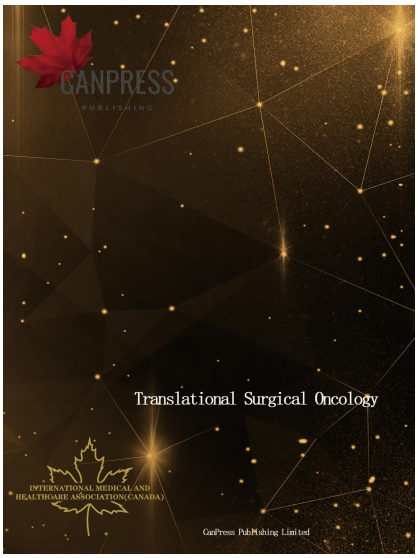
The Potential of Siglec Receptors in Cancer Immunotherapy



Darong Yang[1*]

1. Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN 38105

*Corresponding Authors Email:darong.yang@stjude.org

Abstract

The immune system has the potential to control tumor growth, but the immune responses are often hindered in an intricate tumor microenvironment. Cancer immunotherapy, which includes immune checkpoint blockades to relieve inhibition and cellular therapies to redirect immune cells to attack cancer cells, has improved the treatment for a variety of cancers. However, many types of cancers or partial patients within a sensitive category remain resistant to the currently approved cancer immunotherapies. It is necessary to gain a further comprehensive understanding of immune regulation mechanisms in the tumor, which facilitates the finding of innovative approaches and targets to overcome the resistance. Sialic acid-binding immunoglobulin-type lectins (Siglecs) are a family of immune receptors expressed on most types of immune cells and play vital roles in immune cell signaling. Recent evidence suggests that Siglecs could be a novel type of immune checkpoints and tumor-associated targets for cancer immunotherapy. This review summarizes the latest experimental and clinical evidence on identifying the roles of Siglec receptors in cancer immunotherapy and the ongoing therapeutics to target Siglec receptors and Siglec-sialic acid pathways.

Keywords: Cancer Immunotherapy, Siglec Receptors, Tumor Microenvironment, Immune Checkpoint, CAR T-cell therapy

1. Introduction

A fundamental principle of cancer immunotherapy is that oncogenesis necessitates evasion from immune surveillance [1]. To be efficacious, cancer immunotherapies should surmount cancer immune evasion, either by eliciting a de novo antitumor immune response or by reinvigorating extant effector cells, such as cytotoxic T cells. In the past decades, the field of cancer immunotherapy has experienced notable progress, primarily due to the introduction of immune checkpoint inhibitors that target inhibitory receptors CTLA-4 and PD-1, as well as its ligand PD-L1[2-5]. Additionally, the development of adoptive cellular therapies, such as chimeric antigen receptor (CAR) T-cell therapy, has significantly advanced blood cancer treatment, ultimately resulting in long-term remission for patients[6-9]. Despite monotherapies targeting CTLA-4, PD-1, or PD-L1 have demonstrated anti-tumor effects, the response rate in the treated population was only 20-30%[10]. Furthermore, combination therapies utilizing the blockade of CTLA-4/PD-1 or PD1/LAG3 have led to improved clinical outcomes and increased survival rates [11-14]. Nevertheless, the resistant cancer cells still could evade the antitumor immunity through other immune inhibitory receptors or pathways. Expanding the toolbox of immune checkpoint inhibitor candidates and other immune regulatory targets can facilitate designing and developing next-generation immunotherapy for cancer.

Sialic acid-binding immunoglobulin (Ig)-like lectins (Siglecs) are a family of immune receptors that mediate cell-cell and cell-pathogen interactions to regulate the functions of cells in immune system through recognition of sialic acid-modified ligands (sialoglycans)[15-16]. Since sialic acids are ubiquitously present on mammalian cells, Siglec-sialic acid interactions play crucial roles in self-nonself discrimination[17], regulation of inflammatory responses[18], phagocytosis[19], and allergic pathogenicity[20], determination of bacterial and viral infection

[21,22], and prevention of overactive immune responses as immune checkpoint[23]. In cancer, increasing evidence suggests that Siglec-sialoglycan interactions can have a detrimental impact in the tumor microenvironment, leading to immune suppression[24-26]. Therefore, Siglec receptors and tumor-associated sialoglycan ligands are emerging as new therapeutic targets for cancer immunotherapy. This review provides an overview of the fundamental roles of Siglec receptors and Siglec-sialic acid pathways in immune regulation and highlights recent progress in developing next-generation cancer immunotherapies by targeting Siglec receptors and Siglec-sialoglycan interactions.

2. Siglecs and their ligands

Siglecs are a group of Ig-like type I transmembrane proteins that include 14 members in humans and 9 members in mice (Figure 1). Of them, four Siglecs are highly conserved orthologs among all mammals including Siglec-1 (also known as CD169; Sialoadhesin), Siglec-2 (also known as CD22), Siglec-4 (also known as myelin-associated glycoprotein (MAG)), and Siglec-15. Other Siglecs lacking strict Orthology, referred to as CD33-related Siglecs, are thought to have originated from a duplication of the CD33 gene[27]. Human Siglecs are numbered according to their discovery, whereas murine Siglecs without human homology are assigned alphabetical names. The CD33-related Siglecs in humans include Siglec-3 (also known as CD33), Siglec-5, Siglec-6, Siglec-7, Siglec-8, Siglec-9, Siglec-10, Siglec-11, Siglec-14, and Siglec-16, while in mice, they are Siglec-3, Siglec-E, Siglec-F, Siglec-G, and Siglec-H[18]. Siglec-12 and -13 are excluded from this group because they are nonfunctional in humans[16,27]. Siglec receptors are broadly expressed on a variety of innate and adaptive immune cells and a few cell types out of the immune system including oligodendrocytes, Schwann cells, and placental trophoblasts (Figure 1)[15,18,27].

The extracellular domains (N-terminal) of Siglecs contain varying numbers of C-set Ig-like domains and a single V-set Ig-like domain that recognizes the sialoglycan ligands (Figure 1)[18]. Sialoglycan ligands are glycoproteins or glycolipids masked by sialic acid, a nine-carbon sugar, through a process called sialylation. The level of sialylation is regulated by two types of enzymes, sialyltransferases and sialidases, that add sialic acid residues to or remove sialic acid residues from glycoproteins or glycolipids, respectively[28]. The selectivity and specificity of individual Siglec toward different sialoglycan ligands are dependent on many factors including the linkages of sialic acids (α2-3, α2-6, and α2-8), sialic acid types (N-acetylneuraminic acid (Neu5Ac) or N-glycolylneuraminic acid (Neu5Gc)), and glycoproteins or glycolipids structures[29].

Except for Siglec-1, other Siglecs contain cytoplasmic domains (C-terminal) that are responsible for intracellular signal transduction. Most Siglecs have one or more consensus immunoreceptor tyrosine-based inhibitory motifs (ITIMs) that can potentially contribute to inhibitory signals. These ITIMs include classic ITIM, ITIM-like motif, and immunoreceptor tyrosine-based switch motif ITSM (Figure 1)[27]. The ITIMs of Silegcs can be phosphorylated by Src kinases, which then recruit SHP-1 and/or SHP-2, two phosphatases containing the Src-homology 2 domain (SH2). These two phosphatases subsequently dephosphorylate molecules within the activation complex, thereby inhibiting signaling[27]. In addition to these ITIMs, some Siglecs possess other cytoplasmic motifs, such as a motif for binding Grb2 in Siglec-2, Siglec-10, and Siglec-G, and a Fyn kinase binding site in Siglec-4 that is crucial for myelin function (Figure 1)[15,18,27].

Unlike the inhibitory Siglecs, Siglec-14, Siglec-15, Siglec-16, and Siglec-H are classified as activating Siglecs[27]. These Siglecs don't have an immunoreceptor tyrosine-based activation motif (ITAM) within their minimal cytoplasmic domain. However, upon binding to their sialoglycan ligands, these Siglecs can recruit an adaptor molecule called DAP12, which carries an ITAM and initiates activation signaling to exert an activating function[30-34]. In addition, the association of activating Siglecs and DAP12 is dependent on the positively charged amino acid residues within a transmembrane domain of Siglecs[30-34]. However, there is also evidence showing the immune suppressive role of Siglec-15 in the tumor microenvironment (TME)[35].

3. Siglecs as immune checkpoints

Blocking immune checkpoint pathways, such as PD1/PDL1 and CTLA4/CD80/CD86 pathways, has revolutionized cancer therapy[2-5]. However, only 12.46% of U.S. patients respond to FDA-approved checkpoint inhibitors based on the data in 2018, suggesting other immunosuppressive molecules also contribute to immune evasion in cancer[36]. There is increasing evidence showing that the inhibitory Siglecs can exert immune checkpoint functions in a variety of solid and liquid (blood) tumors (Figure 2)[24,37,38]. Release of Siglec-based inhibition is a promising approach to develop next-generation immune checkpoint inhibitors for cancer treatment. Some monoclonal antibody candidates targeting Siglecs or their ligands are in different phases of clinical trial (Table 1), though the mechanisms are not fully clear.

3.1 Siglec-7, Siglec-9, and Siglec-E

Siglec-7 is mainly expressed on natural killer (NK) cells, myeloid cells, and a proportion of tumor-infiltrating lymphocytes (TILs) including CD4+ and CD8+ T cells[27,39,42]. Siglec-9 is highly homologous to Siglec-7 (about 80% homology in V-set Ig-like domain) and expressed on monocytes, neutrophils, conventional dendritic cells, NK cells, and TILs[27,42-44]. Engaging Siglec-7 and Siglec-9 by tumor-expressed sialoglycan ligands inhibits NK function that could provide protection for tumor cells to prevent killing by NK cells (Figure 2)[45-46]. On T cells, Siglec-7 and Siglec-9 expression attenuates T cell activation and T cell–mediated tumor cell killing (Figure 2) [41-43,47]. Siglec-E is a murine

paralog of human Siglec-9[42]. Siglec-E deficiency could enhance neutrophil killing of tumor cells and in vivo immunosurveillance of autologous tumors, and this effect is reversed by transgenic Siglec-9 expression in myeloid lineage cells[48]. Another study showed that Siglec-E+ CD8+ TILs have a significant terminal exhaustion phenotype with high expression of Eome, PD1, Tim-3, and Lag3, and low expression of T-bet[42].

Tumor cells are commonly hypersialylated. For example, ligands for inhibitory Siglec-7/-9 on intratumoral NK cells and myeloid cells are increased in many types of solid tumors including pancreatic cancer, melanoma, non-small cell lung cancer, and hepatocellular carcinoma, and in hematological malignancies[45,48,49]. Siglec-9 ligands are upregulated in non-small cell lung cancer, squamous cell carcinoma, and melanoma, which can bind to Siglec-9-positive tumor-infiltrating CD8 T cells[42,43]. Notably, sialylated CD43 is a highly specific ligand for Siglec-7 that suppresses NK cell-mediated killing of K562 leukemia cells[50,51]. LGALS3BP is a secreted cancer-associated ligand for Siglec-9, Silgec-5, and Siglec-10, and is enriched in the extracellular matrix of prostate and colorectal cancers[52]. Siglec-9 is upregulated on tumor-infiltrating T cells in patients with melanoma, non–small cell lung cancer (NSCLC), colorectal, and ovarian cancer[42,43]. Siglec-7 overexpression on tumor-associated macrophages is associated with poor outcomes in metastatic colorectal cancer patients[53]. High-level expression of Siglec-9 on T cells correlates with lower survival of NSCLC patients[42].

3.2 Siglec-10 and Siglec-G

Siglec-10 and its murine paralog Siglec-G exhibit a strong affinity with hypersialylated CD24, a key switch in the negative regulation of innate immunity[17,28]. Disruption of the CD24/Siglec-10/G pathway can reverse this immune-suppressive effect[17,19,28]. The expression of Siglec-10/G is widespread across B cells, dendritic cells, and macrophages, and it binds robustly to CD24 in a sialylation-dependent manner[28]. Intracellular components released during tissue injuries are known as damage/danger-associated molecular patterns (DAMPs) that trigger inflammation. The host response to DAMPs, but not pathogen-associated molecular patterns (PAMPs), is negatively regulated by the interaction between CD24 and Siglec-10/G. Notably, CD24-/- mice completely survived from lethal doses of acetaminophen (AAP)-induced hepatotoxicity by suppressing the immune response to HMGB1[17]. Similarly, Siglec-G deletion significantly rescued mice from AAP-induced mortality. Importantly, knockout of CD24 or Siglec-G has no impact on LPS and Poly I:C-induced cytokine production as well as mice survival in LPS shock[17]. Thus, the CD24/Siglec-10/G pathway serves to discern between DAMPs and PAMPs in the host innate immune system. Intriguingly, microbial sialidases can bind to and remove sialic acids from hypersialylated CD24 which disrupts the immunosuppression of CD24/Siglec-G axis, contributing to the escalation of inflammation[54]. Consistently, mutations in either gene worsen the severity of sepsis. Therapeutic sialidase inhibitors are capable of protecting mice from sepsis by engaging a mechanism that encompasses both CD24 and Siglec-G [54].

In macrophage, CD24/Siglec-10/G axis serves as a ‘don’t eat me’ signal, allowing cancer cells to evade macrophage-mediated anti-tumor immunity. For instance, cancer cells expressing the CD24 antigen can trigger an anti-phagocytic signal on macrophages to shield themselves from macrophage-mediated phagocytosis through interaction with the Siglec-10/G receptor on tumor-associated macrophages (TAMs)[19]. In patient samples, CD24 is upregulated in various solid tumors, including ovarian and breast cancers, and is co-expressed with Siglec-10 on TAMs [19,55,56]. Genetic ablation of either CD24 or Siglec-10, as well as blockade of the CD24-Siglec-10 interaction using monoclonal antibodies, significantly enhances the phagocytosis of CD24+ human cancer cells (Figure 2). Furthermore, monoclonal antibody blockade of CD24-Siglec-10 signaling markedly improves the clearance of CD24-expression tumors in preclinical mouse models[19]. Ovarian and breast cancers have exhibited weaker responses to anti-PD-L1/PD-1 immunotherapies compared to other cancers, implying other alternative strategies, such as CD24-Siglec-10 blockade, may be necessary for better immunotherapy of CD24-high tumors.

3.3 Siglec-15

While belonging to the category of activating Siglecs[27], Siglec-15 has been identified as a new type of immune checkpoints in anti-tumor immunity by using a high-throughput T cell activity array that includes over 6,500 human genes encoding more than 90% of transmembrane proteins[35]. Siglec-15 is broadly upregulated on human cancer cells and TAMs though it is typically expressed only on certain myeloid cells in physiological condition[35]. Siglec-15 deficient mice are resistant to B16-GMCSF tumor growth by promoting CD8+ T cell responses, as depletion of CD8+ T cells reversed this phenotype[35]. More importantly, anti-Siglec-15 monoclonal antibody improved tumor control, rescued T cell suppression, and enhanced anti-tumor immunity in several mouse models[35]. One recent study using spatial technology showed that a limited number of CD8+ T cells surrounded Siglec-15+ tumor cells and Siglec-15+ TAMs exhibited close proximity to CD8+ T cells in PD-L1− cells [57]. In addition, Siglec-15+ tumor cells and TAMs were co-localized with more Tregs than Siglec-15- compartments [57]. Interestingly, CD11b (also known as Mac-1) and CD18 integrins on T cells were identified as binding partners of Siglec-15 via a proximity labeling assay[58]. The blockade of CD11b with monoclonal antibody (M1/70) dramatically abrogated the binding of Siglec-15 to human T cells. The presence of α2-6 sialoglycans on CD11b is crucial for the

interaction of Siglec-15 and CD11b/CD18 heterodimer [58]. In a phase 2 clinical trial of anti-Siglec-15 NC318 antibody for NSCLC, 28% of patients (5/18) who have experienced disease progression on/after PD-1 axis inhibitor therapy had durable clinical benefit with combination treatment of anti-Siglec-15 NC318 antibody and Pembrolizumab (NCT04699123). Therefore, Siglec-15 emerges as a promising target in addition to PD-1/PD-L1 for cancer immunotherapy.

4. CAR T-cell therapy targeting Siglecs or ligands

CAR T cells are modified T cells that express a CAR element containing extracellular antigen-binding domains, hinge and transmembrane domains, and intracellular co-stimulation domains including CD28 or/and 4-1BB (also known as CD137 and TNFRSF9) and activation domain CD3ζ[59]. CAR redirects T cells to tumor cells in a major histocompatibility complex (MHC)-independent manner (Figure 2)[60]. CD19-targeted CAR T-cell therapy led to a revolutionary advance for cancer treatment with 70% to 90% of complete response rates for B-cell acute lymphoblastic leukemia (ALL) patients[60,61]. However, about half of CD19-CAR-T-treated patients relapsed eventually after 1 year of treatment [60,62-64]. For patients with higher tumor burden at the time of CD19-CAR-T infusion, the relapse rate elevated to 69%[65]. One of major causes is CD19 antigen downregulation or even complete loss on relapsed leukemia cells[66]. This antigen-loss resistance can be overcome by additional infusion of CAR T cells targeting another antigen or treatment with dual targeting CAR T cells. With a similar expression profile of CD19, CD22 (Siglec-2) is expressed on the majority of pre-B cell ALL and normal B cell lineage[67-70]. In a phase 1 clinical trial, five CD19dim/neg relapsed B-ALL patients after anti-CD19 CAR T-cell therapy achieved a 100% complete response rate in a secondary anti-CD22 CAR T-cell therapy. CD22-CAR T cells induced overall 73% of complete remission in twenty-one CD19-CAR naïve and resistant B-ALL patients [71]. The following multiple CD22-directed CAR-T clinical trials also showed very promising results with 50% to 100% overall response rates (Table 2)[72-76]. Recent clinical trials using bispecific CAR T cells targeting both CD22 and CD19 have demonstrated positive results for B-cell malignancies (Table 2) [77-82]. One of the studies showed 88% of minimal residual disease-negative complete remission (CR) in 17 B-ALL patients and 29% of CR in 21 large B cell lymphoma (LBCL) patients[77].

CD33, also known as Siglec-3, is a myeloid cell surface marker expressed on normal myeloid progenitor cells, monocytes, macrophages, and leukemic blasts in more than 90% of acute myeloid leukemia (AML)[83,84]. In a first CD33-targeted CAR-T preclinical study, the anti-CD33 CAR T cells harboring a second-generation CAR construct with a 4-1BB co-stimulatory domain have been shown to effectively kill leukemic cells in vitro and in vivo[85]. Especially, anti-CD33 CAR T cells can target primary acute myeloid leukemia blasts derived from AML patients as well as normal CD33-positive bone marrow cells, suggesting the potential on-target, off-tumor toxicity should be carefully considered and managed in future clinical applications[85]. Another preclinical study generated a similar second-generation 4-1BB CAR targeting CD33 that shows potent anti-AML activity in vitro and in AML xenografts[86]. This study also emphasized the unacceptable hematopoietic toxicity during anti-CD33 CAR-T treatment in xenograft models. To reduce this side effect, the investigators utilized a transient CAR-expressing strategy instead of lentivirus-mediated stable expression to limit toxicity but keep potency for AML killing[86]. Another study introduced a CD33 knockout approach to overcome this side effect. Human hematopoietic stem and progenitor cells (HSPCs) with CD33 deficiency could differentiate into normal myeloid cells in immunodeficient mice[87]. Long-term multilineage engraftment of CD33-deficient HSPCs was observed after transplantation in rhesus macaques and those CD33-deficient HSPCs and derived myeloid cells were protected from anti-CD33 CAR T-cell attacking[87]. There were several anti-CD33 CAR-T clinical trials conducted for AML patients (Table 2). The response rates are varied, and further recruitment of more patients will help precisely assess its efficacy and safety[88-90]. To avoid single antigen escape or expand the targeting spectrum, multiple preclinical studies have evaluated the efficacy of bispecific CAR-T products that target CD33 and another AML marker. For instance, a CD123×CD33 bicistronic CAR-T approach could clear AML blasts expressing either or both CD123 and CD33 without more hematological toxicity than CD123 or CD33 single-target CAR T-cell therapy in preclinical mouse models [91]. While another CD33/CD123 bispecific CAR-T approach uses an “AND” logic gate only activated by cells expressing both CD33 and CD123, which may reduce on-target, off-tumor effects[92]. CD33 and CLL1 dual targeting CAR T-cell therapy was also assessed in mouse models and in AML patients. A 6-year-old female patient diagnosed with a complex karyotype AML including FLT3-ITD mutation achieved complete remission after two doses of CD33/CCL1 CAR-T infusion[93].

Siglec-6 is the third promising target in the Siglec family that has been evaluated in CAR T-cell therapy against AML and chronic lymphocytic leukemia (CLL)[94,95]. The best characterized AML CAR-T targets, for example, CD33 and CD123, are also expressed on HSPCs which causes unfavorable toxicity and safety issues[96]. Strikingly, Silgec-6 is not expressed on CD34+ HSPCs but highly expressed on primary and relapsed CLL cells and AML blasts including AML stem cells[94,95]. The binding domain of Siglec-6–specific CAR was derived from human monoclonal antibody JML-1. Siglec-6-targeted CAR T cells could eradicate Siglec-6+ AML and CLL tumor cells in xenograft mouse models[94,95], proposing a potential approach for AML and CLL treatment.

Compared to normal tissue cells, tumor cells are largely hypersialylated in many types of cancers[45,48,49]. The

hypersialylated ligands in the cell surface of tumor cells provide selective targets for the design of novel immunotherapies. Based on the features of Siglec-sialoglycan ligand interactions, one study tested the possibility of sialoglycan ligand-targeting CAR T-cell therapy using extracellular portion of either Siglec-7 or Siglec-9 as CAR antigen-binding domain, named S7 CAR or S9 CAR. Human T cells engineered with S7 CAR or S9 CAR exerted antitumor function in vitro and in vivo[97]. However, the side effects and therapeutic efficacy of Siglec-based CAR T-cell therapy should be further evaluated in more preclinical tumor models.

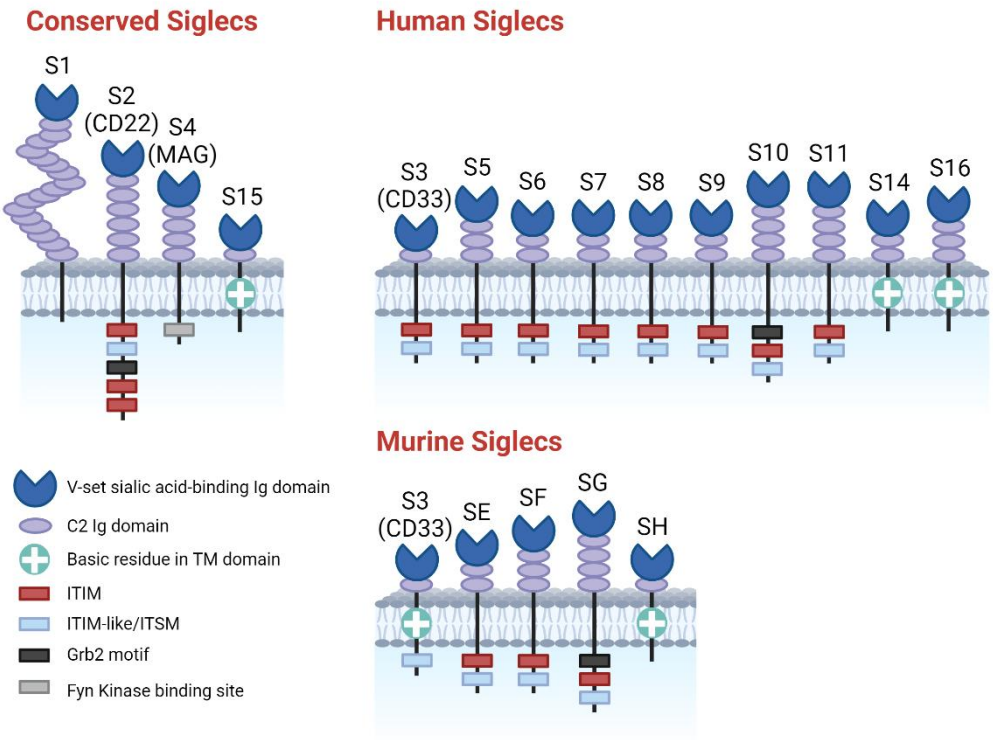
5. Siglec-based ADCs and BiTEs

Antibody-drug conjugates (ADCs) are a type of targeted cancer therapy that combines the specificity of monoclonal antibodies with the cytotoxic effects of chemotherapy drugs. After binding to a ligand, Siglec receptor could mediate the endocytosis of engaged molecule, which provides a strategy to deliver toxins to cancer cells that highly express a specific Siglec (Figure 2)[98,99]. For instance, Siglec-2 (CD22) is expressed on normal B cells and tumor cells of B-cell leukemia and lymphoma[67-70]. Several Siglec-2-targeted ADCs have been used for B-cell malignancies[25,100]. Inotuzumab ozogamicin (brand name Besponsa) is an FDA-approved ADC medication containing a complex of anti-CD22 antibody and calicheamicin toxin for treating relapsed or refractory B-cell precursor ALL (Table 1)[25]. Moxetumomab pasudotox (brand name Lumoxiti) is an ADC-like medication, called anti-CD22 immunotoxin, that includes a fusion protein of anti-CD22 monobody and PE38 Pseudomonas toxin[101]. It was approved by FDA in 2018 for the treatment of adults with relapsed or refractory hairy cell leukemia (HCL) who have received at least two prior systemic therapies, including treatment with a purine nucleoside analog (Table 1). Siglec-3 (CD33) expression is restricted to myeloid cells including AML blasts and AML stem cells[83,84]. Similar ADC approaches targeting CD33 could be used for the treatment of AML patients. Gemtuzumab ozogamicin (brand name Mylotarg) is an FDA-approved ADC medication harboring an anti-CD33 antibody and a calicheamicin toxin for the treatment of newly diagnosed and relapsed or refractory CD33+ AML in adults and pediatric patients (Table 1).

Bispecific T-cell engagers (BiTEs) are composed of two antibody fragments—one that recognizes and binds to a specific protein on the surface of CD3 on T cells and another that binds to a protein expressed on the surface of cancer cells. BiTEs facilitate the activation of T cells by bringing T cells and cancer cells into close proximity, leading to the formation of an immunological synapse and the subsequent killing of cancer cells by the T cells (Figure 2)[102]. Like ADCs targeting Siglec-2 and Siglec-3, many types of Siglec-targeted BiTEs have been tested in preclinical models[25,103-108]. These BiTEs target B-cell and myeloid cancer cells via binding to Siglec-2, Siglec-3, or Siglec-2/CD19 dural antigens[103-108]. Currently, some of them are undergoing Phase I clinical trials (Table 1). The first BiTE Blinatumomab (brand name Blincyto) targeting CD3/CD19 was approved by FDA for patients with Philadelphia negative relapsed or refractory B-cell progenitor ALL[25]. Up to December 2023, there are 11 BiTEs approved by FDA for the treatment of ALL, hemophilia, melanoma, lymphoma, and myeloma. Three of them were approved in 2023 including: 1. Epcoritamab-bysp (brand name Epkinly) binding to both the CD3 receptor on T cells and CD20 on malignant B cells received FDA accelerated approval for patients with relapsed or refractory DLBCL-NOS or HGBCL in May 2023; 2. Talquetamab-tgvs (brand name Talvey) which brings T cells to tumor cells by targeting GPRC5D for the treatment of relapsed or refractory multiple myeloma was approved by FDA in August, 2023; 3. Elranatamab-bcmm (brand name Elrexfio) is bispecific B-cell maturation antigen (BCMA)-directed CD3 T-cell engager approved by FDA in August, 2023 for adults with relapsed or refractory multiple myeloma.

6. Conclusion

The multifaceted interactions between Siglecs and tumor cells, immune cells, and TME have emerged as crucial determinants in shaping the immune response against cancer. By deciphering the complex signaling pathways and immunomodulatory functions of Siglecs, researchers are paving the way for innovative therapeutic strategies. The potential of targeting Siglec receptors to enhance anti-tumor immunity is underscored by the diverse array of preclinical and clinical studies. In this review, we summarized the recent advances of Siglec-based clinical trials in immune checkpoint blockade, BiTEs, ADCs (Table 1), and CAR T-cell therapy (Table 2). Better understanding of the dynamic interplay between Siglec receptors and the immune system holds great promise for refining and optimizing cancer immunotherapies, ultimately contributing to the development of more effective and tailored treatment approaches for cancer patients. Finally, the combination of Siglec-based therapeutics and other FDA-approved immune checkpoint inhibitors or cellular therapies may provide more treatment options for refractory or relapsed cancers.



Conserved Siglecs	S1	S2	S4	S15
Expression cell types	Mac, DC	B, cDC, MC	OD, Schw	Ocl, Mac

Human Siglecs	S3	S5	S6	S7	S8	S9	S10	S11	S14	S16
Expression cell types	Mac, MyP, Mo, Mic	N, Mo, B, MC	B, MC, Troph	NK, Mo, T, MC	Eo, Ba, MC	Mo, N, cDC, NK, T	B, Mo, Eo	Mac, Mic	N, Mo	Mac, Mic

Murine Siglecs	S3	SE	SF	SG	SH
Expression cell types	Mac, Mic	N, Mo, cDC, Mic	Eo, Mac	B, cDC	pDC, Mac, Mic

Figure 1. Siglec receptors.

Conserved Siglecs include Siglec-1 (S1), Siglec-2 (S2), Siglec-4 (S4), and Siglec-15 (S15). Human Siglecs include Siglec-3 (S3), Siglec-5 (S5), Siglec-6 (S6), Siglec-7 (S7), Siglec-8 (S8), Siglec-9 (S9), Siglec-10 (S10), Siglec-11 (S11), Siglec-14 (S14), and Siglec-16 (S16). Murine Siglecs include Siglec-3 (S3), Siglec-E (SE), Siglec-F (SF), Siglec-G (SG), and Siglec-H (SH). Sigelc expression cell types include macrophages (Mac), conventional and plasmacytoid dendritic cells (cDC and pDC), monocytes (Mo), myeloid progenitor (MyP), basophils (Ba), eosinophils (Eo), neutrophils (N), mast cells (MC), natural killer cells (NK), B cells (B), T cells (T), microglia (Mic), osteoclasts (Ocl), oligodendrocytes (OD), Schwann cells (Sch), and placental trophoblasts (Troph).

Table 1. Siglec-targeted mAbs, BiTEs, and ADCs in Clinical Trials.

Siglec	Drug name	Therapeutic Approach	Cancer type	Clinical phase	Trial ID
Siglec-2 (CD22)	Inotuzumab ozogamicin	mAb	ALL, lymphoma, NHL	II, I/II	NCT03441061, NCT03913559, NCT03104491
	Epratuzumab	mAb	CD22-Positive ALL	I/II	NCT00098839
	ADCT-602	mAb	Relapsed/Refractory ALL	I/II	NCT03698552
	JNJ-75348780 (J&J)	BiTE	NHL, CLL	I	NCT04540796
	DT2219	ADC (CD22/CD19)	Refractory B-lineage leukemia or lymphoma	I/II	NCT02370160
	TRPH-222	ADC	Lymphoma, NHL	I	NCT03682796
	Moxetumomab pasudotox-tdfk (Lumoxiti, AstraZeneca)	ADC	Relapsed/Refractory hairy cell leukemia	Approved	NCT03501615
	Inotuzumab ozogamicin (Besponsa, Pfizer)	ADC	Relapsed/Refractory ALL	Approved	NCT01564784
Siglec-3 (CD33)	Lintuzumab	mAb	AML	I/II, II	NCT03867682, NCT03441048
	Eluvixtamab (AMG 330)	BiTE	Relapsed/Refractory AML	I	NCT02520427
	JNJ-67571244 (J&J)	BiTE	AML, myelodysplastic syndromes	I	NCT03915379
	GEM333 (GEMoaB)	BiTE	Relapsed/Refractory AML	I	NCT03516760
	Gentuzumab Ozogamicin (Mylotarg, Pfizer)	ADC	Newly diagnosed and relapsed AML	Approved	NCT03727750
Siglec-9	Gatipotuzumab (PankoMab-GEX)	mAb (Siglec-9 ligand)	Solid Tumors	II, I	NCT01899599, NCT01222624, NCT03360734
Siglec 10	Alemtuzumab	mAb (Siglec-10 ligand)	CLL, SLL	II	NCT01465334
Siglec 15	NC318	mAb	Advanced non-small cell lung cancer	II	NCT04699123

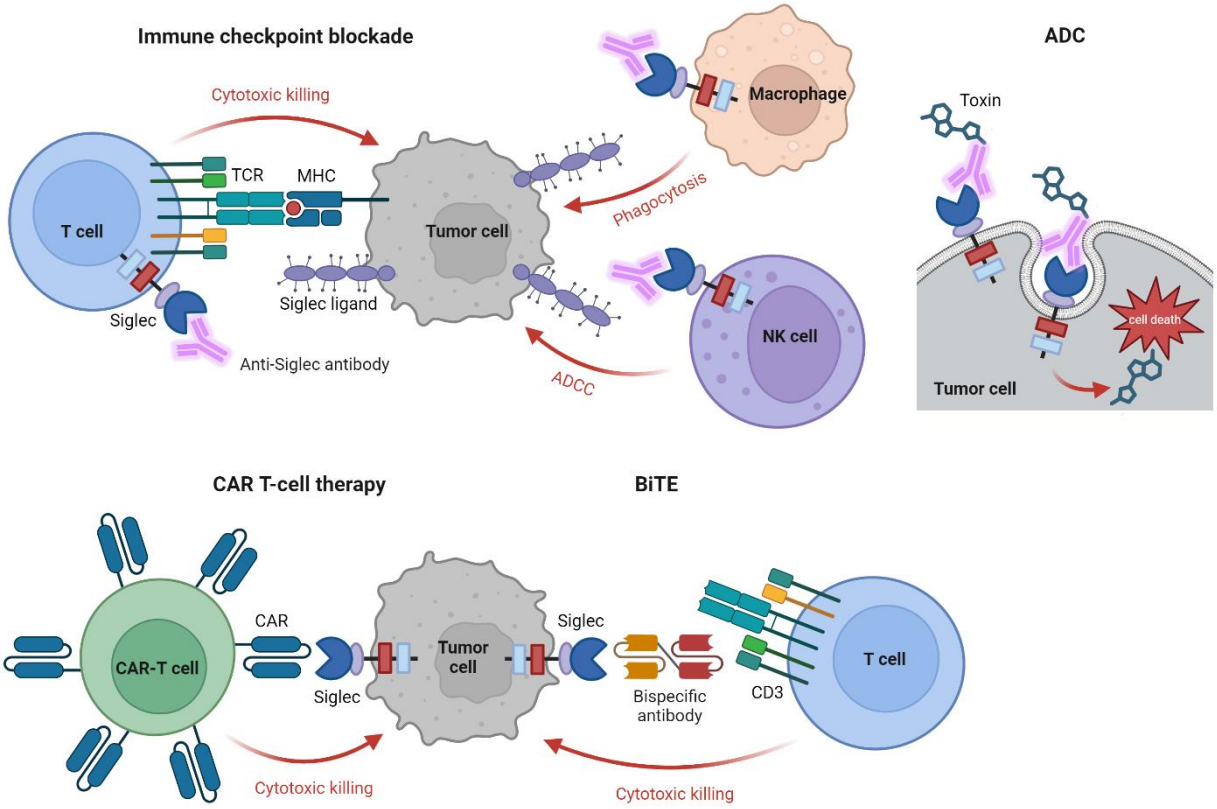


Figure 2. Siglec-based therapeutic approaches.

Four Siglec-based therapeutic approaches: 1. Immune checkpoint blockade by using anti-Siglec antibody. The engagement of Siglec ligands expressed on tumor cells to inhibitory Siglec receptors expressed on T cells, Macrophages, and NK cells leads to an immune suppressive signal which dampens the immune responses. Anti-Siglec antibody binding to the inhibitory Siglec, a new type of immune checkpoint, could release this inhibition. 2. Antibody-drug conjugate (ADC). Siglec-targeted ADC consists of an anti-Siglec antibody and an antibody-conjugated toxin molecule. When binding to the targeted Siglec receptor, the ADC construct is delivered into tumor cell via endocytosis. Then the internalized toxin triggers tumor cell death. 3. Bispecific T-cell engager (BiTE). BiTE is composed of two linked different single-chain variable fragments (scFvs) derived from different antibodies. For Siglec-based BiTE, one scFv targets tumor-associated Sigelc and another scFv targets CD3 on T cell, which brings T cell to tumor cell site to kill the engaged tumor cell. 4. Siglec-targeted CAR T-cell therapy. CAR-T cells are a special type of T cells engineered with a specific CAR that recognizes tumor antigen. Siglec-targeted CAR-T cells could bind to Siglec-positive tumor cells and trigger cytotoxic killing effect to clear tumor.

Table 2. Siglec-targeted CAR T-cell therapies in Clinical Trials.

Siglec	Drug name	Therapeutic Approach	Cancer type	Clinical phase	Trial ID
Siglec-2 (CD22)	CRG-022 (CargoTx)	CAR-T	Relapsed/Refractory lymphoma	II	NCT05972720
	Anti-CD22 CAR-T	CAR-T	Relapsed/Refractory ALL	II	NCT04340167
	JCAR-018 (BMS, NCI)	CAR-T	CD22-expressing B cell malignancies, hairy cell leukemia	I	NCT02315612, NCT04815356
	MendCART (Hrain Biotechnology)	CAR-T	Recurrent lymphoma	I	NCT02721407

	Anti-CD22 CAR-T	CAR-T	Large cell lymphoma, follicular lymphoma, ALL, NHL	I	NCT02315612
	UCART22 (Cellectis)	CAR-T (Allogeneic)	Relapsed/Refractory ALL	I	NCT04150497
	ThisCART22	CAR-T (Allogeneic)	B-cell malignancies	I	NCT05106946
	Anti-CD22 CAR-T	CAR-T	B-cell malignancies	I	NCT04088890
	CART22	CAR-T	Chemotherapy resistant or refractory ALL	I	NCT02650414
	UCART20x22 (Cellectis)	CAR-T (CD22/CD20)	NHL	I/II	NCT05607420
	AUTO1/22 (Autolus Therapeutics)	CAR-T (CD22/CD19)	ALL, NHL	I	NCT02443831
	CTA-101 (Nanjing Bioheng Biotech)	CAR-T (CD22/CD19)	NHL	I	NCT04227015
	CAR-T19/CAR-T22	CAR-T (CD22/CD19)	Relapsed/Refractory ALL and lymphoma	I	NCT04204161
	CD19/CD22-BBz	CAR-T (CD22/CD19, Allogeneic)	Lymphoid leukemia	I	NCT05507827
	CAR20.19.22	CAR-T (CD22/CD20/CD19)	B-cell malignancies	I	NCT05094206
	Anti-CD19/CD20/CD22 CAR-T	CAR-T (CD22/CD20/CD19)	Recurrent ALL, CLL, PLL, NHL	I	NCT05418088
Siglec-3 (CD33)	VOR33 (Vor BioPharma)	CAR-T	AML	I/II	NCT05984199
	Anti-CD33 CAR-T	CAR-T	AML	I/II	NCT03126864
	Enhanced CD33 CAR T	CAR-T	Relapsed/Refractory AML	I/II	NCT04835519
	CD33CART	CAR-T	AML	I/II	NCT03971799
	PRGN-3006 (Precigen)	CAR-T	AML, myelodysplastic syndromes	I	NCT03927261
	SC-DARIC33	CAR-T	Relapsed/Refractory CD33+ AML	I	NCT05105152
	Anti-CD33 CAR-T	CAR-T	AML	I	NCT05445765
	Anti-CD33 CAR-T	CAR-T	Recurrent/Refractory/Secondary AML	I	NCT05672147
	CD33KO-HSPC	CAR-T	AML	I	NCT05945849
	Anti-CD33 CAR-T	CAR-T	AML	I	NCT05473221
	Dual CD33/CLL1 CAR T	CAR-T (CD33/CLL1)	Relapsed/Refractory AML	I	NCT05248685
	CLL1+CD33 CAR-T	CAR-T (CD33/CLL1)	AML	I	NCT05467254
Siglec-6	Anti-siglec-6 CAR-T	CAR-T	Relapsed/Refractory AML	I/II	NCT05488132

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Competing interests

The author declares no conflict of interest.

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