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Review Article

The sialic acid–Siglec immune checkpoint: an opportunity to enhance immune responses and therapy effectiveness in melanoma

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Abstract

Modulation of immune responses through immune checkpoint blockade has revolutionized cutaneous melanoma treatment. However, it is still the case that not all patients respond successfully to these therapies, indicating the presence of as yet unknown resistance mechanisms. Hence, it is crucial to find novel targets to improve therapy efficacy. One of the described resistance mechanisms is regulated by immune inhibitory Siglec receptors, which are engaged by the carbohydrates sialic acids expressed on tumour cells, contributing to programmed cell death protein-1 (PD1)-like immune suppression mechanisms. In this review, we provide an overview on the regulation of sialic acid synthesis, its expression in melanoma, and the contribution of the sialic acid–Siglec axis to tumour development and immune suppressive mechanisms in the tumour microenvironment. Finally, we highlight potential sialic acid–Siglec axis-related therapeutics to improve the treatment of melanoma.

Melanoma is an aggressive type of cancer produced by the aberrant proliferation of melanocytes in the skin, eyes and mucosal tissues. Melanoma is classified into four subtypes, according to the tissue where it develops: cutaneous melanoma, uveal melanoma, acral melanoma and mucosal melanoma. Cutaneous and uveal melanoma have the highest incidence.[1–4]

Targeting tumour-infiltrating lymphocytes (TILs) using immune-checkpoint blockade (ICB) therapies has revolutionized the treatment of hot tumours in the past decade, especially for cutaneous melanoma.[4,5] These therapies interfere with immune-checkpoint molecules present on T cells, which contain inhibitory intracellular domains, leading to immune suppression upon engagement with their ligands.[6] Cutaneous melanoma was one of the first cancers in which ICB therapies were shown to be effective, targeting the immune checkpoints cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and programmed cell death protein-1 (PD1) (or its ligand, PD-L1). However, because around 50% of the patients with melanoma still do not show a beneficial response from ICB, the search for novel targets and combinatorial therapies is ongoing.[4] Conversely, treatment options for uveal melanoma are scarce, rendering it a deadly malignancy at the present time. However, Kimmtrak, a drug that enhances T cell-mediated tumour killing, was recently approved to treat this rare type of melanoma.[2,7,8]

Tumours contain a complex microenvironment in which not only malignant cells, but also other cell types such as endothelial cells, immune cells and stromal cells co-evolve in time. The immune landscape of a tumour can include cells from both the lymphoid lineage [$CD8^+$ T cells, $CD4^+$ T cells, natural killer (NK) cells and B cells] and the myeloid lineage (mainly dendritic cells, macrophages and myeloid-derived suppressor cells).[9] All current ICB therapies focus on reactivating the T cell function.[4] However, the findings of large populations of myeloid cells infiltrating the TME hint at additional resistance mechanisms mediated by the myeloid repertoire.[10] Therefore, there is an urgent need to better understand the complex TME of melanoma and novel molecular pathways that control melanoma progression to increase the response rate to therapy.

A well-characterized molecular pathway known to affect melanoma progression and survival is the metabolic process of glycosylation. Glycosylation is a post-translational modification in which proteins and lipids are decorated with carbohydrates, also called glycans, giving rise to a variety of glycan structures that can bind receptors on immune cells. Sialylation is the process by which a specific glycan called sialic acid is enzymatically attached to these glycan structures.[11,12] The increased expression of sialic acid, or hypersialylation, has been described as a major hallmark in melanoma and other types of cancer, playing a key role in tumour development and antitumour immune responses through engagement of Siglec receptors on immune cells.[13–16] Most Siglecs have inhibitory intracellular domains containing immunoreceptor tyrosine-based inhibitory motifs (ITIM) or ITIM-like motifs similar to PD1 and, upon engagement with sialic acid, trigger immune suppressive mechanisms.[17–19]

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This makes the sialic acid–Siglec axis an emerging glyco-immune checkpoint to be targeted in the TME, in particular because of its wide immune-modulatory effect on both the myeloid and the lymphoid compartments.[14,20]

Sialic acid and the sialylation pathway: a metabolic process

The term sialic acid refers to around 50 different compounds. *N*-acetyl-neuraminic acid (Neu5Ac) is the most abundant sialic acid in the human body.[21] The synthesis of Neu5Ac starts with the hexosamine biosynthetic pathway, which converts glucose into uridine diphosphate-*N*-acetyl-glucosamine (UDP-GlcNAc). From UDP-GlcNAc, the enzyme UDP-GlcNAc 2-epimerase/ManNAc-6-kinase (GNE) synthesizes *N*-acetyl-mannosamine 6-phosphate (ManNAc-6P), which is then converted to Neu5Ac (Figure 1.1). Neu5Ac enters the nucleus where it is activated by the CMP-Neu5Ac synthase (CMAS) enzyme (Figure 1.2). The activated sialic acid donor, CMP-Neu5Ac, is transported to the Golgi apparatus by a CMP antiporter (SLC35A1) (Figure 1.3). In the Golgi apparatus, several enzymes called sialyltransferases (STs) transfer Neu5Ac from the activated donor to glycan chains on either proteins (Figure 1.4a, b, c) or lipids (Figure 1.5). Depending on the type of linkage that STs create between Neu5Ac and its underlying glycan, sialylation is classified into α2,3, α2,6 or α2,8 sialylation. Finally, all sialylated glycoproteins and sialylated glycosphingolipids (also called gangliosides) can be expressed on the cell surface (Figure 1.6). In contrast to STs, there are sialic acid-hydrolysing enzymes called neuraminidases or sialidases, that cut sialic acid off from glycoproteins and glycolipids, enabling its recycling (Figure 1.7).[11,16]

Either an increase in the expression of STs and/or sialic acid transporters, a downregulation or decrease in the activity of neuraminidases, or an increased flux through the sialic acid synthesis pathway can lead to hypersialylation. Thus, the up- and downregulated expression of the genes that encode the sialylation machinery modulate the synthesis of a large variety of sialylated structures that can be expressed on the cell membrane.[22,23]

Hypersialylation in melanoma

Transcriptomic data analysis demonstrated that α2,3 sialylation is particularly increased in melanoma compared with other tumours and mainly STs responsible for α2,3 sialylation are overexpressed in human melanoma.[24,25] In particular, ST3Gal-I expression correlates with cutaneous melanoma progression, as it is upregulated in metastatic melanoma compared with primary melanoma. ST3Gal-I is also overexpressed in *BRAF*-mutant melanoma compared with the wild-type (WT) subtype. Furthermore, ST3Gal-I promoted cell migration and invasion of a human melanoma cell line from lung metastasis.[26] In addition, ST3Gal-III knockout (KO) mouse melanoma cells have less metastatic capacity than their ST3Gal-III-expressing counterparts.[27] Expression of ST3Gal-IV also promotes melanoma invasiveness, as knocking down ST3Gal-IV in a human melanoma cell line reduced proliferation and migration *in vitro*.[28]

STs that are responsible for α2,6 sialylation are also overexpressed in patients with melanoma, such as ST6Gal-I and ST6GalNAc-II.[25] ST6Gal-I expression is upregulated in *BRAF*-mutant melanoma compared with WT melanoma cell lines.[29] Although ST6Gal-I has been associated with progression and metastasis of different cancer types, little is known about the role of ST6Gal-I in melanoma.[30] Downregulation of ST6Gal-I in mouse melanoma cell lines decreased cell adhesion and migration through extracellular matrix components *in vitro*.[31]

The upregulation of STs involved in α2,8 sialylation was associated with melanoma, mainly ST8Sia-I.[25,32,33] In line with this, gangliosides GM3, GD3 and GD2 are overexpressed in cutaneous melanoma.[32–34] Also, different clusters of patients can be identified based on the expression of gangliosides in patients' melanoma cells, which correlate with their survival.[32] Gangliosides are involved in tumour cell proliferation and invasion.[34] Engagement of the ganglioside GD2 with integrin β1 is related to melanoma cell adhesion and a malignant phenotype.[35]

Little is known about the expression of neuraminidases in melanoma and how this can impact sialylation. Only NEU3 was reported to be overexpressed in melanoma cell lines derived from patients. NEU2 is not expressed in melanoma cells, and the expression of NEU1 and NEU4 varies among melanoma cell lines.[32,36] Furthermore, knocking down the transporter SLC35A1 in a mouse melanoma cell line led to a decrease on surface α2,6 sialylation and impaired tumour growth *in vivo*.[37] Also, knocking out the CMAS enzyme in the same cell line led to reduction of surface sialylation and significantly delayed tumour growth *in vivo* (unpublished data).

In contrast to cutaneous melanoma, little is known about the role of sialylation in uveal melanoma. The expression of genes associated with the sialic acid synthesis pathway correlates with lower survival of patients with uveal melanoma.[24] *In vitro* studies demonstrated that uveal melanoma cells express α2,3- and α2,6-linked sialic acid, but at a lower extent compared with cutaneous melanoma cells.[38,39] In particular, ST3Gal-IV is overexpressed in both cutaneous and uveal melanomas.[24] Furthermore, downregulation of ST6Gal-I, ST6GalNAc-II and ST8Sia-I has been correlated with invasiveness of uveal melanoma cells.[40] Also, GM3 and GD3 gangliosides are expressed by uveal melanoma cells, but not GD2.[41]

Sialic acid–Siglec interactions in melanoma

Immune cells express surface receptors for sialic acid called Siglecs (sialic acid-binding immunoglobulin-like lectins) (Figure 2). Most Siglecs contain inhibitory intracellular domains that are activated upon engagement with sialic acid, dampening immune responses. In physiological conditions, sialic acid works as a 'self-associated molecular pattern' by engaging inhibitory Siglecs and preventing immune activation to maintain self-tolerance. However, tumour cells exploit this interaction by increasing the expression of sialic acid on their surfaces.[11,21,42] Hypersialylation enables immune evasion by tumour cells through engagement with Siglecs on both myeloid and lymphoid immune cells that infiltrate the TME.[17,20]

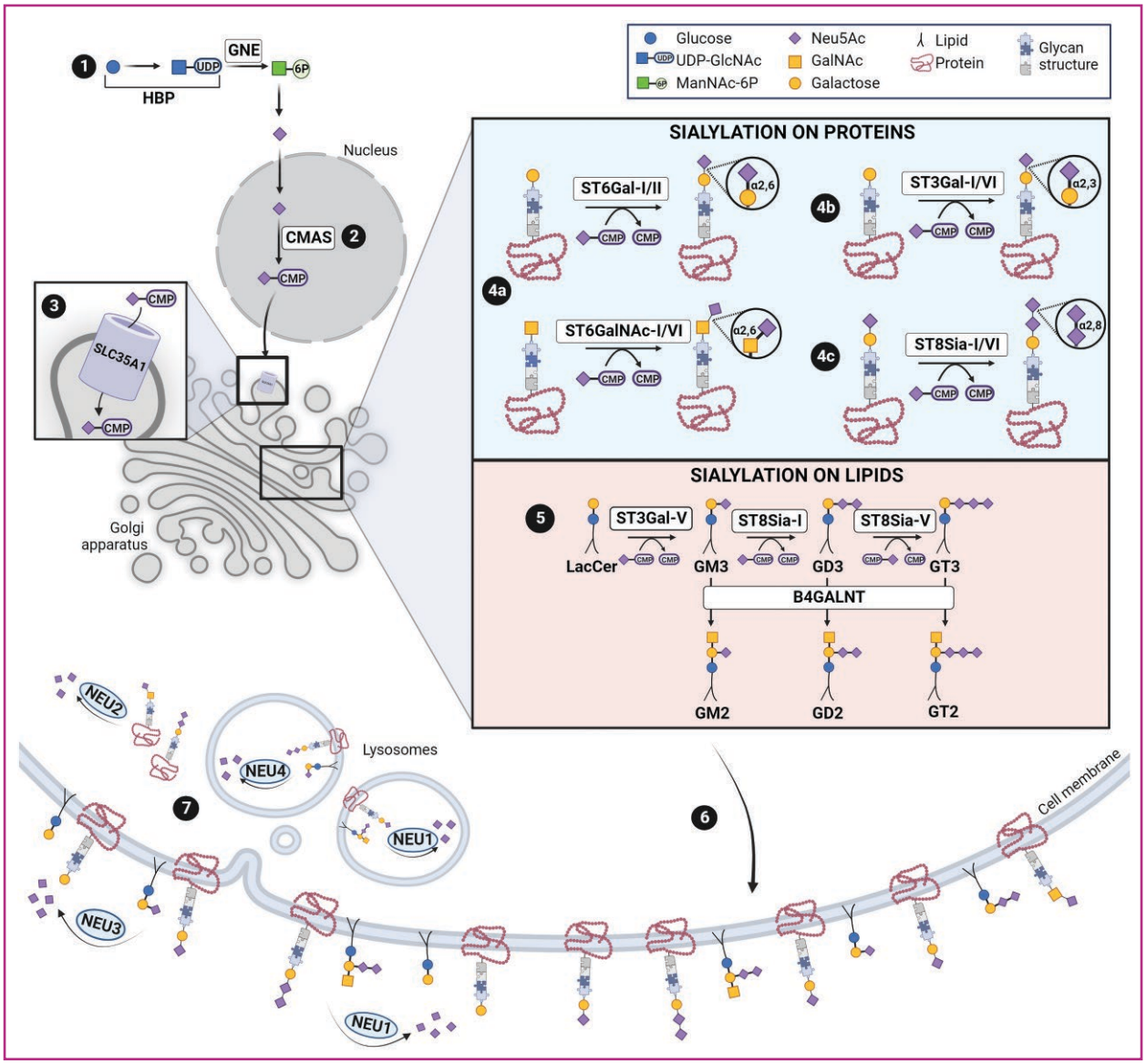

Figure 1 The sialylation pathway. (1) Uptake of glucose that leads to the synthesis of Neu5Ac. (2) Activation of Neu5Ac. (3) Transport of CMP-Neu5Ac into the Golgi apparatus. (4a–c) Sialylation on proteins. (5) Sialylation on lipids. In humans, there are 20 STs that catalyse the α2,3; α2,6 or α2,8 linkage between Neu5Ac and another glycan: (4a) ST6Gal-I and II and ST6GalNAc-I to VI for α2,6 sialylation with galactose (Gal) or *N*-acetyl galactosamine (GalNAc) as underlying glycan, respectively; (4b) ST3Gal-I to VI for α2,3 sialylation with Gal as underlying glycan; and (4c) ST8Sia-I to VI for α2,8 sialylation with sialic acid as underlying glycan. (5) Sialylated glycosphingolipids, or gangliosides, are synthesized by specific STs from the lipid LacCer. (6) Expression of sialylated proteins and gangliosides on the cell surface. (7) There are four neuraminidases in humans: NEU1–4. NEU1 and NEU4 act mainly in lysosomes; NEU1 can be also found on the cell surface; NEU2 is in the cytosol; NEU3 acts mainly on glycolipids on the cell surface. B4GALNT, β1–4 *N*-acetyl-galactosaminyltransferase; CMAS, CMP-Neu5Ac synthase; CMP-Neu5Ac, cytidine monophosphate *N*-acetyl neuraminic acid; GalNAc, *N*-acetyl galactosamine; GNE, UDP-GlcNAc 2-epimerase/ManNAc-6-kinase; HBP, hexosamine biosynthetic pathway; LacCer, lactosyl ceramide; ManNAc-6P, *N*-acetyl-mannosamine 6-phosphate; NEU, neuraminidase; Neu5Ac, *N*-acetyl neuraminic acid; STs, sialyltransferases; UDP-GlcNAc, uridine diphosphate-*N*-acetyl glucosamine. Created with BioRender.com.

Cutaneous melanoma cells express mainly ligands for Siglecs-7 and 9, which is in line with the upregulation of the specific STs mentioned in the previous section, as they are involved in the synthesis of ligands for these two Siglec receptors.[13,43] Regarding the lymphoid compartment (Figure 3, light blue callout), all NK cells express Siglec-7 (Figure 3a), while Siglec-9 is limited to a subset of $CD56^{dim}$ NK cells (Figure 3b). Engagement of Siglecs-7 and 9 on NK cells *in vitro*, with either agonistic antibodies or sialic acid present on tumour cells, led to a more suppressive phenotype.[44] T cells also express Siglec-7 and 9.[17] $CD8^+$ T cells in the TME but not in peripheral blood of patients with melanoma express Siglec-9 (Figure 3c), indicating that expression of this receptor on $CD8^+$ T cells may be induced in the TME.[25]

As for the myeloid cells (Figure 3, orange callout), monocytes, tumour-associated macrophages (TAMs), dendritic cells and neutrophils can also express Siglec-9, or its mouse homologue, Siglec-E (Figure 3d).[17,20,45] Siglec-E expression was related to tumour growth, survival and response to

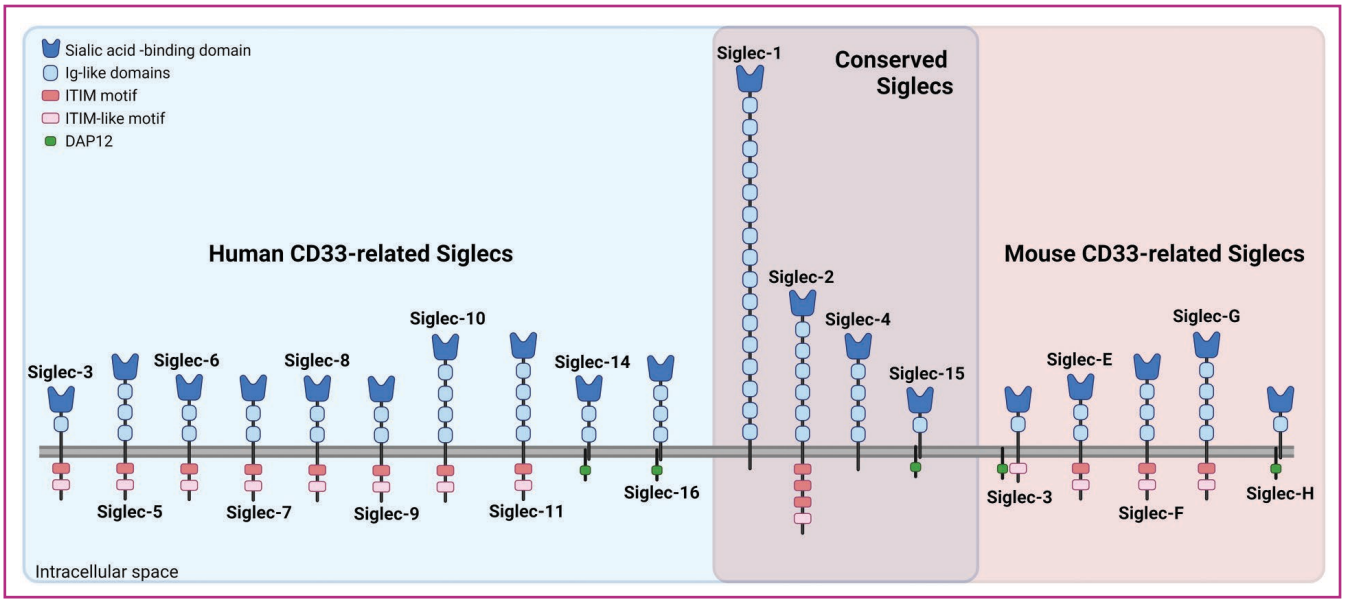

Figure 2 Siglec receptors contain a sialic acid-binding domain and Ig-like domains. They are divided into two groups: Conserved Siglecs (present in both humans and mice) and CD33-related Siglecs. Most of the Siglecs have inhibitory intracellular motifs (ITIM or ITIM-like motifs). The exceptions are Siglec-1 and Siglec-4, which do not present any intracellular motif with immunomodulatory properties; Siglec-3, which is an inhibitory receptor in humans, but in mice, although it contains an ITIM-like motif, it is described as an activatory Siglec as it can interact with DAP12; and Siglecs-14, 15, 16 and H, which are activating receptors as they interact with the protein DAP12, although for Siglec-15 inhibitory functions have been described. Ig, immunoglobulin; ITIM, immunoreceptor tyrosine-based inhibitory motifs; Siglecs, sialic acid-binding immunoglobulin-like lectins. The grey line represents the cell membrane. Created with BioRender.com.

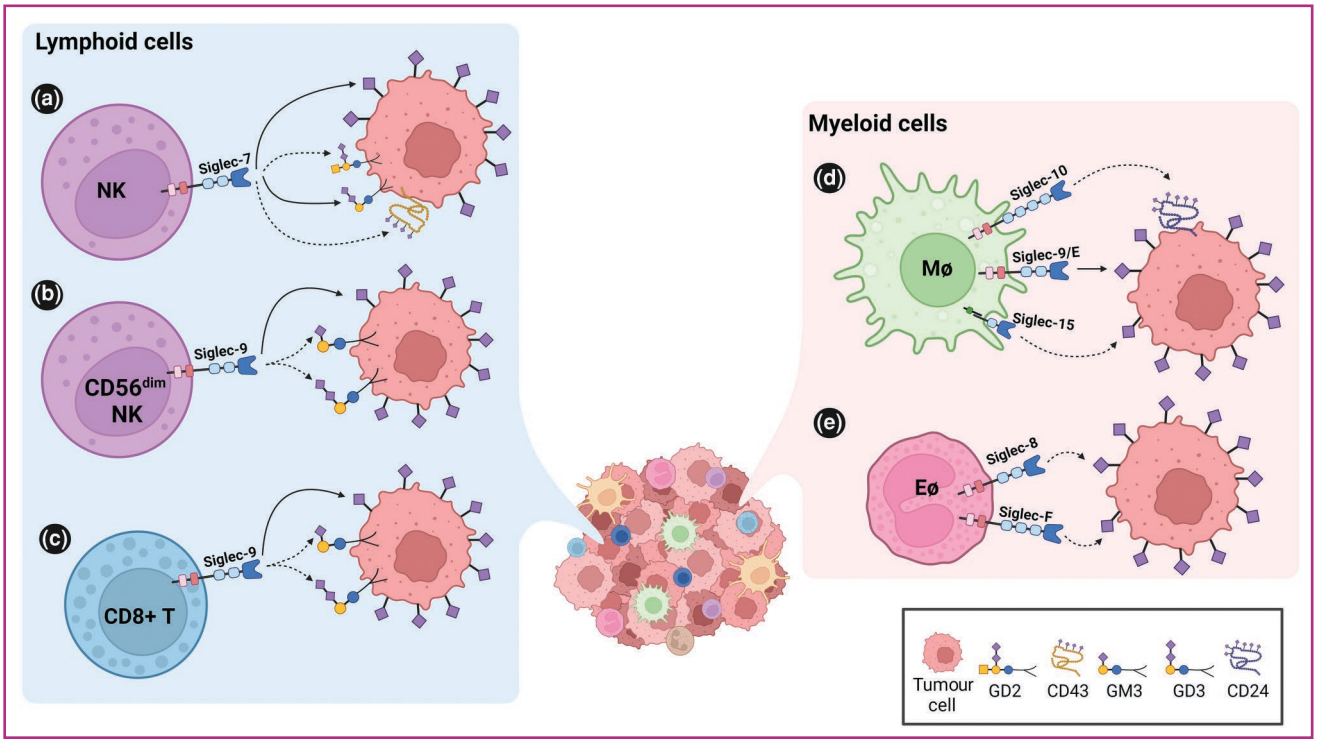

Figure 3 Sialic acid–Siglec interactions in the melanoma TME. Both lymphoid (light blue callout) and myeloid (orange callout) immune cells that are found in the melanoma TME express Siglecs. Sialic acid–Siglec interactions between melanoma cells and (a) NK cells, (b) a subset of NK cells ($CD56^{dim}$ NK cells), (c) $CD8^{+}$ T cells, (d) macrophages (Mø) and (e) eosinophils (Eø). NK, natural killer; Siglecs, sialic acid-binding immunoglobulin-like lectins; TME, tumour microenvironment. Full arrows represent interactions found in the melanoma TME. Dashed arrows represent potential interactions not yet determined in melanoma. Created with BioRender.com.

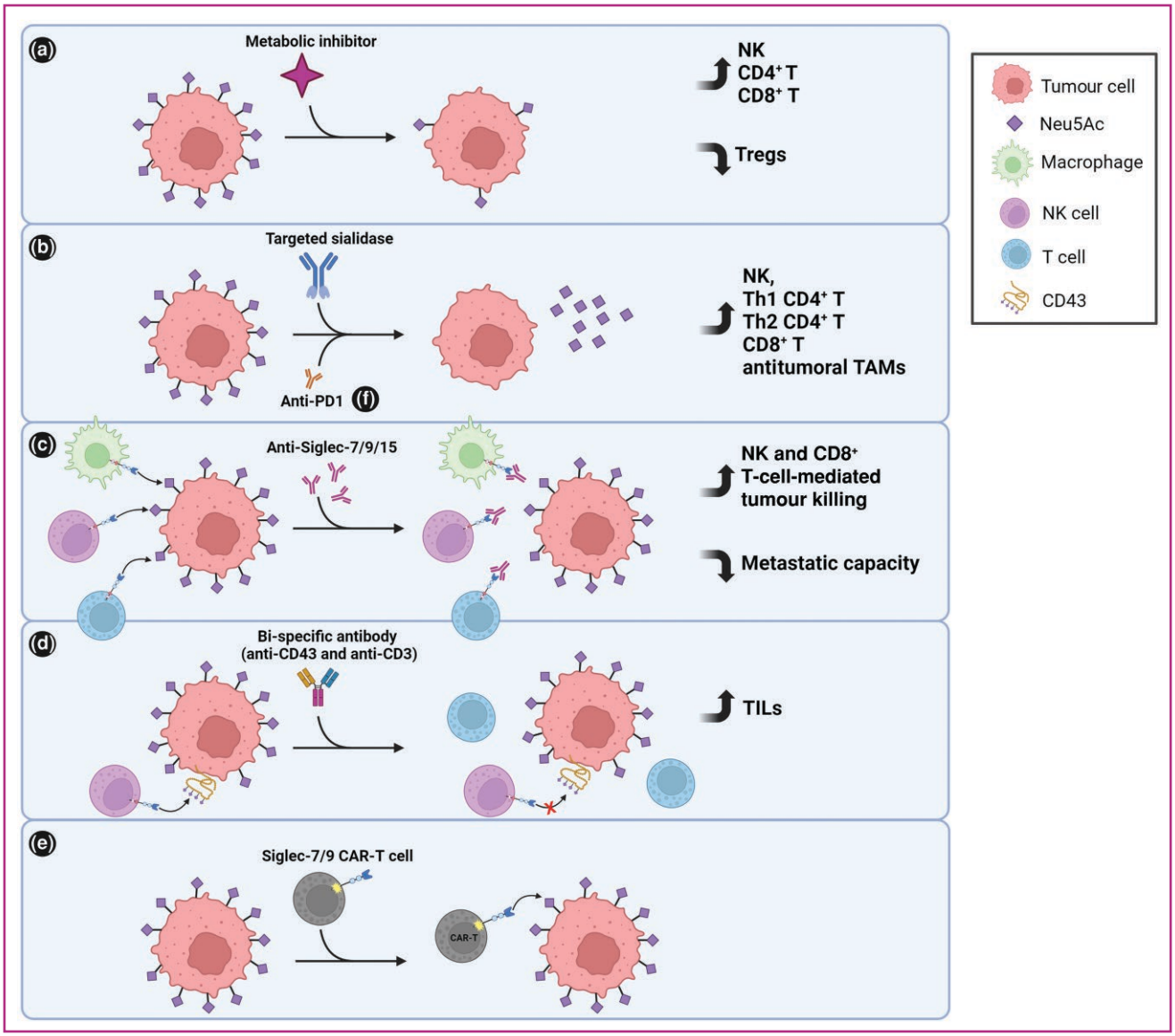

Figure 4 Therapeutic strategies targeting the sialic acid–Siglec axis and their effects *in vivo*. (a) Metabolic inhibitor of sialylation. (b) Targeted sialidase. (c) Anti-Siglec antibodies. (d) Bi-specific antibodies against CD43 and CD3. (e) CAR-T cells expressing the extracellular domains of Siglec-7 and 9, modified with activatory intracellular motifs. (f) The combination of a targeted sialidase with anti-PD1 is currently being tested in a phase I clinical trial (NCT05259696). The main effects in the TME produced by each therapeutic strategy are indicated at the right, as increase or decrease with arrows. CAR, chimeric antigen receptor; Neu5Ac, *N*-acetyl neuraminic acid; NK, natural killer; PD1, programmed cell death protein-1; Siglec, sialic acid-binding immunoglobulin-like lectin; TAMs, tumour-associated macrophages; Th, T helper; TILs, tumour-infiltrating lymphocytes; TME, tumour microenvironment; Tregs, T regulatory cells. Created with BioRender.com.

therapy in subcutaneous mouse models of melanoma.[46,47] Interestingly, expression of Siglec-E in melanoma can change according to the anatomical location of the tumour. When B16 tumours were developed subcutaneously or in the liver of mice, Siglec-E was expressed mainly on TAMs. However, when the tumours were located in the lungs, Siglec-E was mainly expressed on neutrophils.[48] This highlights the importance of studying the expression of Siglecs according to tumour location in order to determine which immune cell subset can be the main contributor to tumour suppression. In addition, sialylated glycans have been shown to be involved in monocyte differentiation to immunosuppressive TAMs, through engagement of Siglec-7 and 9.[49] Whether this plays a role in the melanoma TME remains to be elucidated.

Gangliosides expressed by melanoma cells can also bind Siglec receptors. GD3 binds both Siglec-7 and 9, dampening NK cell cytotoxic activity (Figure 3a).[50,51] GD2 can also interact with Siglec-7. Targeting GD2 has been beneficial for melanoma treatment; however, its dependence on Siglec-7 engagement remains to be determined.[52,53] GM3 can also bind Siglec-9 (Figure 3b, c); however, the impact of this binding in melanoma development has not yet been studied.[32,51] Furthermore, melanoma cells express the sialylated protein CD43, which was identified as a Siglec-7 ligand.[54]

Although Siglecs-7 and 9 are the main Siglecs involved in cutaneous melanoma, other Siglecs are also present on myeloid cells in its TME. Siglec-15 was identified as a T cell suppressor molecule expressed on TAMs in human

melanoma (Figure 3d). Although Siglec-15 has an intracellular domain that interacts with DAP12, its presence in myeloid cells of the TME reduces T cell proliferation and functionality. The mechanism behind this suppressor activity remains to be elucidated.[55] Injection of a B16 melanoma cell line in Siglec-15 KO mice led to delayed tumour growth and increased survival compared with WT mice, and this effect was dependent on CD8$^+$ T cells.[56] Also, expression of CD24, a Siglec-10 ligand, in melanoma cells is related to poor prognosis and enhanced tumour growth and metastasis *in vivo*. This effect could be mediated by Siglec-10, which is present on TAMs.[57–59] In addition, eosinophils have recently been described as important mediators of antitumour immunity and therapy response in melanoma. Although they are usually defined by the expression of Siglec-8 or F (Figure 3e), the role of these Siglecs on eosinophils in the TME has not yet been elucidated.[60,61]

As for uveal melanoma, Siglec-10 expression was associated with poor disease outcome.[62] However, the role of sialic acid–Siglec axis in this type of melanoma remains to be studied.

Targeting the sialic acid–Siglec axis

Stimulation of antitumour immunity

Different therapeutic strategies have been tested in melanoma to disrupt the sialic acid–Siglec axis (Figure 4). Reduction of surface sialic acid on tumour cells using a metabolic inhibitor of sialylation led to slower tumour growth and decreased metastasis in subcutaneous mouse models of melanoma, as well as increased survival (Figure 4a).[63–65] Furthermore, this treatment increased the number of NK cells and CD4$^+$ and CD8$^+$ T cells in the TME, together with a decrease in T regulatory cells. CD8$^+$ T cells in the TME expressed more activation and degranulation markers compared with nontreated tumours. In addition, intratumoral injections with this metabolic inhibitor caused remission in 50% of the mice and even protection against tumour rechallenge. The treatment led to an increased infiltration of major histocompatibility complex class I-restricted, ovalbumin-specific, CD8$^+$ T cells (OT-I T cells), and increased rejection of tumours after adoptive transfer of OT-I T cells, with improved median survival time. A combination of the inhibitor with TLR9 agonist (CpG) treatment reduced tumour growth and increased median survival time compared with single treatments.[63] In a metastatic model of B16F10, intravenous treatment with tumour-targeted particles containing the sialylation inhibitor led to reduced metastatic lesions in the lungs.[64] However, it remains to be determined if the inhibitor treatment effects are Siglec-dependent.

Another therapeutic option tested in melanoma is tumour-targeted sialidases (Figure 4b). Stanczak *et al*. used a targeted antibody-sialidase fusion protein (E-301) to treat B16D5 melanoma tumours expressing the antigen HER2 *in vivo*.[46] After intraperitoneal treatment with E-301, tumour growth was delayed and survival was increased. This effect was dependent on CD8$^+$ T cells. Moreover, there was an increase in NK cells, effector CD8$^+$ T cells and T helper (Th)1 and Th2 CD4$^+$ T cells in the TME, as well as a shift from immunosuppressive TAMs to TAMs with antitumoral properties.[46]

Directly targeting Siglecs or their specific ligands also represents an effective treatment strategy. B16 melanoma cells were injected intravenously in mice expressing human Siglecs-7 and 9, and the treatment with anti-Siglec-7 and anti-Siglec-9 antibodies led to a reduction in the number of metastatic lesions in the lungs (Figure 4c).[48] Co-culture of NK cells with tumour cells containing Siglecs-7 and 9 ligands in the presence of anti-Siglec-7 and anti-Siglec-9 blocking antibodies led to enhanced cytotoxicity, while the opposite effect was seen in the presence of agonistic antibodies.[44] Siglec-9 was also identified on CD8$^+$ T cells in the TME of melanoma, and cytotoxicity *in vitro* was inhibited with agonistic anti-Siglec-9.[25] Siglec-9 appears to be a promising target to enhance both NK cell and CD8$^+$ T cell-mediated killing of melanoma cells.[25,44,66] In addition, treatment with antibodies targeting both CD43 (a Siglec-7 ligand) and CD3 led to more infiltration of T cells in the TME and delayed tumour growth of melanoma *in vivo*, using a humanized mouse model (Figure 4d).[67]

Apart from monoclonal antibodies, other approaches have been used to target Siglecs and their ligands in melanoma. Meril *et al*. produced chimeric antigen receptor (CAR) T cells expressing chimeric receptors with the exodomains of Siglec-7 or Siglec-9 (Figure 4e). They used a tumour xenograft model injecting a primary melanoma cell line, and after three injections of either Siglec-7 or Siglec-9 CAR T cells, tumour growth was delayed and survival was increased.[68]

Improvement of response to current immunotherapies

The synergy between altering the sialic acid–Siglec axis and antibody-based anticancer therapies was proven by genetic approaches in melanoma *in vivo* models. Mice bearing B16F10 tumours lacking the enzyme GNE had increased sensitivity to anti-PD1 and anti-CTLA4 therapy compared with WT tumours.[46] Ibarlucea-Benitez *et al*. inoculated B16 tumour cells subcutaneously in either WT mice, Siglec-E KO mice or mice expressing human Siglecs-7 and 9.[48] Only in Siglec-E KO mice was tumour growth significantly reduced after treatment with a monoclonal antibody against the tumour antigen gp75. They also combined anti-gp75 with anti-PD1 therapy, and the reduction in tumour growth was more pronounced in Siglec-E KO mice than in WT mice. Similar results were obtained using a cell line that expresses more Siglec ligands, B16-FUT3.[48] Furthermore, the expression of Siglec-9 on CD8$^+$ T cells in the TME of melanoma correlated with PD1 expression, and only Siglec-9$^+$ CD8$^+$ T cells, but not Siglec-9$^-$ CD8$^+$ T cells, responded to the anti-PD1 checkpoint inhibitor.[25]

Stanczak *et al*. combined the targeted sialidase E-301 with anti-PD1 and anti-CTLA4 treatment in a B16D5 model, which enhanced the beneficial effects in tumour growth and survival compared with E-301 treatment alone.[46] Currently, a bi-sialidase fusion protein (E-602) is being tested as a single agent and in combination with anti-PD1 therapy in a phase I clinical trial (NCT05259696) for different cancers, including melanoma[69,70] (Figure 4f).

Conclusion

The interaction of tumour sialic acid with Siglec receptors dampens the immune response in the TME of melanoma through not only lymphoid cells but also cells from the myeloid compartment. Hence, the sialic acid–Siglec axis imposes an extensive resistance mechanism including a wide variety of immune cells that are also important players in cancer development. This makes the sialic acid–Siglec axis an attractive target to combine with current ICB therapies, which aim to target only lymphoid cells. Tumour-targeted therapies based on the disruption of the sialic acid–Siglec axis reduced tumour growth, increased survival and enhanced infiltration of antitumour immune cells in melanoma. However, most research has been done *in vitro* and *in vivo* using animal models, with as yet limited applications in humans. Potential efficacy and side-effects need to be explored, which will be achieved in the coming years with the results of ongoing clinical trials. Moreover, we need to understand the variability of the sialic acid–Siglec axis among patients with melanoma and its utility to better predict responders and nonresponders to immunotherapy.

In addition, more research needs to be done to unveil the implication of sialic acid in uveal melanoma progression and the potential benefit of therapies against the sialic acid–Siglec axis for this type of melanoma. This arises as an opportunity to explore the treatment of both primary and metastatic disease, for which effective therapies are still lacking.

Finally, it is important to highlight that the role of the sialic acid–Siglec axis can be different between tumours and during different stages of the same tumour, so it is crucial to study this pathway in each context. One question to be answered is whether the sialic acid–Siglec axis is involved in the development of hot vs. cold and altered tumours, which could ultimately help us to understand how sialylation is involved in cutaneous vs. uveal melanoma, and how it can be modulated to drive the conversion of cold or altered tumours into hot tumours, potentially increasing the efficacy of current immunotherapies and contributing to personalized therapies.

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability

No new data were generated.

Ethics statement

Not applicable.

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