectin-9 regulates immune homeostasis and tumor cell survival through its interaction with its receptor Tim-3. In the review, based on a brief description of the signaling mechanisms and immunomodulatory activities of galectin-9 and Tim-3, we summarize the targeted expression patterns of galectin-9 in a variety of malignancies and the promising mechanisms of anti-galectin-9 therapy in stimulating anti-tumor immune responses.

Introduction

Galectins are an interesting family of β-galactoside-binding proteins that are widely present not only in animals but also in bacteria and fungi at varying levels. They are a highly conserved core sequence, defined by the evolutionarily conserved carbohydrate recognition domain (CRD)[1]. Their biological role was initially understood to be limited to recognition of endogenous ("self") carbohydrate ligands during embryogenesis and early development, but it was later discovered that they also play a key role in tissue repair, adipogenesis, cancer development, and the

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regulation of immune homeostasis[2,3]. The galectins protein family has two typical characteristics: (1) sharing significant similarities in a conserved amino acid sequence; (2) a high affinity for β-galactoside sugars. Currently, 15 galectins have been identified in mammals and 11 are found in humans, acting both intracellularly and extracellularly. Galectins can be classified into three subtypes based on the protein structure: (1) proto-type, is a protein containing one CED, which usually form homodimers, including galectin-1, galectin-2, galectin-5, galectin-7, galectin-10, galectin-11, galectin-13, galectin-14, and galectin-15; (2) chimera-type, galectin-3, is the only chimeric galectin which contains a non-lectin domain linked to a CRD. It contains two domains, a C-terminal CRD and a non-carbohydrate binding N-terminal domain, which self-associate into an oligomer; (3) tandem-repeat-type, has two CRDs with different binding specificity, which are conjugated by ligating peptides, including galectin-4, galectin-6, galectin-8, galectin-9, and galectin-12 (Fig. 1)[4,5]. Galectins have cytoplasmic properties and can only be synthesized in the cytoplasm. It is not related to the traditional endoplasmic reticulum/Golgi trafficking mechanism and is secreted through non-classical pathways. Multiple pathways have been proposed to explain the mechanisms by which galectins leave the cell via the non-classical pathways, including the dependence of galectins secretion on its oligomerization, the use of direct translocation pathways for translocating across the plasma membrane, and the release of galectins in extracellular vesicles[6]. Additional studies support a model of galectins release either through exosomes or lysosomes[7]. Many galectins are located outside of the cell and participate in interactions with other extracellular proteins. This family is widely distributed in human tissues and has a wide range of biological functions.

Galectin-9 (Gal-9), also known as LGALS9, was first identified as a transmembrane urate transporter in 1997 from rat embryonic kidney[8], an eosinophil attractant in T-lymphocytes[9], and an auto-antigen in human Hodgkin's lymphoma tissue[10]. Recently, it has gained much attention due to its therapeutic potential in numerous pathological disorders and strong immunomodulatory effects. Thus, the expression level of Gal-9 was involved in a variety of physiological functions, such as cell growth, differentiation, adhesion, communication, and cell death. In addition, aberrant expression of Gal-9 in solid tumors is associated with the tumor occurrence or metastasis[11–14]. For instant, high expression level of Gal-9 in tumor cells promoted the colony formation of melanoma cells, whereas down-regulation of Gal-9 in these cells correlated with cell proliferation without colony formation[11].

Moreover, accumulating evidences have demonstrated that galectins can directly influence both innate and adaptive anticancer immunity by glycan receptors. It is worth nothing that Gal-9, unlike other galectin members, is essentially an immune system inhibitor: it promotes differentiation of regulatory T cells (Tregs), and reduces T-helper 17 (Th17) and Th1 cells, resulting in suppression of excessive immunity and inflammation[15,16]. Gal-9 specifically interacts with one of its receptors, T-cell immunoglobulin and mucin-domain containing-3 (Tim-3), and induces $CD8^+$ T cell apoptosis[17]. Furthermore, Gal-9 stimulates IL-12 production by ligating Tim-3 to initiate adaptive immune responses[15,18]. It is possible that the Gal-9-Tim-3 complex may play critical role in different physiological conditions. However, it was shown that Gal-9 activity was incompletely eliminated by antagonistic Tim-3 antibodies in T-cell lines[19]. The role of Gal-9 in immunoregulation seems to be complex. At present, Gal-9/Tim-3 is an emerging immune checkpoint inhibitor (ICI) following PD-1/PD-L1. The use of ICIs stimulates the immune microenvironment and enhances the anticancer immune responses, thus improving the therapeutic effect of patients. Compared with radiotherapy and chemotherapy, immunotherapy has better anticancer effects and fewer side effects, and has expanded the options for patients with relapse-resistant drugs after first-line treatment[20]. However, primary and acquired resistance significantly reduce the success of ICIs, so there is an urgent need to develop new strategies to enable more

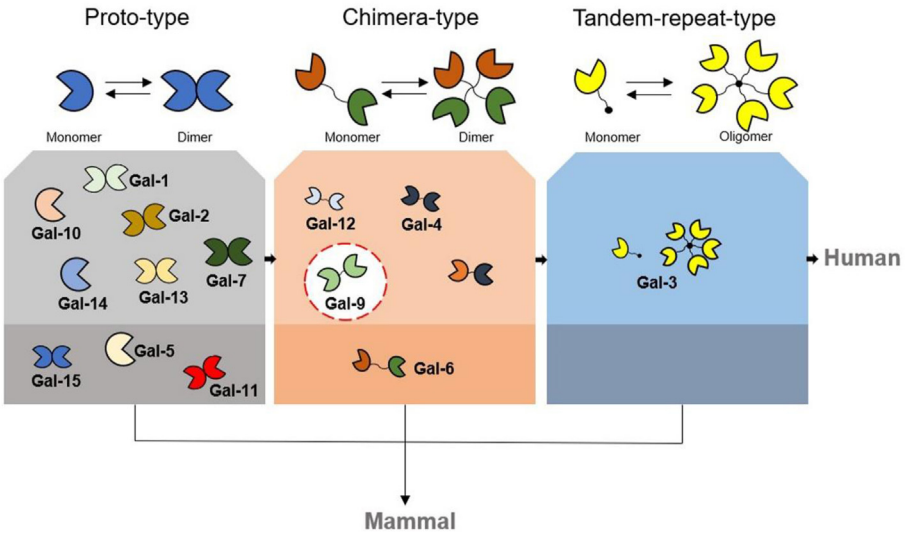

Figure 1 Galectin family in mammals. Fifteen galectins have been described in mammals, 11 of which have also been found in humans (located in the light-colored area at the top of the diagram). This figure shows the structural variation and classification of different subtypes of galectins.

patients to achieve long-term remission through cancer immunotherapy. We focus on an emerging biomarker, Gal-9, which, unlike other galectins, plays a role in both promoting and inhibiting tumor growth, depending on interactions with T cells, antigen-presenting cells, or receptors on tumor cells[21].

In this review, we discuss the interaction mechanism of Gal-9/Tim-3, focus on the expression pattern of Gal-9 in different malignant tumors and the potential mechanism, and provide a theoretical basis for the therapeutic potential of Gal-9 in the immunotherapy of malignant tumors.

Molecular structure and distribution of galectin-9

Molecular structure of Gal-9

Gal-9 is encoded by the *LGALS9* gene and located on human chromosome 17. It has a characteristic amino acid sequence and is a highly conserved sugar recognition domain consisting of approximately 130 amino acid sequences. Gal-9 belongs to tandem-repeat-type galectins which containing two separated homologous CRD domains, namely N-terminal CRD (N-CRD) and C-terminal CRD (C-CRD). It is an integral membrane protein that exists as two informs, a long form and a short form, which differ by an internal stretch of 32 amino acids[22,23].

Cell distribution of Gal-9

In human cells, Gal-9 is primarily localized to the cytosol, which is based on antibodies targeting proteins from multiple genes. Hofbauer cells, Kupffer cells, and Paneth cells show single-cell type-specific enhancement, which is generally expressed in immune cells[24–26]. The expression of gal-9 is significantly enhanced in HMC-1, THP-1, U-937, MOLT-4, and REH cell lines, all cancer cell lines, mainly derived from myeloid and lymphoid (data from *The Human Protein Atlas* https://www.proteinatlas.org/).

Tissue distribution of gal-9

Gal-9 is expressed in almost all organ systems, and its protein and RNA expression levels varied. The protein and RNA encoded by the *LGALS9* gene are highly expressed in bone marrow and lymphoid tissues. At the protein level, Gal-9 is highly expressed in the brain, lung, endocrine tissue, gastrointestinal tract, liver, gallbladder, kidney, urinary bladder, male tissues, female tissues, and muscle tissue. At the RNA level, it is expressed relatively high in the blood (data from *The Human Protein Atlas* https://www.proteinatlas.org/).

Physiological function of galectin-9

Many studies have shown that Gal-9 is involved in many physiological processes and is an indispensable key element in maintaining normal physiological functions of the body. Gal-9 plays an important role in growth and development[27], angiogenesis[28], central nervous system stability[29], T-cell homeostasis[30], maintenance of liver homeostasis[24], regulation of lysosomes and autophagy, and maintenance of intestinal stability[25]. A growing body of evidence suggests that Gal-9 plays a variety of roles in maintaining and regulating immunity. The function of Gal-9 as an eosinophilic chemoattractant helps in the recruitment of eosinophils through T cells, trigging eosinophils activation[31]. Gal-9 is involved in Th17/Treg immunoregulation by mediating immunosuppression and differentiation[32]. The effect of Gal-9 on activated T cells is concentration-dependent. At higher concentrations, Gal-9 induces apoptosis of activated $CD8^+$ and $CD4^+$ T cells, but at lower concentrations, it increases the cytokine production by activated T cells. At higher concentrations, bivalent Gal-9 can be oligomerized, and therefore may aggregate more signaling proteins or induce binding with more ligands, which is important for apoptotic signaling[33]. Besides, Gal-9 inhibits B-cell signaling by binding to B-cell receptors[34]. Gal-9 promotes interactions between B cells and vascular endothelial cells while transmitting anergic signals to control B cells reactivity[35]. Maturation of dendritic cells (DCs) is a key step to initiate the immune response, and Gal-9 can promote the maturation of DCs through upregulated expression of co-stimulatory molecules such as CD40, CD54, CD80, etc., and HLA-DR. IL-1β and interferon (IFN)-γ also increase the expression of Gal-9 while promoting the maturation of DCs[36]. Recent evidence has shown that Gal-9 in the cytosol regulates the phagocytosis of DCs by regulating the plasma membrane structure. Gal-9 is an evolutionally conserved lectin required to maintain the structure and function of the cortical cytoskeleton in DCs, and Gal-9 plays an important role in modulating the function of human DCs. DCs lacking Gal-9 showed a significant decrease in cytokine secretion upon infection, suggesting a poor initiation of the immune response[37].

Galectin-9-Tim-3 signaling

Gal-9 can interact with various extracellular matrix proteins and cell surface ligands as well as Tim-3 receptor. Table 1 lists the roles of Gal-9 and their binding partners in the regulation of immune response and tumor biology.

Tim-3 is the most famous Gal-9 binding receptor, and it is the most closely related among the multiple receptors of Gal-9. A large number of data suggest that Tim-3 is an immune checkpoint receptor that promotes immune homeostasis by regulating innate and adaptive immunity, and negatively regulates T cell response by inducing Th1 cell apoptosis[50]. To date, many studies have investigated the interaction between Gal-9 and Tim-3. Under physiological conditions, the binding between Gal-9 and Tim-3 induces the exhaustion or apoptosis of effector T cells, which affects T cell tolerance, negatively regulates IFN-γ secretion, and induces apoptosis of Th1 and Th17 cells; hence plays an important role in the regulation of Th1/Th17 polarization[32]. When associated with the plasma membrane, the Tim-3-Gal-9 complex triggers downstream signaling that promotes cell renewal and forms an autocrine toop[51]. Tim-3-Gal-9 autocrine loop is activated in human acute myeloid leukemia cells through protein kinase C (PKC)/mTOR pathway, which induces high levels of Gal-9 secretion and the release of soluble Tim-3, leading to reduced immune surveillance and disease progression[52]. A growing body of evidence suggests that the interaction of Tim-3-Gal-9 has different phenotypes and functions during the progression of multiple tumors. The Tim-3-Gal-9 pathways contributes to the suppressive tumor microenvironment (TME) in human through Tregs promotion

Table 1 Galectin-9 and its binding partners in the regulation of immune response and tumor biology.

Ligand	Targeted cells	Biological function	Refs.
Tim-3	Dendritic cells, monocytes	Maturation promotion and cytokine secretion	36,38
Tim-3	T cells	Exhaustion or apoptosis	32,39
Tim-3	Natural killer cells	Regulating cell function at the maternal—fetal interface in early pregnancy	40
PD-1	T cells	Suppressing Gal-9/TIM-3-induced T cell apoptosis	41
Dectin-1	Macrophages	Tolerogenic macrophage programming and adaptive immune suppression	42
CD206	Macrophages	Driving angiogenesis and producing chemokines to support tumor growth	43
CD40	T cells	Suppressing proliferation and inducing cell death	44
4-1BB	T cells	Transducing signal and controlling functional activity	45
VISTA	T cells	Apoptosis	46
DR3	Regulatory T cells	Facilitating the activity of DR3 with respect to promoting Treg function that limits inflammatory disease	47
CD44	Regulatory T cells	Increasing cell stability and function, and enforcing cell differentiation and maintenance	48
TLR-4	Microglia	Alleviating brain injury and promoting neuronal restoration	49

Abbreviations: PD-1, programmed cell death protein 1; VISTA, V-domain Ig-containing suppressor of T cell activation; TLR-4, Toll-like receptor-4

and TCR activation[53]. Both Gal-9 and Tim-3 can inhibit anti-cancer immune surveillance[52]. Gal-9 and Tim-3 are also expressed in some solid tumors, and tumor cells use these proteins to escape immune attack from the host[54,55]. The interaction of Tim-3-Gal-9 can induce apoptosis of $CD4^+$ and $CD8^+$ tumor-infiltrating lymphocytes (TILs) and inhibit T cells immune response. Blocking the Tim-3-Gal-9 pathway can restore the function of TILs. An anti-Tim-3 antibody blocks their interaction to increase the proliferation and cytokine production of Tim-3-positive T cells in humans and mice. Besides, an anti-Tim-3 antibody shows anticancer effects in a mouse tumor model (including restoration of their ability to express IFN-γ, IL-2, perforin, and granzyme B)[56—59]. The latest study further highlights Gal-9 as a target for cancer immunotherapy. In growing tumors, IFN-β is produced by DCs and cancer cells, while IFN-γ is released by activated $CD8^+$ T cells. The synergistic effect of IFN-β and IFN-γ upregulates the expression and secretion of Gal-9 in antigen-presenting cells (APC) and cancer cells, thereby inhibiting the anti-tumor response by inducing T cell death[41] (Fig. 2). Currently, blocking the interaction of Tim-3-Gal-9 is considered to be a promising therapy in human malignancies.

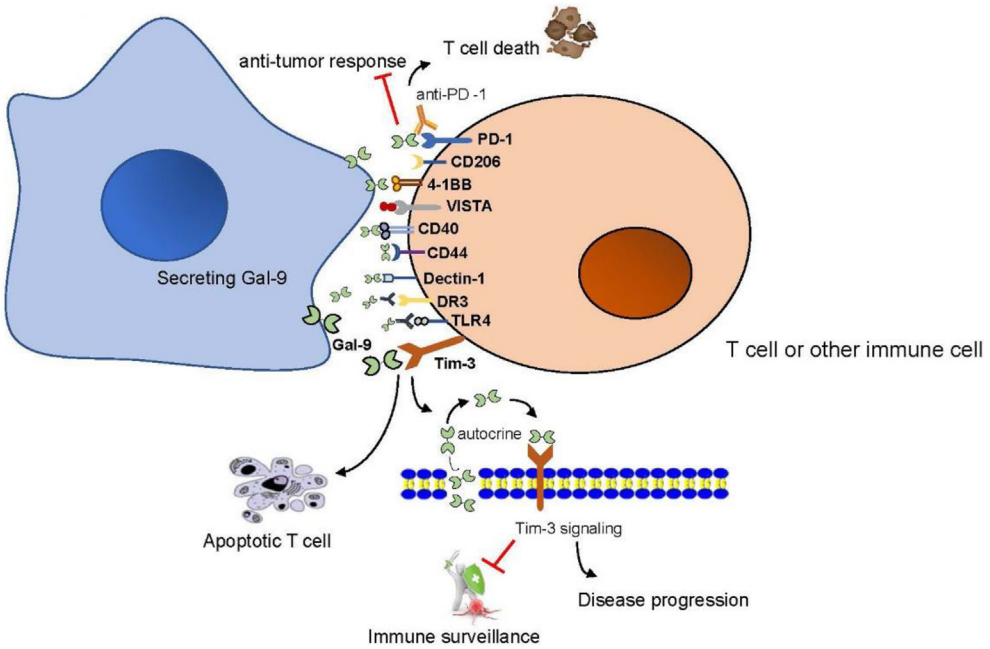

Figure 2 The binding partners of Gal-9. Gal-9 binds to multiple receptors on the cell surface. Binding of Gal-9 to Tim-3 induces T cell apoptosis under physiological conditions, and forms autocrine loop with Tim-3 under pathological conditions, leading to reduced immune surveillance and promoting disease progression. PD-1 binding to Gal-9 plays a key role in inhibiting anti-tumor response and promoting T cell death.

Galectin-9 expression in cancer

The expression of Gal-9 in cancer has been extensively studied, and the levels of Gal-9 in tumor cells or tumor tissues are different compared to normal controls. Thus far, in many types of malignancies, such as breast cancer[54], human papilloma virus (HPV)-associated cervical carcinoma[59], pancreatic carcinoma[60], glioblastoma multiforme[61], cutaneous T-cell lymphoma[62], chronic lymphocytic leukemia[63], tumor cells or tumor tissue contained high or increased levels of Gal-9 compared to normal para-cancerous tissues. On the other hand, in some malignancies, such as gastric cancer, colon cancer, esophageal carcinoma, melanoma, hepatocellular carcinoma, lung cancer, renal cell carcinoma, adrenal carcinoma, and prostate cancer cells, the Gal-9 expression has been observed to be down-regulated in corresponding healthy counterparts[11,64–68]. The expression level of Gal-9 in different tumors not only affects the occurrence and progression of tumors, but also affects their prognosis (Table 2).

Double-edged sword role of Galectin-9 in tumors

Unlike other galectins, Gal-9 both promotes and inhibits tumor activity, showing a "double-edged sword" effect in malignant tumors. Expression of Gal-9 in tumors is not only related to tumor cell adhesion or metastasis, but also involved in the regulation of immune response through interaction with different receptors. Considering the importance of Gal-9 in cancer, targeting Gal-9 expression holds promise in cancer therapy. The following sections discuss the specific role of Gal-9 in various cancers (Table 2).

Galectin-9 and breast cancer

Interestingly, Gal-9 has been shown to have anti-metastatic potential in breast cancer, possibly because Gal-9 induces tumor cell aggregation and reduced adhesion of breast cancer cells to the extracellular matrix, thereby preventing metastasis and improving patients' survival[13,69]. In a recent study, researchers found that breast tumor cells expressed

Table 2 Expression and role of galectin-9 in different types of cancer.

Type of cancer	Expression of Gal-9	Activity	Possible mechanism	Prognosis	Refs.
Gastric cancer	Down-regulated	Tumor suppressor	Inducing tumor cell apoptosis	Favorable	71,72
Colorectal cancer	Down-regulated	Tumor suppressor	Inducing tumor cell apoptosis and inhibiting proliferation, reducing tumor escape immune surveillance, and inducing autophagy	Favorable	64,75-77
Lung cancer	Down-regulated	Tumor suppressor	Suppressing tumor cell adhesion and invasion, and activating NK cells	Favorable	78,80,81
Hepatocellular carcinoma	Down-regulated	Tumor suppressor	Inducing tumor cell apoptosis, and inhibiting the anti-tumor immune response by inducing T cell senescence in Kupffer cells and other APCs	Favorable	66,83,84
Esophageal carcinoma	Down-regulated	Tumor suppressor	Inducing tumor cell apoptosis	Favorable	68,85,86
Melanoma	Down-regulated	Tumor suppressor	Inducing tumor cell apoptosis	Favorable	11,94,95
Breast cancer	Up-regulated	Tumor promotor	Inhibiting immune surveillance through the Tim-3-Gal-9 pathway, and inducing tumor cell aggregation to reduce metastasis	Favorable	13,54,69
Glioma	Up-regulated	Tumor promotor	Tumor immunosuppression or immune escape through the Tim-3-Gal-9 pathway	Unfavorable	61,88
Pancreatic carcinoma	Up-regulated	Tumor promotor	Reprogramming macrophages and exerting immunosuppressive effects	Unfavorable	42,60,91
Cervical carcinoma	Up-regulated	Tumor promotor	Tumor immune escape through activation of the Tim-3-Gal-9 pathway	Unfavorable	59,93
Lymphoma	Up-regulated	Tumor promotor	Reducing CD8+ T cell infiltration	Unfavorable	62
Leukemia	Up-regulated	Tumor promotor	Immune escape through the Tim-3-Gal-9 pathway	Unfavorable	51,52,101

levels of Gal-9 and Tim-3, especially Gal-9, compared with healthy breast tissues from the same patients, and that these proteins were co-localized. Furthermore, it was further found that breast cancer cells activated the Tim-3-Gal-9 pathway by expressing LPHN1 and its ligand FLRT3, and transferred Gal-9 to the cell surface to protect breast cancer cells against cytotoxic immune attack while inhibiting host anti-cancer immune surveillance[54].

Galectin-9 and gastric cancer

Previous studies have shown that higher levels of Gal-9 expression were observed in gastric cancer patients without lympho-vascular invasion, lymph node metastasis, or distant metastasis, and Gal-9 expression is closely related to better survival rates of gastric cancer[70]. A meta-analysis involving 2093 patients with gastric cancer, including eight studies, also showed that low expression of Gal-9 was significantly associated with a poorer prognosis[71]. Down-regulation of Gal-9 mRNA levels was observed in gastric cancer tissues[67]. Further, an *in vitro* study showed that recombinant human Gal-9 (rh-Gal-9) suppresses gastric cancer cell lines proliferation by inducing apoptosis, regulating receptor tyrosine kinases (RTK) pathways and angiogenesis-related molecules, and altering miRNA expression profiles[72]. The Gal-9 promotor region could bind to peroxisome proliferator-activated receptor γ (PPARγ), and Gal-9 activity increased in gastric cancer cells with overexpression of PPARγ. PPARγ inhibited gastric cancer cell invasion, migration, and epithelial-mesenchymal transformation by up-regulation of Gal-9[73]. Tim-3 was significantly expressed in TILs of patients with gastric cancer and was an independent prognostic factor of gastric cancer. Gal-9 was mainly expressed in gastric cancer cells[74]. The mechanism of Tim-3 in gastric cancer and its interaction with Gal-9 is not yet clear, and further studies are needed in the future.

Galectin-9 and colorectal cancer

Gal-9 expression in colon cancer tumor tissues was lower than that in para-cancerous tissues, and low levels of Gal-9 expression were positively correlated with a poor histological grade and lymph node metastasis of colon cancer ($P < 0.05$). Patients with high Gal-9 expression had a longer overall survival at long-term follow-up ($P < 0.05$). The infiltration rate of $CD56^+$ natural killer (NK) cells in tissues with high Gal-9 expression was significantly increased. Both Gal-9 secreted by colon cancer cells and rh-Gal-9 increased the recruitment of NK cells by increasing the expression of Rho/ROCK1 signaling, an essential part of many cytoskeleton-dependent processes, suggesting that tumors could escape immune surveillance through a regulatory mechanism that reduced Gal-9 expression[64]. Another study showed that overexpression of Gal-9 promoted apoptosis and inhibited proliferation in colon cancer cells. MiR-455-5p was identified as the upstream regulatory microRNA (miRNA) of Gal-9 and demonstrated that miR-455-5p reduced Gal-9 expression by directly targeting its 3'-untranslated region[75]. In a mouse colon cancer model, most tumor-infiltrating $CD8^+$ T cells expressed Tim-3, and Gal-9 secreted by tumor cells increased apoptosis of tumor-infiltrating $CD8^+$ T cells. Anti-Tim-3 antibody blockaded the Tim-3-Gal-9 signaling, reduced $CD8^+$ T cells apoptosis, and also inhibited tumor growth in mice[55]. Using pharmacological inhibitors induced mitochondrial dysfunction would not affect the ability of human colorectal cancer cells to secrete Gal-9, but could significantly reduce Gal-9 exocytosis and the presence of Gal-9 on the cell surface, thus reducing the Tim-3-Gal-9 mediated tumor immune escape, suggesting that targeted de-functionalization of mitochondria in malignant cells may be a novel strategy for anti-cancer immunotherapy[76]. Rh-Gal-9 has potent antitumor activity against refractory KRAS mutant colorectal cancer cells and induces fatal frustrated autophagy, depending on the increased basal autophagic flux, but has no effect on BRAF mutant[77].

Galectin-9 and lung cancer

Gal-9 expression was detected in all pathological types of non-small cell lung cancer (NSCLC), and Gal-9 levels were only associated with Tim-3 levels on tumor cells, while Gal-9 was widely associated with other immune checkpoints (including PD-1, PD-L1, and Tim-3) on TILs. High Gal-9 expression on tumor cells suggested a possible longer survival in patients, while high Gal-9 expression on TILs might indicate early tumor relapse[78]. However, a recent study on small cell lung cancer (SCLC) has shown that low Gal-9 expression with TILs can predict early recurrence of patients with stage I-III SCLC. Patients with SCLC with high immune risk score showed lower Gal-9 expression, and Gal-9 expression was significantly associated with changes in the tumor-immune microenvironment and immune infiltration[79]. Limited studies have investigated the role of Gal-9 in lung cancer. Increased cytoplasmic Gal-9 expression in tumor cells may inhibit lung metastasis, which may be related to the suppressive effect of Gal-9 on adhesion and invasion of tumor cells[80]. Gal-9 induced macrophages to differentiate into plasmacytoid DC-like macrophages, which may enhance the activation of NK cells that prolonged the survival of lung cancer-bearing mice[81]. The interaction of Tim-3-Gal-9 not only enhances anticancer immunity but also suggests that the Tim-3-Gal-9 is the key mechanism of primary or secondary resistance to anti-PD-1 in metastatic NSCLC patients, which may become a new target for immunotherapy combinations[82].

Galectin-9 and hepatocellular carcinoma

Gal-9 has also been shown to have an antitumor effect in hepatocellular carcinoma (HCC), and its main thrust is to bring apoptosis. Gal-9 induced apoptosis of HCC cells independently of Tim-3. miR-1246 was a miRNA associated with the antitumor effect of Gal-9 in HCC, which enhanced the apoptotic effect of Gal-9 through the miR-1246-DYRK1A-caspase-9 axis[83]. Low expression of Gal-9 and low $CD8^+$ TILs count were significantly associated with poor prognosis in HCC patients[84]. In hepatitis B virus (HBV)-associated HCC microenvironment, TILs-derived IFN-γ stimulated the expression of Gal-9 on APC (especially Kupffer cells). Tim-3 positive T cells in HCC were co-localized with Gal-9 positive Kupffer cells. Tim-3-Gal-9 signaling pathway T cell senescence, and blocking this pathway could restore T cell

effector function[56]. This means that Gal-9 behaves dichotomous behaviors in HCC. Further studies showed that IFN-γ significantly induced the up-regulation of Gal-9 expression in HCC cells, and Gal-9 overexpression inhibited tumor growth and metastasis *in vitro* and *in vivo*[66].

Galectin-9 and esophageal carcinoma

Esophageal carcinoma includes two histological subtypes: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). Rh-Gal-9 induced mitochondrial dysregulation in ESCC cells (considered to be an irreversible step in apoptosis), thereby mediating tumor cell apoptosis and inhibiting proliferation through JNK and p38 activation[85]. Similarly, Gal-9 showed antitumor effects in EAC cells by inducing apoptosis. The increased expression level of IL-8 in EAC cells treated with Gal-9[86]. The elevated expression of IL-8 has been associated with a poor prognosis of esophageal cancer patients[87]. Thus, suggesting that Gal-9 resistance in EAC cells maybe attribute to IL-8 expression induced by Gal-9. Data from a Chinese study showed that low Gal-9 expression level was associated with a poor prognosis in ESCC patients[68].

Galectin-9 and glioma

Gal-9 and Tim-3 were expressed at an increased level in glioma tissues and TILs, respectively, and were correlated with tumor malignancy. The interaction of Tim-3 on TILs with Gal-9 expressed by tumors induced dysfunction or exhaustion of TILs. Blockading the Tim-3-Gal-9 pathway might delay glioma progression and improve KPS socre[88]. A study involving 1,027 patients with glioma suggests that Gal-9 plays a key role in the malignant progression of glioblastoma multiforme (GBM), with the highest expression in GBM, especially in the mesenchymal GBM subtype. Patients with high Gal-9 expression had a significantly lower survival rate than those with low Gal-9 expression. Further analysis of immune function suggested that Gal-9 might exert tumor immunosuppression or immune escape by positively regulating M2 tumor-associated macrophages[61]. In the tumor microenvironment of glioma, the Tim-3-Gal-9 pathway is affected by isocitrate dehydrogenase (IDH) mutant. IDH mutant reduced the interaction rate between Tim-3 positive T cells and Gal-9 positive microglia/macrophages, suggesting that IDH mutant could convey resistance to immune checkpoint inhibition and may be one of the ways that patients benefit from immunotherapy[89].

Galectin-9 and pancreatic carcinoma

Gal-9 was highly expressed in human pancreatic carcinoma, and patients' blood immune cells also showed higher Gal-9 expression. The study showed that neoadjuvant chemotherapy significantly reduced Gal-9 expression on pancreatic carcinoma cells. Gal-9 serum levels were significantly lower for the long-term (>12 months) compared with short-term survivors. Gal-9 promoted the phenotypic and functional polarization of macrophages towards M2 phenotype, and significantly reduced the secretion of TNF-α and INF-γ by T cells, which might support the further growth of tumors[60]. Gal-9 binds to one of its receptors, Dectin 1 (which is highly expressed on macrophages in pancreatic ductal adenocarcinoma), leading to tolerogenic macrophage programming and adaptive immunosuppression. The blockade of Gal-9 was also found to cause tumor regression and prolong survival[42]. A study of the efficacy of chimeric antigen receptor (CAR) T cells in pancreatic ductal adenocarcinoma (PDA) showed that the combination of CAR T cells with a biological inhibitor of Gal-9 significantly enhanced CAR T cell cytotoxicity against PDA cells, suggesting an immunosuppressive effect of Gal-9.[90] PDA-infiltrating γδ T cells (a subset of $CD3^+$ $CD4^-$ $CD8^-$ lymphocytes) not only release IL-10 and IL-17, but also express Gal-9 and PD-L1, hence establishing a powerful immunosuppressive microenvironment that promotes the development and progression of PDA.[91] However, exogenous rh-Gal-9 shows an opposite effect to endogenous Gal-9. Rh-Gal-9 may induce apoptosis through cytochrome release, which is associated with changes in miRNAs, thereby inhibiting tumor cell proliferation, suggesting that exogenous rh-Gal-9 may be a new therapeutic agent for pancreatic cancer[92].

Galectin-9 and cervical carcinoma

Cervical carcinoma is known to be mainly caused by HPV infection. The latest study found that Gal-9 and Tim-3 were highly expressed in cervical carcinoma patients, and their levels were significantly higher in HPV-positive cervical carcinoma patients than in HPV-negative cervical carcinoma and cervical intraepithelial neoplasia (CIN) patients, and were associated with tumor malignancy and disease. It was further found that the activation of the Tim-3-Gal-9 pathway was involved in the immune escape of cervical carcinoma by promoting the secretion of TGF-β and IL-10 by Treg cells, thereby inhibiting the cytotoxic function of Th1 and $CD8^+$ T cells[59]. Gal-9 and Tim-3 are overexpressed in cervical carcinoma tissues, and the biological effect is mediated through their epigenetics (hypo-methylation in the promoter regions). SUV39H1 (H3K9me3-specific histone methyltransferase) contributes to the changes of the methylation state of Gal-9 and Tim-3 by up-regulating the H3K9me3 (trimethylation of histone 3 lysine 9 at gene promoter region is an important epigenetic mechanism for silencing gene expression) level at the DNMT3A promoter region, and reverses the expression of Gal-9 and Tim-3, thus helping to improve the immune-imbalanced tumor microenvironment[93].

Galectin-9 and melanoma

In malignant melanoma, Gal-9 has a tumor suppressor function, and the weakening to loss of Gal-9 is closely related to the progression of metastasis[11]. High Gal-9 expression is associated with better prognosis[11,94]. Gal-9 inhibits the invasion and metastasis of malignant melanoma by blocking adhesion to endothelium and extracellular matrix[80]. Subsequent studies have shown that Gal-9 has direct apoptotic activity toward melanoma cells[95]. In patients with metastatic malignant melanoma, Gal-9 binding to CD206 on M2 macrophages promotes angiogenesis and chemokine production, which supports tumor growth and leads to poor prognosis in patients, suggesting that targeting the

interaction between this interaction may be a novel approach to improve the local antitumor effects of macrophages[43]. Accumulation of Gal-9 positive melanoma cells was observed in melanoma metastases *ex vivo*[96]. DNA methylation plays a central role in the pathogenesis and disease progression of melanoma[97]. Studies have shown a significant correlation between Gal-9 and Tim-3 methylation and mRNA expression and immune cell infiltration. High expression of Gal-9 and Tim-3 mRNA levels are significantly associated with increased overall survival, suggesting the feasibility of combining immune checkpoint molecules such as Gal-9 and Tim-3 as predictive biomarkers[94].

Galectin-9 and lymphoma

Cutaneous T-cell lymphoma (CTCL) is a type of peripheral T-cell lymphoma. A study showed that CTCL tumor cells expressed Gal-9, while Gal-9 expression was almost undetectable in normal skin, and high Gal-9 expression was associated with reduced $CD8^+$ T cell infiltration. Serum Gal-9 levels were increased in patients with advanced CTCL and correlated with disease severity markers, suggesting that Gal-9 expression in tumor cells might be related to disease progression in CTCL. On the other hand, exogenous administration of rh-Gal-9 could induce CTCL cell lines apoptosis by activating caspase-3 and caspase-9 independently of Tim-3, and inhibit tumor growth in tumor-bearing mice *in vivo*, and rh-Gal-9 combined with anti-Tim-3 blocking antibody had a synergistic effect on tumor inhibition *in vivo*[62]. The genetic status of Gal-9 may have an impact on cancer development. According to the COSMIC database, the mutant rate of Gal-9 in different cancers ranges from 0% to 2.5%, and the mutation frequency of Gal-9 is higher in diffuse large B-cell lymphoma (DLBCL) patients (3.2%), which may interfere with the interaction between Gal-9 and Tim-3[98]. At present, there are few studies on Gal-9 in various subtypes of lymphoma, and the effect of Gal-9 on the pathogenesis and prognosis of various subtypes remains to be further studied.

Galectin-9 and leukemia

Acute myeloid leukemia (AML) is derived from self-renewing leukemic stem cells (LSCs). Studies have confirmed that the serum Gal-9 was significantly elevated in human AML cells and a variety of preleukemic diseases, such as myelodysplastic syndromes (MDSs) and myeloproliferative neoplasms (MPNs), and myeloproliferative neoplasms (MPNs). Gal-9 and Tim-3 constitute an autocrine stimulatory loop for LSC self-renewal and human AML development, and Gal-9/Tim-3 autocrine signaling co-activates NF-κB and β-catenin signaling to facilitate expansion and transformation of malignant bone marrow clones[51]. Another study found that the Tim-3-Gal-9 autocrine loop was activated in AML cells through PKC/mTOR pathways[52]. Gal-9 is overexpressed in AML, especially when relapse after hematopoietic stem cell transplantation (HSCT), suggesting a poor prognosis. There is a strong positive correlation between Gal-9 and proteasome subunit beta type-8 (PSMB8), which plays a synergistic role in the progression of AML[99]. Drug resistance is a real barrier to chemotherapy in patients with many cancers, including AML, and has multiple forms, namely, multidrug resistance (MDR). The development of MDR in AML is related to changes in the Tim-3-Gal-9 signaling pathway[100]. Gal-9 and Tim-3 are significantly elevated in patients with chronic lymphocytic leukemia (CLL) and are closely associated with disease progression. Blocking the Tim-3/Gal-9 pathway weakens Treg cell's function and promotes CLL cell clearance[63,101]. Gal-9 may produce immune dysfunction, characterized by accumulation of non-functional and exhausted $CD8^+$ T cells[102]. Gal-9 is more expressed in advanced clinical stages of CLL patients[103]. These results suggest the importance of Gal-9 as a prognostic factor for leukemia and may be a promising molecular target for the treatment of leukemia, which may provide more combined therapy options.

According to the above discussion, Gal-9 plays a different role in different tumors, which can promote and inhibit cancer. In the role of promoting cancer, it mainly induces apoptosis of anti-tumor immune cells by binding to T cell surface receptors (especially Tim-3), which reduces anti-tumor immunity and promotes tumor growth. Gal-9 can directly induce tumor cell apoptosis, inhibit tumor cell migration, invasion, epithelial-mesenchymal transformation, and induce tumor cell cycle arrest. Gal-9 is involved in both immune escape and anti-tumor immune response. However, to date, it is not clear how changes in Gal-9 expression amplification in patients affect anti-tumor immune response. The correlation between the expression of Gal-9 and the characteristics of tumor-infiltrating immune cells needs more research (Fig. 3).

Immune potential of galectin-9 in cancer therapy

As mentioned above, the expression and function of Gal-9 vary dramatically in different types of cancers. Detection of Gal-9 expression in cancer has guiding significance for treatment selection and prognosis. For example, Gal-9 plays an important role as a tumor suppressor in gastric cancer, colorectal cancer, lung cancer, hepatocellular carcinoma, esophageal carcinoma, and melanoma[14,64,70,72,78,86]. In this case, inducing tumor cells to express more Gal-9 may benefit tumor therapy, while targeting Gal-9 may lead to tumor progression and metastasis. At present, several studies have shown that exogenous administration of human recombinant Gal-9 can significantly inhibit the growth of such tumors, which is effective new preparation. On the other hand, we will focus on cancer with Gal-9 as a tumor promoter that may benefit from anti-Gal-9 targeted therapy.

By reviewing a large number of relevant studies, we concluded that the increased expression of Gal-9 in patients with glioma, pancreatic carcinoma, cervical carcinoma, ovarian carcinoma, leukemia, and lymphoma is often indicative of malignant tumor behavior and poor prognosis. Specifically, higher levels of Gal-9 have been associated with increased susceptibility of glioma patients to develop malignant brain tumors, and Gal-9 levels were negatively associated with overall survival. Gal-9 was highly expressed in serum, intra-tumoral, and peripheral blood monocytes of patients with pancreatic carcinoma, which was associated with a low survival rate after metastasis. Increased expression of Gal-9 in epithelial tissue predicted an adverse response to treatment of high-grade serous ovarian

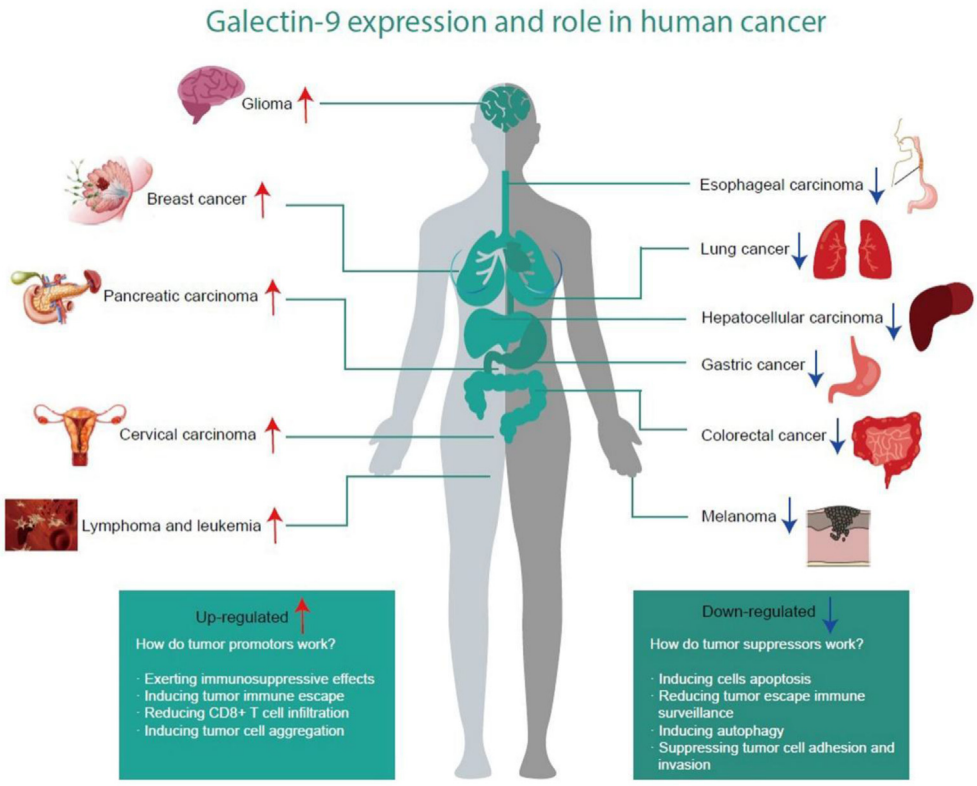

Figure 3 Different expression of Gal-9 in various types of human cancers. Gal-9 expression can be abnormally up-regulated or down-regulated during tumor-genesis to promote or inhibit tumor progression. Red arrow: Gal-9 increases with tumor progression. Blue arrow: Gal-9 decrease during tumorigenesis.

carcinoma. Leukemia patients with higher levels of Gal-9 showed treatment failure. Higher levels of Gal-9 on TILs predicted shorter relapse-free survival[18,60,63,104,105]. The use of anti-Gal-9 targeted therapy is likely to greatly improve clinical outcomes in these patients, and the combination of anti-Gal-9 therapy and ICIs may play a more powerful immunomodulatory role. These beneficiaries have the following important characteristics, suggesting that Gal-9 is an ideal tumor immunotherapy target. 1) Gal-9 is widely expressed in tumor cells, which is higher than that in adjacent tissues. Gal-9 can protect tumor cells against cytotoxic cell-dependent killing, and an increased level of Gal-9 can help tumor cells avoid phagocytosis and killing by the host immune system. 2) Gal-9 participates in tumor immune escape in such tumor cells by inducing apoptosis of Tim-3-positive T cells, and regulating T cell activity through the death receptor 3 signaling pathway, suggesting that targeting Gal-9 can block Gal-9-induced host immune surveillance escape. 3) The increased expression of Gal-9 in such tumors is often closely related to malignant proliferation. Gal-9 is involved in tumor progression, tumor angiogenesis, and other processes, contributing to the distant aggregation and adhesion of tumor cells. Therefore, targeting Gal-9 has significant therapeutic potential in inhibiting its malignant behavior. 4) Increased expression and secretion of Gal-9 drive the production of chemokines and inflammatory cytokines. Chemokines support tumor growth and poor prognosis, while inflammatory cytokines are involved in inhibiting TME formation and the Th2 inflammatory state that supports tumor growth. These characteristics suggest the reasons why this population may benefit from anti-Gal-9 targeted therapy[21,42,43,54,106–108].

Gal-9 is a widely expressed protein in immune cells, and its expression is involved in the regulation of various normal physiological functions. So, how can anti-Gal-9 targeted therapy avoid the off-target effects of normal tissues to achieve precision therapy? First, the expression pattern of Gal-9 in malignant tumors is different from that of normal cells. Studies have shown that Gal-9 is expressed mainly inside T cells, and its secreted form is barely detected under physiological conditions[30]. Serum Gal-9 levels can be significantly increased due to increased production of cancer cells, and cellular or tissue damage[109,110]. Excessive circulating Gal-9 competes for the availability of receptors outside their cell vicinity, such as Tim-3, PD-1, CD44, Dectin-1, etc., which may trigger unwanted signals, such as immunosuppression and apoptosis[107,110]. Meanwhile, studies and public database analysis of *The Human Protein Atlas* found that Dectin-1 and CD44 were almost unexpressed or low expressed in normal tissues and organs, while significantly expressed in malignancies[42,111,112]. Anti-Gal-9 targeted therapy works by competitively blocking the abnormal binding signal between Gal-9 and its receptors in tumors and manipulating its secretory pathways to normalize Gal-9 levels. Second, Gal-9-Tim-3 in tumor biology is different from normal cells. In many hematopoietic lineage tumors and solid cancers, the interaction between Gal-9 and Tim-3 may play a role in regulating tumor cell expansion and survival. The secretion of Gal-9 and Tim-3 was not detected in normal hematopoietic stem cells but significantly increased in myeloid LSCs. The co-secretion

of Gal-9 and Tim-3 promoted the expansion and transformation of malignant myeloid clones[51]. Third, Gal-9 contains two CRDs in the N- and C-terminal regions, with different activity characteristics. N-CRD is involved in the regulation of innate immune cells, and C-CRD is more effective in inducing T-cell apoptosis[113]. One study found that anti-Gal-9 specific antibodies targeting C-CRD significantly inhibited tumor growth and improved survival in tumors with high Gal-9 expression, and reduced differentiation of tumor-associated macrophages into protumor phenotypes. However, its binding to N-CRD was significantly lower than that of C-CRD, which also proved that it has no direct effect on monocyte differentiation, suggesting that anti-Gal-9 antibodies did not interfere with the regulation of innate immune function[114]. Finally, Gal-9, expressed in various normal tissues and organs, binds to cell surface receptors to elicit transient signals that participate in the regulation of T or B cell homeostasis in normal tissues. It also can be used to distinguish non-self from self by reading different glycan signals on pathogens and regulating host immune response, as well as facilitating timely shutdown of adaptive immunity to prevent autoimmune diseases[115,116].

Synergistic treatment of anti-galectin-9/Tim-3 in the context of existing immune checkpoint blockade

Immune checkpoint blockade (ICB) therapy has recently emerged as a significant clinical breakthrough in the treatment of advanced malignancies[117,118]. Although a series of ICIs, such as PD-1 and CTLA-4 inhibitors, have proven successful in a range of malignancies, there are subsets of patients that do not respond to these agents due to the upregulation of adaptive and innate resistance mechanisms by the tumor and its surrounding microenvironment[119]. Thus, significant challenges remain, including understanding pathways of resistance, optimizing patient selection, improving the management of immune-related adverse events, and identifying rational therapeutic combinations[120]. As new immunotherapeutic strategies are developed, there is a need for rational implementation of novel immunotherapy combinations that target complementary mechanisms of immunotherapy resistance intrinsic to each patient and tumor type.

Gal-9 and Tim-3 do not have an exclusive ligand-receptor as they have multiple binding partners involved in different signaling pathways[121]. Gal-9 is highly positively correlated with PD-1/PD-L1 in glioma patients and can be used as a biomarker for PD-1/PD-L1 tumor therapy[104]. A recent study showed that Gal-9 is a PD-1-binding protein, and the binding of Gal-9 to PD-1 is mainly mediated by the CRD of Gal-9 and the *n*116-linked glycan of PD-1. The two T cell inhibitory receptors PD-1 and Tim-3 are co-expressed on exhausted T cell differentiation, and PD-1 interacts with Gal-9 and Tim-3 to attenuate Gal-9/Tim-3-induced apoptosis of PD-1$^+$ Tim-3$^+$ T cells in cancers[41]. In addition, PD-1 expression desensitizes T cells to cell death mediated by the interaction of Gal-9 with Tim-3, and Gal-9 expression and secretion is regulated by IFNs[122]. These evidences confirm that Gal-9 as an important regulator of tumor immune response that can be targeted for cancer immunotherapy. Combined blockade of Gal-9 and PD-1 synergistically protects and promotes T cell activation in orthotopic pancreatic ductal adenocarcinoma[42], strongly suggesting that anti-Gal-9 therapy may also be used in conjunction with ICIs for cancer treatment. Furthermore, anti-Gal-9 therapy reduces the resistance and increases the sensitivity of PD-1 inhibitors in tumor immunotherapy. Tim-3 expression in CD8$^+$ T cells and Gal-9 expression in myeloid-derived suppressor cells are involved in lung cancer drug resistance to nivolumab, a monoclonal antibody (mAb) targeting PD-1. Anti-Tim-3 blocking antibody interferes with the Gal-9/Tim-3 pathway, thereby reversing resistance to PD-1 in lung cancer patients[82]. Tim-3$^+$ PD-1$^+$ TILs show a significant decrease in IL-2, TNF, and IFN-γ production, blocking Gal-9 enhanced the expression of these cytokines, thereby increasing the therapeutic effect of PD-1[123]. The expression of PD-L1 and Gal-9 in triple-negative breast cancer is increased by nuclear activation of NF-κB after treatment with taxane and anthracyclines. Cytotoxic agents, which increase immune checkpoint expression in tumor cells may increase the efficacy of ICIs therapy[124].

Galectin-9-Tim-3 targeting agents under development

There is increasing evidence that Gal-9-Tim-3 interaction plays a critical role in the context of cancer. In human AML, the secretion of Gal-9 and Tim-3 induces AML cells to evade immune surveillance and form an autocrine Gal-9-Tim-3 loop to drive the self-renewal of AML stem cells[51]. Gal-9 and Tim-3 expression levels were higher in the blood and bone marrow of AML patients who failed chemotherapy, and targeting the Gal-9-Tim-3 axis could be effectively combined with induction chemotherapy to increase the likelihood of complete remission in AML patients[105]. Furthermore, transgenic overexpression of Tim-3 has been shown to lead to increased frequency of CD11b$^+$ Ly-6G$^+$ myeloid suppressor cells, as well as a similar effect with Gal-9 overexpression, and loss of Tim-3 reversed this expansion and restored normal immune responses[125]. In breast cancer models, anti-Tim-3 antibody administration increased granzyme B expression in CD8$^+$ T cells and elicited an immune-mediated response to chemotherapy. Anti-Tim-3 and anti-Gal-9 antibodies showed equal effectiveness in improving the ability to respond to paclitaxel chemotherapy[126]. Collectively, these data suggest that the Gal-9-Tim-3 interactions inhibit immune responses and facilitate tumor growth. Therefore, the development of small-molecule inhibitors targeting Gal-9-Tim-3 has therapeutic potential for cancers whose growth is potentially mutually regulated by the Gal-9-Tim-3 signaling.

In cancer, Tim-3 expression specifically marks the most dysfunctional or terminally exhausted subset of tumor-infiltrating CD8$^+$ T cells. High expression of Tim-3 is associated with poor prognosis of solid malignancies and accumulated preclinical models suggest therapeutic benefits of Tim-3 blockade in regulating TME and limiting tumor growth, especially in combination with PD-1 blockade[41,127,128]. In addition to Tim-3$^+$ CD8$^+$ T cells, Tim-3$^+$ Treg cells are also targeted by anti-Tim-3 antibodies. Tim-3$^+$ Treg cells account for more than 50% of total Treg cells in malignant solid tumors, and their expression is correlated with tumor severity and progression. Anti-Tim-3 antibodies have been developed

Table 3 Anti-Tim-3 clinical trials.

Reagent name (manufacturer)	ClinicalTrials.gov Identifier	Phase	Cancer type	Co-blockade	Primary outcome	Secondary outcome	Recruitment status
Sym023 (Symphogen A/S)	NCT03489343	I	Solid tumors and lymphoma	Monotherapy	MTD; RP2D	Immunogenicity; ORR; SD; TTP	Completed
Sym023 (Symphogen A/S)	NCT03311412	I	Solid tumors and lymphoma	Anti-PD-1 (Sym021)	Treatment emergent AEs meeting DLT criteria (safety and tolerability)	Immunogenicity; ORR; SD; TTP; maximum concentration	Recruiting
TSR-022 (Tesaro)	NCT03680508	II	Locally advanced or metastatic liver cancer	Anti-PD-1 (TSR-042)	ORR	DOR; TTP; PFS; alpha-fetoprotein (AFP) response	Recruiting
TSR-022 (Tesaro)	NCT02817633	I	Advanced solid tumors	Anti-PD-1 (TSR-042)	DLT; AEs; ORR	DOR; DCR; OS; t1/2 of TSR-022 in combination with TSR-042	Recruiting
TSR-022 (Tesaro)	NCT04139902	II	Melanoma	Anti-PD-1 (TSR-042)	MPR	AEs; relapse-free survival; OS; frequency of cancellations of surgery	Recruiting
LY3321367 (Eli Lilly and Company)	NCT03099109	Ia/Ib	Advanced relapsed/ refractory solid tumors	Anti-PD-1 (LY3300054)	Number of participants with DLTs	Cmax of LY3321367; Cmax of LY3321367 in combination with LY3300054; PFS; DOR; TTR; DCR	Active, no recruiting
RO7247669 (Hoffmann-La Roche)	NCT04785820	II	Advanced or metastatic esophageal squamous cell carcinoma	Targets both Tim-3 and PD-1	OS	AEs; DCR; PFS; serum concentrations of RO7247669; CD8+Tim-3+	Recruiting
AZD7789 (AstraZeneca)	NCT04931654	I/IIa	NSCLC	Targets both Tim-3 and PD-1	AEs; DLT; preliminary anti-tumor activity of AZD7789	ORR; DCR; DOR; OS; C_{max}; immunogenicity; clearance;	Not yet recruiting
MBG453 (Novartis Pharmaceuticals)	NCT03961971	I	Glioblastoma multiforme	Anti-PD-1 (spartalizumab)	Number of participants with serious AEs	PFS; OS; ORR	Recruiting
MBG453 (Novartis Pharmaceuticals)	NCT04823624	II	Myelodysplastic syndromes	Monotherapy	ORR	OS; PFS; TTP; DOR	Not yet recruiting

MBG453 (Novartis Pharmaceuticals)	NCT02608268	I-Ib/II	Advanced malignancies	Anti-PD-1 (PDR001)	Safety and tolerability; ORR; DLTs	BOR; presence and concentration of anti-MBG453 antibodies; C_{max}; OS; DOR; PFS;	Active, not recruiting
MBG453 (Novartis Pharmaceuticals)	NCT03946670	II	Myelodysplastic syndromes	Randomized; HMA (decitabine or azacitidine)	CR rate; PFS	OS; event free survival; leukemia-free survival; response rate; time to CR	Active, not recruiting
INCAGN02390 (Incyte Corporation)	NCT03652077	I	Advanced malignancies	Monotherapy	Number of treatment-emergent adverse events; maximum tolerated dose of INCAGN02390	ORR; DOR; DCR; PFS; immunogenicity; level of binding of INCAGN02390 to Tim-3	Active, not recruiting
BMS-986258 (Bristol-Myers Squibb)	NCT03446040	I-II	Advanced cancer	Anti-PD-1 (Nivolumab), human recombinant hyaluronidase	AEs; serious AEs; incidence of AEs leading to discontinuation or to death	ORR; PFS; C_{max}; DOR; incidence of anti-drug antibody to BMS-986258	Recruiting
RO7121661 (Hoffmann-La Roche)	NCT03708328	I	Solid tumors, metastatic melanoma; NSCLC	Targets both Tim-3 and PD-1	DLT; ORR; DCR; DOR; PFS	C_{max}; total clearance; Volume of Distribution at Steady State; terminal half-life	Active, not recruiting
BGB-A425 (BeiGene)	NCT03744468	I	Solid tumors	Anti-PD-1 (Tislelizumab)	Safety and tolerability (MTD, RP2D and MAD)	DOR; DCR; PFS; C_{max}; terminal half-life; immunogenicity	Recruiting

Abbreviations: MTD, maximum tolerated dose; RP2D, recommended phase 2 dose; OR, objective response rate; SD, stable disease; TTP, time to progression; DLT, dose-limiting toxicities; AE, adverse event; PFS, progression free survival; DOR, duration of response; DCR, disease control rate; MPR, major pathologic response; Cmax, maximum concentration; BOR, best overall response; NSCLC, non-small cell lung cancer; MAD, maximum administered dose.

with high affinity and selectivity for Tim-3, specifically blocking the binding of Tim-3 to one or more its ligands, such as Gal-9, phosphatidylserine (PtdSer), carcinoembryonic antigen cell adhesion-related molecule 1 (CEACAM1), and high mobility group protein B1 (HMGB1). Antagonistic ligand-blocking anti-Tim-3 antibodies have the potential to eliminate T cell inhibition, activate antigen-specific T cells, and enhance anti-tumor immunity[121,129,130].

To date, many human phase 1/2 clinical trials of Tim-3 antibodies have been initiated (Table 3), as well as ongoing clinical trials targeting Gal-9. The first trial data to be reported was TSR-022, a humanized anti-Tim-3 IgG4 antibody. TSR-022 monotherapy resulted in stable disease in 31 patients with advanced solid tumors and one partial response in a patient with leiomyosarcoma, with no dose-limiting toxicity observed[131]. This provides support for combination therapy with anti-PD-1 antibodies (such as TSR-042). The clinical data of TSR-022, in monotherapy or in combination with TSR-042 in patients who progressed after anti-PD-1 treatment indicated that the combination of TSR-022 and TSR-042 (500 mg) was generally well-tolerated in both NSCLC and melanoma patients, and clinical activities have been observed in combination therapy. Particularly, at high doses of TSR-022 (300 mg) with an objective response rate (ORR) of 15% (3/20) and stable disease of 40% (8/20)[132]. Eli Lilly and Company reported that their anti-Tim-3 antibody, LY3321367, was preliminarily well-tolerated and passed phase I clinical trials[133]. According to the latest trial data, LY3321367 monotherapy had an ORR of 0%, disease control rate (DCR) of 35%, and progression-free survival (PFS) of 1.9 months in anti-PD-1/L1 refractory NSCLC patients ($n = 23$), ORR of 7%, DCR of 50%, and PFS of 7.3 months in PD-1/L1 responders ($n = 14$). In patients treated in combination with PD-L1 antibody, LY300054, ORR was 4% and DCR was 42% ($n = 91$). Data from this study indicate that LY3321367 has an acceptable safety profile and modest anti-tumor activity, and further studies in large cancer populations with assessable tumor samples for biological evaluation are needed to determine its efficacy[134]. Novartis' anti-Tim-3 antibody MBG453 in combination with spartalizumab, an anti-PD-1 antibody, also showed preliminary signs of antitumor activity and calculated a recommended phase II dose of 800 mg Q4W[135]. These data are consistent with data from preclinical models showing that the combination of anti-Tim-3 and anti-PD-1/PD-L1 is superior to monotherapy. Since December 2020, a Phase 1/2 clinical trial (NCT04666688) has been conducted to evaluate the safety and anti-tumor activity of LYT-200 (a mAb targeting Gal-9 protein) alone and in combination with chemotherapy or anti-PD-1 in patients with metastatic solid tumors (such as pancreatic cancer, cholangiocarcinoma, etc.). This is the first clinical trial to investigate targeting Gal-9 mAb, and we look forward to reporting the data from subsequent studies. Overall, existing human clinical trials data suggest that targeting Gal-9/Tim-3 and inhibiting Tim-3 can enhance the antitumor effect of PD-1 blockade. These results offer new insights into cancer patients who are unable to benefit from anti-PD-1 antibodies.

Conclusions

Gal-9 has attracted much attention in recent years due to its immunomodulatory role in various cancers. Gal-9 is expressed in virtually all organ systems and regulates cellular signaling by binding to its receptor, namely Tim-3, PD-1, CD44, CD40, 4-1BB, DR3, VISTA, and TLR-4. Gal-9 is involved in many physiological functions, such as growth, differentiation and death, cell adhesion, communication, and immune regulation. At present, many studies have agreed that the expression of Gal-9 is related to human cancer. The expression of Gal-9 plays different roles in different solid tumors and hematological tumors. Gal-9 has been established as a reliable, sensitive, and easily measured non-invasive biomarker, which can be combined with other disease parameters to jointly define disease activity and severity under various pathological conditions and can also be used as a prognostic monitoring indicator. Subsequent studies will focus on the specific role of dynamic changes in Gal-9 levels in monitoring disease progression and facilitating individualized treatment decisions. How to normalize the level of Gal-9 should be considered in subsequent studies, possibly through competitive blocking, interference of secretion pathways, and traditional treatment strategies. There are currently no obvious inhibitors of Gal-9, and the development of novel Gal-9 inhibitors to reduce the risk of cancers associated with high levels of Gal-9 may have great therapeutic potential in improving the clinical outcomes of cancer patients.

The interaction between Gal-9 and Tim-3 plays a key role in tumor immunity, and blocking the Gal-9/Tim-3 pathway induces anti-tumor immune responses and inhibits tumor growth. Therefore, activating cell costimulatory molecules by combining anti-Gal-9 antibodies or anti-Tim-3 antibodies and other immune checkpoint inhibitors may be a new type of immunotherapy for cancer treatment in the near future. Novel immunotherapy is a latent approach with great clinical benefits. Meanwhile, it is necessary to explore the synergistic or antagonistic relationship between different immune checkpoints to provide more scientific and reasonable individualized treatment, to open up a new path for cancer treatment.

Author contributions

Yan Lv, Xiao Ma, Yuxin Ma conceived and designed the study and also managed the collected data. All authors contributed to data collection and wrote the manuscript. Yuxin Du fully revised the manuscript, designed, and presented the figures and supported the work along with Jifeng Feng and Yan Lv.

Conflict of interests

The authors promise no potential conflicts of interest.

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Data availability statement

Research data are not shared unless the corresponding authors agree.

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