Glycobiology, 2018, vol. 28, no. 9, 640–647
doi: 10.1093/glycob/cwx108
Advance Access Publication Date: 27 January 2018
Review

Review

Targeting sialic acid–Siglec interactions to reverse immune suppression in cancer

Olivia Joan Adams[2], Michal A Stanczak[3], Stephan von Gunten[2,1], and Heinz Läubli[3,4,1]

[2]Institute of Pharmacology, University of Bern, Inselspital INO-F, 3010 Bern, Switzerland, [3]Laboratory of Cancer Immunology, Department of Biomedicine, and [4]Medical Oncology, Department of Internal Medicine, University Hospital Basel, Petersgraben 4, 4031 Basel, Switzerland

[1]To whom correspondence should be addressed: Tel: +41-61-265-5074; Fax: +41-61-265-5316; e-mail: heinz.laeubli@unibas.ch (H.L.); Tel: +41-31-632-32-98; Fax: +41-31-632-49-94; e-mail: stephan.vongunten@pki.unibe.ch (S.v.G.)

Received 14 October 2017; Revised 25 November 2017; Editorial decision 18 December 2017; Accepted 19 December 2017

Abstract

Changes in sialic acids in cancer have been observed for many years. In particular, the increase of sialoglycan density or hypersialylation in tumors has been described. Recent studies have identified mechanisms for immune evasion based on sialoglycan interactions with immunoregulatory Siglec receptors that are exploited by tumor cells and microorganisms alike. Siglecs are mostly inhibitory receptors similar to known immune checkpoints including PD-1 or CTLA-4 that are successfully targeted with blocking antibodies for cancer immunotherapy. Here, we summarize the known changes of sialic acids in cancer and the role Siglec receptors play in cancer immunity. We also focus on potential ways to target these Siglec receptors or sialoglycans in order to improve anti-cancer immunity.

Key words: blocking antibodies, cancer immunotherapy, hypersialylation, immune evasion, *N*-acetyl-neuraminic acid, *N*-glycolyl-neuraminic acid

Introduction

The first descriptions of altered glycosylation in cancer were already published several decades ago (Hakomori 1984; Amon et al. 2014). One of the major observations from these early investigations was the increase of sialylation in many tumor types. The direct influence of this so-called hypersialylation on cancer progression remained elusive for many years after its initial discovery (Schauer 2000; Büll et al. 2014). Several lines of evidence demonstrate that interactions with sialic acid-binding receptors, including selectins, can significantly influence cancer progression (also see the review article by Lubor Borsig in the same issue). Hypersialylation can lead to a change in the physical properties of the tumor cell (Schultz et al. 2012), potentiate evasion of apoptosis of cancer cells (Büll et al. 2014) and influence signaling platforms via clustering of sialoglycans in lipid rafts or the immunological synapse (Nicoll et al. 2003; Krengel and Bousquet 2014). Earlier analysis has also shown that hypersialylation enhances immune evasion by interference with factor H and inhibition of the complement system (Fedarko et al. 2000). Recent studies elucidated that hypersialylation can enhance immune evasion through the engagement of sialic acid-binding immunoglobulin-like lectins (Siglec) receptors (Hudak et al. 2014; Jandus et al. 2014; Läubli et al. 2014b; Beatson et al. 2016). Similar to successfully targeted immune checkpoints including PD-1 and CTLA-4 (Sharma and Allison 2015; Topalian et al. 2015), Siglecs are mostly inhibitory immune-modulatory receptors (Macauley et al. 2014; Fraschilla and Pillai 2017). Here, we summarize the observed changes of sialic acids in cancer and the role of hypersialylation in cancer immune evasion. In particular, we highlight possible strategies to reverse sialic acid-mediated immune suppression for cancer immunotherapy.

Changes of sialoglycans in cancer

The diversity of glycans and glycoconjugates can be attributed to the variety of biosynthetic pathways involved, governed by an array of glycosyltransferases, glycosidases and other glycan-modifying enzymes (Li and Chen 2012). The mechanism of glycan biosynthesis, unlike

protein biosynthesis, is not template-based (Dall'Olio et al. 2014; Boligan et al. 2015) but dependent on multiple interactions derived from gene expression (processing enzymes), substrate availability (Tachibana et al. 1994), the cellular environment (Borys et al. 1993) and the underlying protein structure (Berger et al. 1969; Clark and Baum 2012). Aberrant tumor cell surface glycosylation significantly influences tumorigenesis and cancer progression (Fuster and Esko 2005; Rabinovich and Croci 2012; Boligan et al. 2015; Pinho and Reis 2015), while changes of sialic acids comprise alterations of the carbohydrate itself, as well as the amount or density of sialylation/sialic acid that is present (Pearce and Läubli 2016). Hypersialylation and xenosialylation (introduction of the nonhuman sialic acid *N*-glycol-neuraminic acid, Neu5Gc into human glycans) are commonly observed phenomena important for cancer progression (Tangvoranuntakul et al. 2003; Hedlund et al. 2008; Läubli et al. 2014b; Samraj et al. 2015). Hypersialylation in cancer came to the fore early on when different groups reported that sialidase treatment adversely effected tumor transplantation in vivo (Sedlacek et al. 1975; Schultz et al. 2012; Büll et al. 2014). It can be attributed to various pathways including the proto-oncogene driven upregulation of sialyltransferases. Oncogenes such Ras and c-Myc have been implicated in these processes (Kannagi et al. 2010). Overabundance of substrates, upregulation of sialic acid transport systems, enhanced glycan branching and the accumulation of sialic acid acceptor molecules also support tumor hypersialylation (Boligan et al. 2015). Microdomains of sialoglycans on the tumor cell surface may result in cancer immune evasion leading to increased tumor growth, invasion and metastasis. This mechanism serves as an explanation for the early results obtained in sialidase treated tumor transplantation studies (Sedlacek et al. 1975; Büll et al. 2014). Tumor cells pretreated with neuraminidase of bacterial source were implanted in mice and growth inhibition of tumors was observed (Sedlacek et al. 1975; Büll et al. 2014). The field rapidly expanded to clinical trials in humans, most of which was performed in acute myeloid leukemia (AML) patients (Sanford 1967; Currie and Bagshawe 1968). These trials failed to deliver the expected promising patient outcomes. The disappointing clinical results have post-hoc been attributed to the lack of understanding of underlying mechanisms and improper patient stratification (Urbanitz et al. 1987; Büll et al. 2014). Given the molecular tools currently available, the scientific community is now better equipped to rationally design and exploit sialic acid modulating therapeutic strategies in human cancer.

The predominant sialic acid found in mammalian cells, including humans, is *N*-acetylneuraminic acid (Neu5Ac) and humans are not able to synthetize *N*-glycolylneuraminic acid (Neu5Gc), but can take up Neu5Gc from food sources (Tangvoranuntakul et al. 2003; Carlin et al. 2009a; Varki 2009a; Samraj et al. 2014). Various reports document the presence of Neu5Gc on the cell surface glycans of several human cancer types, including melanoma, retinoblastoma, colon cancer and breast cancer (Malykh et al. 2001; Hedlund et al. 2008). Although tumors accumulate Neu5Gc-containing glycans (Hedlund et al. 2008), the exact mechanism remains unclear but might involve a general increase in sialic acid metabolism. Antibodies against Neu5Gc-containing glycans are thought to induce a low-grade inflammatory state that is considered to be pro-tumorigenic and provides a potential hypothesis to explain the well-documented phenomenon that increased red meat intake correlates with increased cancer risk (Samraj et al. 2015; Alisson-Silva et al. 2016). An increased abundance of Neu5Gc instead of Neu5Ac could also change the binding properties to Siglec receptors, since some Siglec receptors have an increased affinity to Neu5Gc (Redelinghuys et al. 2011; Naito-Matsui et al. 2014; Padler-Karavani et al. 2014; Macauley et al. 2015).

In addition to hypersialylation and the uptake of Neu5Gc, other alterations with respect to sialic acids in cancer have been described. These changes include C5-hydroxyl modification of sialic acid, which leads to the generation of 2-keto-3-deoxy-D-glycerol-D-galacto-nononic acid (KDN) (Go et al. 2006). KDN was associated in its free form with ovarian cancer (Inoue et al. 1998) and more recently with carcinoma of the head and neck (Wang et al. 2015). In addition, *O*-acetylation of sialic acid and in particular 9-*O*-acetylation has been shown to be altered in some cancer (Corfield et al. 1999; Shen et al. 2004). While these changes have been described, their functional consequences and influence on tumorigenesis and cancer progression require further investigation.

Siglec receptors

Siglecs represent a family of immune-modulatory receptors that belong to the I-type lectin family (Crocker et al. 2007; von Gunten and Bochner 2008; Pillai et al. 2012; Macauley et al. 2014; Varki et al. 2015). Similar to the currently targeted immune checkpoints such as PD-1 or CTLA-4, Siglecs are mostly inhibitory immune receptors. The family can further be subdivided into two groups. The first group of Siglecs comprises a set of receptors conserved across many mammalian species, although orthologues exhibit a rather low sequence similarity of approximately 25%. The Siglecs of the conserved group in humans include Siglec-1 (sialoadhesin), Siglec-2 (CD22), Siglec-4 (myelin-associated glycoprotein, MAG) and Siglec-15. The genes encoding for these Siglecs are located on different chromosomes, which is in contrast to the second group (Macauley et al. 2014). The genes of the second group of Siglecs are clustered on chromosome 19 and were all derived from one another via gene duplication. These are the CD33-related Siglecs, which in humans include Siglec-3 (CD33), Siglec-5, Siglec-6, Siglec-7, Siglec-8, Siglec-9, Siglec-10, Siglec-11, Siglec-14 and Siglec-16 (Cao and Crocker 2011; Macauley et al. 2014; Schwarz et al. 2015a). The CD33-related Siglecs underwent rapid evolutionary diversification via gene duplication, conversion and deletion as well as exon shuffling. Other mechanisms described include pseudogenization and altered expression. Single-nucleotide changes resulting in amino-acid substitutions within the carbohydrate recognition domain (CRD) altered ligand binding, which drove differences in ligand specificity and furthered diversification (Angata et al. 2004; Altheide et al. 2006). Rapid evolution led to significant differences of CD33-related Siglecs between mammalian species, a fact mirrored by Siglecs from mice are being denoted by letters instead of numbers (Angata 2006). Interestingly, some human CD33-related Siglecs have a paired activating receptor. For example, inhibitory Siglec-5 pairs with activating Siglec-14, similarly as inhibitory Siglec-11 pairs with activating Siglec-16 (Ali et al. 2014; Schwarz et al. 2017). These paired receptors are thought to counterbalance exploitation of the Siglec axis by pathogens that bind to inhibitory Siglecs (Ali et al. 2014; Schwarz et al. 2017). In general, the polymorphisms found in CD33-related Siglecs are believed to derive from pathogen–host interactions (discussed later).

The expression of Siglecs on leukocytes is cell-type and differentiation-dependent (Jandus et al. 2011). Structurally, Siglecs are single-pass transmembrane proteins, which possess an extracellular portion characterized by a V-set immunoglobulin-like domain, containing the CRD, and one or more C2-set immunoglobulin-like domains. The majority of Siglecs possess immunoreceptor tyrosine-based inhibitory motifs

(ITIMs) in their intracellular domain (Schwarz et al. 2015a). After ligand binding, these ITIMs can be phosphorylated by Src family kinases, which leads to the generation of a high affinity docking sites for Src homology region 2 domain-containing phosphatase-1 (SHP-1) and SHP-2 (Crocker et al. 2007). SHP-1/-2 can then dephosphorylate nearby tyrosine-phosphorylated receptors and thereby influence intracellular signaling (Crocker et al. 2007). For example, Siglec-E inhibits integrin-mediated signaling in murine neutrophils (McMillan et al. 2013). Activating CD33-related Siglec receptors lack ITIMs, instead a positively charged amino acid is located within the transmembrane domain that allows binding to DNAX-activating protein of 12 kDa (DAP12) (Macauley et al. 2014; Schwarz et al. 2017). Once DAP12 is bound to activating Siglecs, the immunoreceptor tyrosine-based activating motifs (ITAMs) of DAP12 are phosphorylated and downstream Syk is activated (Crocker et al. 2007). Syk can activate or inhibit signaling pathways depending on the cell type (Linnartz-Gerlach et al. 2014). In plasmacytoid dendritic cells, Siglec-H has no activating function but can inhibit TLR-9 mediated immune cell activation (Takagi et al. 2011). In general, downstream signaling of Siglecs is diverse and cell type specific (Chen et al. 2013; Siddiqui et al. 2017).

The intricacies of Siglecs and their downstream functions are also linked to their ligand binding. Siglec ligands are predominantly sialic acid-containing glycans (Crocker et al. 2007; Varki 2009b; Carlin et al. 2009b; Macauley et al. 2014; Schleimer et al. 2016). It is important to differentiate between carbohydrate moieties that mediate binding to Siglecs and the carriers of these carbohydrates, which can be proteins or lipids (Varki et al. 2015). Siglec ligands can be presented on the cell on which the Siglec is expressed (*cis* ligands), on other cells or on glycans in the extracellular matrix (*trans* ligands) (Macauley et al. 2014). While the CRDs of most Siglecs have some specificities towards certain ligands, several Siglecs, including Siglec-9, have quite broad binding spectrums (Läubli et al. 2014b; Padler-Karavani et al. 2014). In line with that, other nonsialylated glycans such as hyaluronan were found to bind to human Siglec-9 (Secundino et al. 2015). In addition, not only sialylated glycans, but also protein–protein interactions have been described (Carlin et al. 2009a). With regard to their binding to glycan-ligands several Siglecs, including human Siglec-9, were shown to bind ligands across species (Läubli et al. 2014b; Yu et al. 2017), which is relevant for the use of mouse models overexpressing Siglec-9 (Läubli et al. 2014b). Similarly, in a transgenic mouse model expressing human CD22 under the murine CD22 promoter, inhibition of B-cell activation could be observed and was comparable to that by murine CD22 (Bednar et al. 2017). A recent publication has helped to understand CD22 ligand binding by elucidating the structural details (Ereno-Orbea et al. 2017). Several cancer-associated Siglec ligands have been described. For example, the cancer-associated glycoform of MUC1 that has truncated, sialylated O-linked carbohydrates was shown to engage Siglec-9 and influence macrophage polarization (Beatson et al. 2016). LGALS3BP is another cancer-associated glycoprotein that binds several CD33-related Siglecs and could thereby modulate anti-tumor immune responses (Läubli et al. 2014a).

Immune escape through Siglecs in cancer

Mammalian cells have a relatively high density of sialoglycans on their surface in comparison to most pathogens. This high density of sialoglycans, which can be considered as self-associated molecular patterns (SAMPs), can lead to inhibition of immune responses against self (Varki 2011). Broadly binding CD33-related Siglecs, such as Siglec-9, expressed on immune cells can in fact be considered as pattern recognition receptors (PRRs) for sialoglycan-SAMPs (Padler-Karavani et al. 2014). During immune activation, Siglecs could counter-regulate overshooting immune reactions upon immune stimulation by damage associated molecular patterns (DAMPs). One study has found that murine Siglec-G and human Siglec-10 can inhibit overshooting immune reactions and limit tissue damage during inflammation by binding to properly sialylated CD24 (Chen et al. 2009). Other work has shown that the sialylated glycoform of CD52 can engage Siglec-10 on T cells and mouse models have demonstrated an influence on the generation of auto-immune diseases including type 1 diabetes mellitus (Bandala-Sanchez et al. 2013). It is important to note that with respect to these examples, it is not the protein backbone that mediates binding to Siglecs and subsequent immunological effects, but rather the particular glycoform of these proteins.

Several clinically relevant pathogens have evolved mechanisms of molecular mimicry by displaying sialoglycan-SAMP-like structures on their surface. Additionally, the density and avidity of those pathogen sialoglycans which is needed to overcome the *cis* interactions of Siglecs on the surface of immune cells is of importance. Group B streptococci (GBS) (Carlin et al. 2009a; Carlin, Uchiyama et al. 2009), several strains of *Escherichia coli*, that induce meningitis in newborns (Chang and Nizet 2014), and other pathogens such as *Haemophilus influenza*, *Neisseria* species, protozoan *Tryponasoma brucei* (Nagamune et al. 2004) and most enveloped viruses including HIV (Mikulak et al. 2017) are sialylated, bind to Siglecs and thereby modulate the immune response.

Similar to pathogens that can mimic self via Siglec engagement, it is hypothesized that the well-known hypersialylation of the tumor microenvironment is an attempt to upregulate SAMPs (Figure 1) in aid of host immune evasion, potentially leading to cancer progression

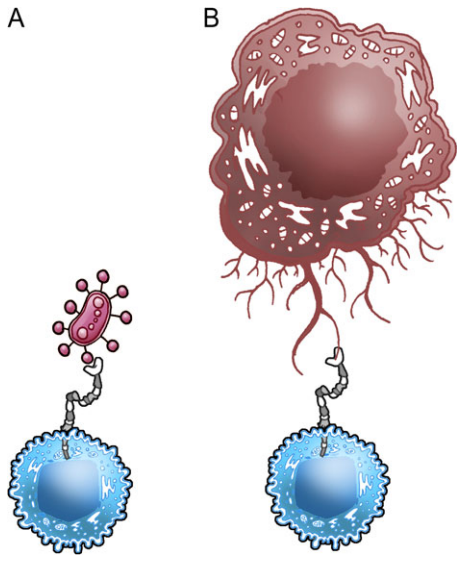

Fig. 1. Hypothesis comparing immune evasion through engagement of inhibitory Siglecs by tumor cells and pathogens (**A**) Pathogens such as group B streptococci (GBS) can mimic sialoglycan-SAMPs on their surface, engage inhibitory Siglecs and thereby dampen the anti-pathogen immune response, which allows immune evasion. (**B**) Similarly, tumor cells are thought to upregulate sialoglycan-SAMPs during cancer progression and evade immune-control by engagement of inhibitory Siglecs including Siglec-9 on myeloid cells and Siglec-7 and Siglec-9 on NK cells. (The depicted diagram is a cartoon and is not meant to reflect the actual structures of proteins and glycans. Drawn by Emmanuel Traunecker.)

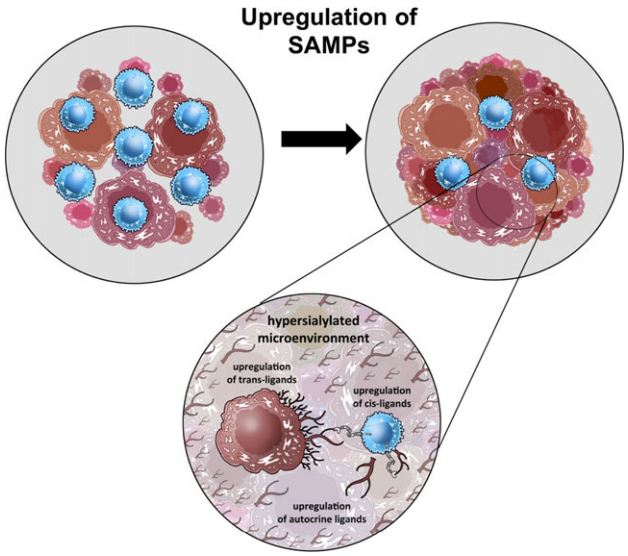

Fig. 2. How upregulation of sialoglycan-SAMPs in the tumor microenvironment could support immune evasion by tumor cells. The upregulation of sialic acid-containing glycans leads to an increased presence of SAMPs, which could inhibit immune cells and drive cancer progression. In the magnification, potential interactions between Siglecs on immune cells and Siglec ligands are shown. Sialoglycan-SAMPs can potentially be presented on the surface of tumor cells, on secreted glycoproteins (*trans* ligands) but also on immune cells themselves (*cis* ligands, cartoon drawn by Emmanuel Traunecker).

(Figure 2). In line with this hypothesis, it has been shown that sialic acid-terminated glycans exhibit lower immunogenicity for antibody responses in healthy individuals (Schneider et al. 2015). The inhibition of immune cells by cancer-associated Siglec ligands has primarily been demonstrated in vitro (Hudak et al. 2014; Jandus et al. 2014; Läubli et al. 2014b; Beatson et al. 2016; Xiao et al. 2016). Data from in vivo mouse experiments and human genetic association studies are at present limited and current efforts are ongoing to improve our understanding of the role of sialic acid–Siglec interactions in cancer progression (Jandus et al. 2014; Läubli et al. 2014b). Here we highlight the current supporting evidence for sialic acid–Siglec interactions pertaining to cancer immune evasion.

Human NK cells constitutively express inhibitory Siglec-7 and a considerable fraction also expresses inhibitory Siglec-9 (Crocker et al. 2007). In addition, it has been found that in cancer Siglec-9 is upregulated on peripheral NK cells, mainly on $CD56^{dim}$ $CD16^{+}$ NK cells (Jandus et al. 2014). The in vitro cytotoxicity of these NK cells against tumor cells (K562) was increased when Siglec-7 or Siglec-9 were blocked by Fab fragments (Jandus et al. 2014). In another study by Bertozzi and colleagues, artificially increasing the density of sialoglycan-SAMPs on the surface of cancer cells lead to Siglec-7 engagement on NK cells and the subsequent inhibition of both their antibody-dependent and -independent cytotoxic capacity (Hudak et al. 2014). An in vivo model in immunodeficient mice with transferred human NK cells and human tumor cells demonstrated a sialoglycan-dependent inhibition of NK cell mediated tumor cell killing (Jandus et al. 2014).

A genetic mouse model for Siglec-E deficiency (McMillan et al. 2013) was used to study the role of Siglecs on myeloid cells (Läubli et al. 2014b). In a metastasis model, neutrophil-associated Siglec-E was shown to promote extravasation and colony formation of tumor cells in the lungs. Depletion of neutrophils demonstrated that the reduced metastasis formation in Siglec-E deficiency was indeed neutrophil dependent (Läubli et al. 2014b). Myeloid-specific re-expression of human Siglec-9, which binds to murine sialoglycans, reversed the protective effect of Siglec-E deficiency in the metastasis model. The strong expression of this broadly binding inhibitory CD33-related Siglec even led to a tendency of enhanced metastasis formation (Läubli et al. 2014b). However, killing of tumor cells by neutrophils has been shown to be enhanced in vitro when Siglec-E was lacking (Läubli et al. 2014b). Similarly, addition of the cancer-associated *N*-glycosylated protein LGALS3BP, which strongly binds to several CD33-related Siglecs, including Siglec-5 and Siglec-9, to human neutrophils was able to inhibit their activation (Läubli et al. 2014a). Some in vitro data demonstrates an enhanced reactive oxygen species (ROS) production in Siglec-E deficient neutrophils (Läubli et al. 2014b; Schwarz et al. 2015b). In contrast, other studies report an inhibition of ROS production in Siglec-E deficient neutrophils when binding to integrins (McMillan et al. 2013, 2014). In addition, several experiments have shown that engagement of Siglecs on neutrophils can induce apoptosis of the immune cells (von Gunten et al. 2005, 2006). It seems that the role of inhibitory Siglecs on neutrophils is context specific and further analysis is required to determine their exact function during cancer progression.

Tumor-associated macrophages (TAMs) express Siglec-9 in humans (Beatson et al. 2016) and strongly express Siglec-E in mice (Läubli et al. 2014b). TAMs in Siglec-E deficient mice had a higher propensity to differentiate into tumor-promoting M2 macrophages and enhanced subcutaneous tumor growth in Siglec-E deficient mice due to this polarization (Läubli et al. 2014b). Recently, Beatson and colleagues demonstrated that Siglec-9 interaction with the cancer-associated glycoform of the mucin MUC1 is potentiating a pro-tumorigenic phenotype in macrophages in vitro (Beatson et al. 2016).

Other immune cells involved in anti-tumor immunity express Siglec receptors as well. There is experimental evidence that inhibitory Siglecs can influence the presentation of sialylated antigens by dendritic cells (DCs) (Perdicchio et al. 2016a, 2016b). For example, published findings of Ding and colleagues showed that Siglec-G inhibits DC cross-presentation by impairing MHC class I-peptide complex formation, which attenuated cytotoxic T lymphocyte (CTL) responses. Furthermore, tumor growth, as well as intracellular bacterial infection, was inhibited in mice lacking Siglec-G (Ding, et al. 2016). Interestingly, another recent study demonstrated that desialylated human DCs loaded with tumor antigens exhibited a higher anti-tumor immune function which ultimately led to increased tumor cell death (Silva et al. 2016). Tumor hypersialylation has been shown to enhance murine tumor growth through an increased presence of regulatory T cells, although the mechanism is not yet elucidated (Perdicchio et al. 2016a). In addition, a carcinogen induced tumor model using 3-meythylcholanthrene (MCA) in Siglec-E deficient mice has shown a delayed appearance of subcutaneous sarcomas indicating an improved adaptive immune response in Siglec-E deficient mice (Läubli et al. 2014b).

Association studies of Siglec associated polymorphisms have provided insights regarding the receptors' roles in cancer immunity. For example, in an analysis of the African-specific Siglec-9 polymorphism K131Q (rs16988910), which mediates reduced binding to ligands, an improved survival was seen during the first two years of patients with non-small cell lung cancer (NSCLC) when the allele was present (Läubli et al. 2014b). Other polymorphisms that have been associated with disease states such as the Siglec-5/14 deletion polymorphism have not yet been studied in the context of cancer.

The mounting evidence of Siglec engagement by cancer cells and the tumor microenvironment to evade and suppress anti-tumor

immune responses make sialic acid–Siglec interactions attractive candidates for improving anti-cancer immunity.

Targeting sialoglycans and Siglecs for cancer therapy

Sialic acids and sialic acid-containing glycoconjugates are attractive targets for anti-cancer immunotherapies as aberrant glycosylation patterns are predominantly associated with tumor tissue. The xeno-antigen Neu5Gc and its conjugates are particularly promising as they would in theory allow for even higher target specificity (Vazquez et al. 2012). Vazquez et al. developed an idiotypic antibody against NGcGM3, a Neu5Gc-containing tumor-associated ganglioside (Vazquez et al. 2012). The safe use of and the immunogenicity of Racotumomab (previously known as 1E10 or Vaxira) were subsequently demonstrated in several cancer patient cohorts, including those diagnosed with melanoma, breast cancer and NSCLC (Alfonso et al. 2002; Diaz et al. 2003; Hernandez et al. 2008). Targeted delivery of a sialic acid-synthesis blocking glycomimetic (P-3Fax-Neu5Ac) was effective in reducing metastasis formation in a murine model of lung metastasis (Büll et al. 2015).

Nanoparticle-based technologies involving sialic acid–Siglec interactions have also been developed. Chen et al. demonstrated that nanoparticles decorated with CD22 (Siglec-2) ligands could effectively deliver chemotherapeutics to lymphoma cells in a mouse model (Chen et al. 2010, 2012). In addition, lipid antigens have been delivered to macrophages via Siglec-1 targeted nanoparticles and induced a robust NKT cell activation (Kawasaki et al. 2013), which could be potentially also used for anti-tumor immune reactions.

Antibody-based cell depletion therapies for lymphomas and leukemias using anti-CD33 and anti-CD22 antibodies are clinically relevant strategies involving Siglecs (Jabbour et al. 2015). For example, clinical trials involving the CD33-specific immunotoxin, gemtuzumab ozogamicin, for the treatment of AML patients (Ravandi et al. 2012) have been conducted as well as those focusing on CD22-immunotoxins such as inotuzumab ozogamicin in acute lymphocytic leukemia (ALL) patients (Jabbour et al. 2017). Beyond targeted and antibody-based approaches, sialic acid–Siglec interactions have also come to the fore as targets with respect to efforts to reverse cancer-associated immune suppression.

Small molecules or glycomimetics that block Siglec–ligand interactions are one potential approach. Bertozzi and co-workers have paved the way for another approach (Xiao et al. 2016), by coupling a sialidase to a tumor-targeting antibody such as anti-HER2 trastuzumab, which could be used to desialylate the sialoglycans and Siglec ligands on tumor tissue (Xiao et al. 2016). Indeed, in vitro studies demonstrated improved killing of HER2 expressing breast cancer cells by NK cells (Xiao et al. 2016). Thus, this approach of targeting a sialidase to the tumor could lead to broad reduction of ligands for various sialic acid-binding lectins including most Siglecs. The presentation of multiple novel glycan structures, including terminal galactose, could however lead to engagement of other immuno-modulatory lectins such as galectins and consequences of in vivo tumor-desialylation require intense investigation prior to clinical implementation.

Concluding remarks

Siglecs are cell surface receptors, which recognize sialoglycans. They are mostly expressed on immune cells and the majority of them mediate inhibitory signals upon recognition of self. Many promising avenues to exploit sialic acid–Siglec interactions to advance cancer therapy are currently under investigation. From targeted approaches to antibody cell depletion therapies to engineering the tumor microenvironment to allow a more immunopermissive state and other immunotherapeutic strategies. While challenging to study, it is of great relevance to continue to investigate the role of sialic acid–Siglec interactions in the context of cancer-associated immune suppression in order to advance clinical applications.

Funding

This work was supported by funding from the Schoenmakers Foundation (to H.L.), the Goldschmidt-Jacobson Foundation (to H.L) and the Promedica Foundation (to M.S.). The laboratory of S.v.G. is supported by the Swiss National Science Foundation (SNSF) (Grant 310030_162552/1) and the Swiss Cancer League/Swiss Cancer Research (Grants KFS-3941-08-2016 and KFS-3248-08-2013).

Conflict of interest statement

H.L. is part of the scientific advisory board of Palleon Pharmaceuticals that produces agents to target the Siglec–sialoglycan interaction in cancer.

Abbreviations

ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; CRD, carbohydrate recognition domain; ITIM, immunoreceptor tyrosine-based inhibitory motif; MAG, myelin-associated glycoprotein; NSCLC, non-small cell lung cancer; PRR, pattern recognition receptor; ROS, reactive oxygen species; SAMP, self-associated molecular pattern; TAM, tumor-associated macrophage.

References

Alfonso M, Diaz A, Hernandez AM, Perez A, Rodriguez E, Bitton R, Perez R, Vazquez AM. 2002. An anti-idiotype vaccine elicits a specific response to N-glycolyl sialic acid residues of glycoconjugates in melanoma patients. *J Immunol*. 168:2523–2529.

Ali SR, Fong JJ, Carlin AF, Busch TD, Linden R, Angata T, Areschoug T, Parast M, Varki N, Murray J et al. 2014. Siglec-5 and Siglec-14 are polymorphic paired receptors that modulate neutrophil and amnion signaling responses to group B Streptococcus. *J Exp Med*. 211:1231–1242.

Alisson-Silva F, Kawanishi K, Varki A. 2016. Human risk of diseases associated with red meat intake: Analysis of current theories and proposed role for metabolic incorporation of a non-human sialic acid. *Mol Aspects Med*. 51:16–30.

Altheide TK, Hayakawa T, Mikkelsen TS, Diaz S, Varki N, Varki A. 2006. System-wide genomic and biochemical comparisons of sialic acid biology among primates and rodents: Evidence for two modes of rapid evolution. *J Biol Chem*. 281:25689–25702.

Amon R, Reuven EM, Leviatan Ben-Arye S, Padler-Karavani V. 2014. Glycans in immune recognition and response. *Carbohydr Res*. 389: 115–122.

Angata T. 2006. Molecular diversity and evolution of the Siglec family of cell-surface lectins. *Mol Divers*. 10:555–566.

Angata T, Margulies EH, Green ED, Varki A. 2004. Large-scale sequencing of the CD33-related Siglec gene cluster in five mammalian species reveals rapid evolution by multiple mechanisms. *Proc Natl Acad Sci U S A*. 101: 13251–13256.

Bandala-Sanchez E, Zhang Y, Reinwald S, Dromey JA, Lee BH, Qian J, Bohmer RM, Harrison LC. 2013. T cell regulation mediated by interaction of soluble CD52 with the inhibitory receptor Siglec-10. *Nat Immunol*. 14:741–748.

Beatson R, Tajadura-Ortega V, Achkova D, Picco G, Tsourouktsoglou TD, Klausing S, Hillier M, Maher J, Noll T, Crocker PR et al. 2016. The mucin MUC1 modulates the tumor immunological microenvironment

through engagement of the lectin Siglec-9. *Nat Immunol*. 17(11): 1273–1281.

Bednar KJ, Shanina E, Ballet R, Connors EP, Duan S, Juan J, Arlian BM, Kulis MD, Butcher EC, Fung-Leung WP et al. 2017. Human CD22 inhibits murine B cell receptor activation in a human CD22 transgenic mouse model. *J Immunol*. 199:3116–3128.

Berger J, Yaneva H, Antoine B. 1969. Application of immunofluorescence to renal pathology. II. Immunohistochemical study of glomerular lesions. *J Urol Nephrol (Paris)*. 75:269–281.

Boligan KF, Mesa C, Fernandez LE, von Gunten S. 2015. Cancer intelligence acquired (CIA): Tumor glycosylation and sialylation codes dismantling antitumor defense. *Cell Mol Life Sci*. 72:1231–1248.

Borys MC, Linzer DI, Papoutsakis ET. 1993. Culture pH affects expression rates and glycosylation of recombinant mouse placental lactogen proteins by Chinese hamster ovary (CHO) cells. *Biotechnology (N Y)*. 11: 720–724.

Büll C, Boltje TJ, van Dinther EA, Peters T, de Graaf AM, Leusen JH, Kreutz M, Figdor CG, den Brok MH, Adema GJ. 2015. Targeted delivery of a sialic acid-blocking glycomimetic to cancer cells inhibits metastatic spread. *ACS Nano*. 9:733–745.

Büll C, Stoel MA, den Brok MH, Adema GJ. 2014. Sialic acids sweeten a tumor's life. *Cancer Res*. 74:3199–3204.

Cao H, Crocker PR. 2011. Evolution of CD33-related siglecs: Regulating host immune functions and escaping pathogen exploitation? *Immunology*. 132:18–26.

Carlin AF, Chang YC, Areschoug T, Lindahl G, Hurtado-Ziola N, King CC, Varki A, Nizet V. 2009a. Group B Streptococcus suppression of phagocyte functions by protein-mediated engagement of human Siglec-5. *J Exp Med*. 206:1691–1699.

Carlin AF, Uchiyama S, Chang YC, Lewis AL, Nizet V, Varki A. 2009b. Molecular mimicry of host sialylated glycans allows a bacterial pathogen to engage neutrophil Siglec-9 and dampen the innate immune response. *Blood*. 113:3333–3336.

Chang YC, Nizet V. 2014. The interplay between Siglecs and sialylated pathogens. *Glycobiology*. 24:818–825.

Chen WC, Completo GC, Sigal DS, Crocker PR, Saven A, Paulson JC. 2010. In vivo targeting of B-cell lymphoma with glycan ligands of CD22. *Blood*. 115:4778–4786.

Chen W, Han C, Xie B, Hu X, Yu Q, Shi L, Wang Q, Li D, Wang J, Zheng P et al. 2013. Induction of Siglec-G by RNA viruses inhibits the innate immune response by promoting RIG-I degradation. *Cell*. 152:467–478.

Chen WC, Sigal DS, Saven A, Paulson JC. 2012. Targeting B lymphoma with nanoparticles bearing glycan ligands of CD22. *Leuk Lymphoma*. 53: 208–210.

Chen GY, Tang J, Zheng P, Liu Y. 2009. CD24 and Siglec-10 selectively repress tissue damage-induced immune responses. *Science*. 323: 1722–1725.

Clark MC, Baum LG. 2012. T cells modulate glycans on CD43 and CD45 during development and activation, signal regulation, and survival. *Ann N Y Acad Sci*. 1253:58–67.

Corfield AP, Myerscough N, Warren BF, Durdey P, Paraskeva C, Schauer R. 1999. Reduction of sialic acid O-acetylation in human colonic mucins in the adenoma-carcinoma sequence. *Glycoconj J*. 16:307–317.

Crocker PR, Paulson JC, Varki A. 2007. Siglecs and their roles in the immune system. *Nat Rev Immunol*. 7:255–266.

Currie GA, Bagshawe KD. 1968. The role of sialic acid in antigenic expression: Further studies of the Landschutz ascites tumour. *Br J Cancer*. 22: 843–853.

Dall'Olio F, Malagolini N, Trinchera M, Chiricolo M. 2014. Sialosignaling: Sialyltransferases as engines of self-fueling loops in cancer progression. *Biochim Biophys Acta*. 1840:2752–2764.

Diaz A, Alfonso M, Alonso R, Saurez G, Troche M, Catala M, Diaz RM, Perez R, Vazquez AM. 2003. Immune responses in breast cancer patients immunized with an anti-idiotype antibody mimicking NeuGc-containing gangliosides. *Clin Immunol*. 107:80–89.

Ding Y, Guo Z, Liu Y, Li X, Zhang Q, Xu X, Gu Y, Zhang Y, Zhao D, Cao X. 2016. The lectin Siglec-G inhibits dendritic cell cross-presentation by impairing MHC class I-peptide complex formation. *Nat Immunol*. 17: 1167–1175.

Ereno-Orbea J, Sicard T, Cui H, Mazhab-Jafari MT, Benlekbir S, Guarne A, Rubinstein JL, Julien JP. 2017. Molecular basis of human CD22 function and therapeutic targeting. *Nat Commun*. 8:764.

Fedarko NS, Fohr B, Robey PG, Young MF, Fisher LW. 2000. Factor H binding to bone sialoprotein and osteopontin enables tumor cell evasion of complement-mediated attack. *J Biol Chem*. 275:16666–16672.

Fraschilla I, Pillai S. 2017. Viewing Siglecs through the lens of tumor immunology. *Immunol Rev*. 276:178–191.

Fuster MM, Esko JD. 2005. The sweet and sour of cancer: Glycans as novel therapeutic targets. *Nat Rev Cancer*. 5:526–542.

Go S, Sato C, Furuhata K, Kitajima K. 2006. Oral ingestion of mannose alters the expression level of deaminoneuraminic acid (KDN) in mouse organs. *Glycoconj J*. 23:411–421.

Hakomori S. 1984. Tumor-associated carbohydrate antigens. *Annu Rev Immunol*. 2:103–126.

Hedlund M, Padler-Karavani V, Varki NM, Varki A. 2008. Evidence for a human-specific mechanism for diet and antibody-mediated inflammation in carcinoma progression. *Proc Natl Acad Sci U S A*. 105:18936–18941.

Hernandez AM, Toledo D, Martinez D, Grinan T, Brito V, Macias A, Alfonso S, Rondon T, Suarez E, Vazquez AM et al. 2008. Characterization of the antibody response against NeuGcGM3 ganglioside elicited in non-small cell lung cancer patients immunized with an anti-idiotype antibody. *J Immunol*. 181:6625–6634.

Hudak JE, Canham SM, Bertozzi CR. 2014. Glycocalyx engineering reveals a Siglec-based mechanism for NK cell immunoevasion. *Nat Chem Biol*. 10: 69–75.

Inoue S, Lin SL, Chang T, Wu SH, Yao CW, Chu TY, Troy FA II, Inoue Y. 1998. Identification of free deaminated sialic acid (2-keto-3-deoxy-D-glycero-D-galacto-nononic acid) in human red blood cells and its elevated expression in fetal cord red blood cells and ovarian cancer cells. *J Biol Chem*. 273:27199–27204.

Jabbour E, O'Brien S, Ravandi F, Kantarjian H. 2015. Monoclonal antibodies in acute lymphoblastic leukemia. *Blood*. 125:4010–4016.

Jabbour E, Ravandi F, Kebriaei P, Huang X, Short NJ, Thomas D, Sasaki K, Rytting M, Jain N, Konopleva M et al. 2017. Salvage chemoimmunotherapy with inotuzumab ozogamicin combined with mini-hyper-CVD for patients with relapsed or refractory philadelphia chromosome-negative acute lymphoblastic leukemia: A Phase 2 Clinical Trial. *JAMA Oncol*. doi:10.1001/jamaoncol.2017.2380.

Jandus C, Boligan KF, Chijioke O, Liu H, Dahlhaus M, Demoulins T, Schneider C, Wehrli M, Hunger RE, Baerlocher GM et al. 2014. Interactions between Siglec-7/9 receptors and ligands influence NK cell-dependent tumor immunosurveillance. *J Clin Invest*. 124:1810–1820.

Jandus C, Simon HU, von Gunten S. 2011. Targeting siglecs – a novel pharmacological strategy for immuno- and glycotherapy. *Biochem Pharmacol*. 82:323–332.

Kannagi R, Sakuma K, Miyazaki K, Lim KT, Yusa A, Yin J, Izawa M. 2010. Altered expression of glycan genes in cancers induced by epigenetic silencing and tumor hypoxia: Clues in the ongoing search for new tumor markers. *Cancer Sci*. 101:586–593.

Kawasaki N, Vela JL, Nycholat CM, Rademacher C, Khurana A, van Rooijen N, Crocker PR, Kronenberg M, Paulson JC. 2013. Targeted delivery of lipid antigen to macrophages via the CD169/sialoadhesin endocytic pathway induces robust invariant natural killer T cell activation. *Proc Natl Acad Sci U S A*. 110:7826–7831.

Krengel U, Bousquet PA. 2014. Molecular recognition of gangliosides and their potential for cancer immunotherapies. *Front Immunol*. 5:325.

Läubli H, Alisson-Silva F, Stanczak MA, Siddiqui SS, Deng L, Verhagen A, Varki N, Varki A. 2014a. Lectin galactoside-binding soluble 3 binding protein (LGALS3BP) is a tumor-associated immunomodulatory ligand for CD33-related Siglecs. *J Biol Chem*. 289:33481–33491.

Läubli H, Pearce OM, Schwarz F, Siddiqui SS, Deng L, Stanczak MA, Deng L, Verhagen A, Secrest P, Lusk C et al. 2014b. Engagement of myelomonocytic Siglecs by tumor-associated ligands modulates the innate immune response to cancer. *Proc Natl Acad Sci U S A*. 111:14211–14216.

Li Y, Chen X. 2012. Sialic acid metabolism and sialyltransferases: Natural functions and applications. *Appl Microbiol Biotechnol*. 94:887–905.

Linnartz-Gerlach B, Kopatz J, Neumann H. 2014. Siglec functions of microglia. *Glycobiology*. 24:794–799.

Macauley MS, Crocker PR, Paulson JC. 2014. Siglec-mediated regulation of immune cell function in disease. *Nat Rev Immunol*. 14:653–666.

Macauley MS, Kawasaki N, Peng W, Wang SH, He Y, Arlian BM, McBride R, Kannagi R, Khoo KH, Paulson JC. 2015. Unmasking of CD22 co-receptor on germinal center B-cells occurs by alternative mechanisms in mouse and man. *J Biol Chem*. 290:30066–30077.

Malykh YN, Schauer R, Shaw L. 2001. N-glycolylneuraminic acid in human tumours. *Biochimie*. 83:623–634.

McMillan SJ, Sharma RS, McKenzie EJ, Richards HE, Zhang J, Prescott A, Crocker PR. 2013. Siglec-E is a negative regulator of acute pulmonary neutrophil inflammation and suppresses CD11b beta2-integrin-dependent signaling. *Blood*. 121:2084–2094.

McMillan SJ, Sharma RS, Richards HE, Hegde V, Crocker PR. 2014. Siglec-E promotes beta2-integrin-dependent NADPH oxidase activation to suppress neutrophil recruitment to the lung. *J Biol Chem*. 289:20370–20376.

Mikulak J, Di Vito C, Zaghi E, Mavilio D. 2017. Host immune responses in HIV-1 infection: The emerging pathogenic role of siglecs and their clinical correlates. *Front Immunol*. 8:314.

Nagamune K, Acosta-Serrano A, Uemura H, Brun R, Kunz-Renggli C, Maeda Y, Ferguson MA, Kinoshita T. 2004. Surface sialic acids taken from the host allow trypanosome survival in tsetse fly vectors. *J Exp Med*. 199:1445–1450.

Naito-Matsui Y, Takada S, Kano Y, Iyoda T, Sugai M, Shimizu A, Inaba K, Nitschke L, Tsubata T, Oka S et al. 2014. Functional evaluation of activation-dependent alterations in the sialoglycan composition of T cells. *J Biol Chem*. 289:1564–1579.

Nicoll G, Avril T, Lock K, Furukawa K, Bovin N, Crocker PR. 2003. Ganglioside GD3 expression on target cells can modulate NK cell cytotoxicity via siglec-7-dependent and -independent mechanisms. *Eur J Immunol*. 33:1642–1648.

Padler-Karavani V, Hurtado-Ziola N, Chang YC, Sonnenburg JL, Ronaghy A, Yu H, Verhagen A, Nizet V, Chen X, Varki N et al. 2014. Rapid evolution of binding specificities and expression patterns of inhibitory CD33-related Siglecs in primates. *FASEB J*. 28:1280–1293.

Pearce OM, Läubli H. 2016. Sialic acids in cancer biology and immunity. *Glycobiology*. 26(2):111–128.

Perdicchio M, Cornelissen LA, Streng-Ouwehand I, Engels S, Verstege MI, Boon L, Geerts D, van Kooyk Y, Unger WW. 2016a. Tumor sialylation impedes T cell mediated anti-tumor responses while promoting tumor associated-regulatory T cells. *Oncotarget*. 7:8771–8782.

Perdicchio M, Ilarregui JM, Verstege MI, Cornelissen LA, Schetters ST, Engels S, Ambrosini M, Kalay H, Veninga H, den Haan JM et al. 2016b. Sialic acid-modified antigens impose tolerance via inhibition of T-cell proliferation and de novo induction of regulatory T cells. *Proc Natl Acad Sci U S A*. 113:3329–3334.

Pillai S, Netravali IA, Cariappa A, Mattoo H. 2012. Siglecs and immune regulation. *Annu Rev Immunol*. 30:357–392.

Pinho SS, Reis CA. 2015. Glycosylation in cancer: Mechanisms and clinical implications. *Nat Rev Cancer*. 15:540–555.

Rabinovich GA, Croci DO. 2012. Regulatory circuits mediated by lectin-glycan interactions in autoimmunity and cancer. *Immunity*. 36:322–335.

Ravandi F, Estey EH, Appelbaum FR, Lo-Coco F, Schiffer CA, Larson RA, Burnett AK, Kantarjian HM. 2012. Gemtuzumab ozogamicin: Time to resurrect? *J Clin Oncol*. 30:3921–3923.

Redelinghuys P, Antonopoulos A, Liu Y, Campanero-Rhodes MA, McKenzie E, Haslam SM, Dell A, Feizi T, Crocker PR. 2011. Early murine T-lymphocyte activation is accompanied by a switch from N-Glycolyl- to N-acetyl-neuraminic acid and generation of ligands for siglec-E. *J Biol Chem*. 286:34522–34532.

Samraj AN, Läubli H, Varki N, Varki A. 2014. Involvement of a non-human sialic acid in human cancer. *Front Oncol*. 4:33.

Samraj AN, Pearce OM, Läubli H, Crittenden AN, Bergfeld AK, Banda K, Gregg CJ, Bingman AE, Secrest P, Diaz SL et al. 2015. A red meat-derived glycan promotes inflammation and cancer progression. *Proc Natl Acad Sci U S A*. 112:542–547.

Sanford BH. 1967. An alteration in tumor histocompatibility induced by neuraminidase. *Transplantation*. 5:1273–1279.

Schauer R. 2000. Achievements and challenges of sialic acid research. *Glycoconj J*. 17:485–499.

Schleimer RP, Schnaar RL, Bochner BS. 2016. Regulation of airway inflammation by Siglec-8 and Siglec-9 sialoglycan ligand expression. *Curr Opin Allergy Clin Immunol*. 16:24–30.

Schneider C, Smith DF, Cummings RD, Boligan KF, Hamilton RG, Bochner BS, Miescher S, Simon HU, Pashov A, Vassilev T et al. 2015. The human IgG anti-carbohydrate repertoire exhibits a universal architecture and contains specificity for microbial attachment sites. *Sci Transl Med*. 7: 269ra261.

Schultz MJ, Swindall AF, Bellis SL. 2012. Regulation of the metastatic cell phenotype by sialylated glycans. *Cancer Metastasis Rev*. 31:501–518.

Schwarz F, Fong JJ, Varki A. 2015. Human-specific evolutionary changes in the biology of siglecs. *Adv Exp Med Biol*. 842:1–16.

Schwarz F, Landig CS, Siddiqui S, Secundino I, Olson J, Varki N, Nizet V, Varki A. 2017. Paired Siglec receptors generate opposite inflammatory responses to a human-specific pathogen. *EMBO J*. 36:751–760.

Schwarz F, Pearce OM, Wang X, Samraj AN, Läubli H, Garcia JO, Lin H, Fu X, Garcia-Bingman A, Secrest P et al. 2015b. Siglec receptors impact mammalian lifespan by modulating oxidative stress. *Elife*. 4:e06184.

Secundino I, Lizcano A, Roupe KM, Wang X, Cole JN, Olson J, Ali SR, Dahesh S, Amayreh LK, Henningham A et al. 2015. Host and pathogen hyaluronan signal through human siglec-9 to suppress neutrophil activation. *J Mol Med (Berl)*. 94(2):219–233.

Sedlacek HH, Meesmann H, Seiler FR. 1975. Regression of spontaneous mammary tumors in dogs after injection of neuraminidase-treated tumor cells. *Int J Cancer*. 15:409–416.

Sharma P, Allison JP. 2015. Immune checkpoint targeting in cancer therapy: Toward combination strategies with curative potential. *Cell*. 161:205–214.

Shen Y, Kohla G, Lrhorfi AL, Sipos B, Kalthoff H, Gerwig GJ, Kamerling JP, Schauer R, Tiralongo J. 2004. O-acetylation and de-O-acetylation of sialic acids in human colorectal carcinoma. *Eur J Biochem*. 271:281–290.

Siddiqui S, Schwarz F, Springer S, Khedri Z, Yu H, Deng L, Verhagen A, Naito-Matsui Y, Jiang W, Kim D et al. 2017. Studies on the detection, expression, glycosylation, dimerization, and ligand binding properties of mouse siglec-E. *J Biol Chem*. 292:1029–1037.

Silva M, Silva Z, Marques G, Ferro T, Goncalves M, Monteiro M, van Vliet SJ, Mohr E, Lino AC, Fernandes AR et al. 2016. Sialic acid removal from dendritic cells improves antigen cross-presentation and boosts anti-tumor immune responses. *Oncotarget*. 7:41053–41066.

Tachibana H, Taniguchi K, Ushio Y, Teruya K, Osada K, Murakami H. 1994. Changes of monosaccharide availability of human hybridoma lead to alteration of biological properties of human monoclonal antibody. *Cytotechnology*. 16:151–157.

Takagi H, Fukaya T, Eizumi K, Sato Y, Sato K, Shibazaki A, Otsuka H, Hijikata A, Watanabe T, Ohara O et al. 2011. Plasmacytoid dendritic cells are crucial for the initiation of inflammation and T cell immunity in vivo. *Immunity*. 35:958–971.

Tangvoranuntakul P, Gagneux P, Diaz S, Bardor M, Varki N, Varki A, Muchmore E. 2003. Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. *Proc Natl Acad Sci U S A*. 100: 12045–12050.

Topalian SL, Drake CG, Pardoll DM. 2015. Immune checkpoint blockade: A common denominator approach to cancer therapy. *Cancer Cell*. 27: 450–461.

Urbanitz D, Buchner T, Pielken H, Koch P, Ludwig WD, Maschmeier G, Heinecke A, van de Loo J. 1987. Neuraminidase-treated allogeneic blasts for maintenance in acute myelogenous leukemia: Results of a prospective randomized trial. *Haematol Blood Transfus*. 30:64–68.

Varki A. 2009a. Multiple changes in sialic acid biology during human evolution. *Glycoconj J*. 26:231–245.

Varki A. 2009b. Natural ligands for CD33-related Siglecs? *Glycobiology*. 19: 810–812.

Varki A. 2011. Since there are PAMPs and DAMPs, there must be SAMPs? Glycan "self-associated molecular patterns" dampen innate immunity, but pathogens can mimic them. *Glycobiology*. 21:1121–1124.

Varki A, Schnaar RL, Crocker PR. 2015. I-Type lectins. In: Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, Darvill AG, Kinoshita T, Packer NH, Prestegard JH et al, editors. *Essentials of Glycobiology*. New York: Cold Spring Harbor.

Vazquez AM, Rodreguez-Zhurbenko N, Lopez AM. 2012. Anti-ganglioside anti-idiotypic vaccination: More than molecular mimicry. *Front Oncol*. 2:170.

von Gunten S, Bochner BS. 2008. Basic and clinical immunology of Siglecs. *Ann N Y Acad Sci*. 1143:61–82.

von Gunten S, Schaub A, Vogel M, Stadler BM, Miescher S, Simon HU. 2006. Immunologic and functional evidence for anti-Siglec-9 autoantibodies in intravenous immunoglobulin preparations. *Blood*. 108:4255–4259.

von Gunten S, Yousefi S, Seitz M, Jakob SM, Schaffner T, Seger R, Takala J, Villiger PM, Simon HU. 2005. Siglec-9 transduces apoptotic and nonapoptotic death signals into neutrophils depending on the proinflammatory cytokine environment. *Blood*. 106:1423–1431.

Wang F, Xie B, Wang B, Troy FA II. 2015. LC-MS/MS glycomic analyses of free and conjugated forms of the sialic acids, Neu5Ac, Neu5Gc and KDN in human throat cancers. *Glycobiology*. 25:1362–1374.

Xiao H, Woods EC, Vukojicic P, Bertozzi CR. 2016. Precision glycocalyx editing as a strategy for cancer immunotherapy. *Proc Natl Acad Sci U S A*. 113:10304–10309.

Yu H, Gonzalez-Gil A, Wei Y, Fernandes SM, Porell RN, Vajn K, Paulson JC, Nycholat CM, Schnaar RL. 2017. Siglec-8 and Siglec-9 binding specificities and endogenous airway ligand distributions and properties. *Glycobiology*. 27:657–668.