The clinical impact of glycobiology: targeting selectins, Siglecs and mammalian glycans

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Abstract | Carbohydrates — namely glycans — decorate every cell in the human body and most secreted proteins. Advances in genomics, glycoproteomics and tools from chemical biology have made glycobiology more tractable and understandable. Dysregulated glycosylation plays a major role in disease processes from immune evasion to cognition, sparking research that aims to target glycans for therapeutic benefit. The field is now poised for a boom in drug development. As a harbinger of this activity, glycobiology has already produced several drugs that have improved human health or are currently being translated to the clinic. Focusing on three areas — selectins, Siglecs and glycan-targeted antibodies — this Review aims to tell the stories behind therapies inspired by glycans and to outline how the lessons learned from these approaches are paving the way for future glycobiology-focused therapeutics.

Glycoproteins
Proteins that are covalently conjugated to one or more glycans. These glycans may be either N-linked (connected to asparagine) or O-linked (connected to serine or threonine).

Glycans are involved in fundamental aspects of cell and organismal biology, such as the receptor-mediated cell to cell interactions that underlie both normal and pathological processes. Indeed, the dense layer of glycans on the cell surface (the glycocalyx) can extend more than 30 nm from the plasma membrane on some cells. Cell surface proteins are therefore embedded in a matrix of glycans.

The varied functions of glycans are matched by their diverse structures. Glycans can be conjugated to proteins (to form glycoproteins, proteoglycans and glycosylphosphatidylinositol (GPI)-anchored proteins) and lipids (to form glycolipids), or they can be secreted without conjugation to other macromolecules (in the form of glycosaminoglycans such as hyaluronan). In humans, glycans are primarily constructed from ten monosaccharides: glucose (Glc), galactose (Gal), N-acetylgalactosamine (GalNAc), N-acetylgalactosamine (GalNAc), fucose (Fuc), xylose (Xyl), sialic acid (Neu5Ac), glucuronic acid (GlcA), mannose (Man) and iduronic acid (IdoA). The assembly of these monosaccharides into glycans is performed by enzymes associated with the endoplasmic reticulum and Golgi apparatus. Monosaccharides are linked together through a glycosidic bond between the anomeric carbon of one sugar and a hydroxyl group of the other. The orientation of the glycosidic bond relative to the anomeric carbon (α versus β) affects the overall shape of the glycan. Therefore, the notation for lactose, Galβ1–4Glc, for example, refers to a galactose linked through a β-glycosidic bond to the hydroxyl group on C4 of glucose. Considering these factors alone, there are 20 different ways of linking together glucose and galactose in their ring forms through a glycosidic bond, 19 of which do not make lactose. Additional complexity arises from modification of glycans by sulfation, methylation, phosphorylation, acetylation and O-acetylation.

Glycosylation has three broad functions. First, some glycans form structures with unique physical properties. Second, glycans can regulate the function or properties of the entity to which they are attached, for instance by controlling protein stability or receptor dimerization. Last, certain glycans are themselves ligands for lectins, which are carbohydrate-specific receptors.

Because glycans are essential for organism health, defects in glycosylation are important contributors to human disease. However, the development of glycan-targeted therapies has been hindered by many factors, beginning with our lack of tools for understanding basic glycobiology. Research in the field hinges on accurate methods for identifying and quantifying glycans in a sample. These profiling experiments typically rely on mass spectrometry, fluorophore-conjugated lectins or antibodies. For the few glycan species with high-affinity anti-glycan antibodies, the pace of discovery is higher than for those without such reagents. However, relatively few antibodies against defined glycans are available. Mass spectrometry-based glycomics provides an inventory of glycans present in the sample, but requires liberating glycans from their underlying scaffold. As a consequence, information is limited about which glycan is attached to the particular glycosites on any given protein or lipid. Glycoproteomics is becoming...
Leukocyte extravasation

The process by which cells move from the circulation, across the blood vessel wall and into tissue parenchyma. In this movement, leukocytes (L-selectin) roll along the vessel wall rather than stop and form an aggregate as they do in diapedesis, where cells cross the endothelial barrier.

Proteoglycans

Glycosaminoglycans are long, linear chains of repeating disaccharides that are frequently sulfated. The sugars constituting this repeating unit are characteristic of different glycosaminoglycans. For instance, repeating N-acetylgalactosamine (GalNAc) and glucuronic acid (GlcA) distinguish chondroitin sulfate, whereas heparan sulfate begins as repeating N-acetylgalactosamine (GlcNAc) and GlcA before undergoing heavy modifications, such as epimerization to IdoA and sulfation.

Catch bonds

Bonds that prolong their lifetime in response to increased tensile forces. By contrast, the lifetime of a slip bond decreases in response to higher tension.

Leukocyte extravasation

The process by which cells move from the circulation, across the blood vessel wall and into tissue parenchyma. Extravasation is a stepwise process beginning with rolling adhesion, then tight binding of leukocytes to the endothelium and, finally, diapedesis, where cells cross the endothelial barrier.

A viable alternative as technologies for sample preparation, database searching and data processing continue to improve.

Experiments aimed at identifying the set of ligands for a biologically relevant lectin are complicated by the nature of the interactions between lectins and their glycosylated ligands. First, monovalent interactions tend to be of low affinity; multivalent presentation of both lectin and ligand is often needed to achieve physiologically relevant binding affinities. Second, lectins may have binding affinities that are not only higher but directed to structurally different glycans when those glycans are presented on a multivalent scaffold as compared with a monovalent interaction in solution. Third, in vitro assays may not capture in vivo complexity; indeed, shear stress is required to observe catch bonds, which are characteristic of selectin–ligand interactions.

Glycan synthesis is also a non-templated process, meaning that glycan sequences are not directly coded in the genome. Instead, glycans are produced by the coordinated activity of hundreds of biosynthetic enzymes. Therefore, a particular glycan cannot easily be genetically deleted or altered in order to explore its function. At best, pathways for glycan biosynthesis can be inhibited or engineered, and synthetic glycans on polymeric carriers can be added into a system, leaving researchers to deconvolute the pleiotropic effects of these treatments.

Despite these difficulties, several investigators have discovered how glycans contribute to disease and have successfully converted these findings into therapies. Perhaps the best known drugs that target glycans are the influenza medications zanamivir and oseltamivir. These drugs are sialic acid mimetics that function as inhibitors of the neuraminidase of influenza viruses. The context in which these glycan inhibitors are presented alters their affinity for any given binding lectin. For instance, the primary ligand for P-selectin is P-selectin glycoprotein ligand 1 (PSGL1), a homodimeric mucin decorated with sLex (REF. 12), but P-selectin also has high-affinity interactions with sulfatides, non-sialylated glycophospholipids and CD24 (REF. 13). P-selectin has a preference for binding to glycoproteins, such as CD34, glycosylation-dependent cell adhesion molecule 1 (GlyCAM1), mucosal addressin cell adhesion molecule 1 (MadCAM1) and PSGL1, which contain sulfated sLeα incorporated into N-linked and O-linked glycans (REF. 14). Interestingly, both L-selectin and P-selectin display affinity for the glycosphingolipid VIM2 epitope, which contains internally fucosylated sialyllactosamine (REF. 15). The variation within these ligand repertoires suggests that, although sLeα-related glycans are important, they are not the sole determinants of selectin binding. Rather, the selectins recognize a molecular surface comprising both glycan and scaffold.

Selectins

The selectins are a family of calcium-dependent (C-type) lectins best known for their role in mediating immune cell adhesion to the endothelium to facilitate entry to secondary lymphoid organs and sites of inflammation. Therapies targeting selectin–ligand interactions have been inspired by the importance of selectins in mediating cell adhesion, and have been investigated in sickle cell disease, cancer cell metastasis and bone marrow transplantation.

The selectin family comprises three members, named after their expression patterns: those expressed on platelets (P-selectin), on endothelial cells (E-selectin) and on leukocytes (L-selectin). These three selectins also differ in biological activities and preferred ligands (FIG. 1). L-selectin is constitutively expressed on all circulating leukocytes and is shed from the cell surface. E-selectin is constitutively expressed on endothelial cells in postcapillary venules of the bone marrow and skin; expression on the endothelium in other organs requires exposure to inflammatory stimuli such as tumour necrosis factor (TNF), interleukin 1β (IL-1β) and lipopolysaccharide (LPS). P-selectin is expressed by endothelial cells and activated platelets (REF. 16).

Among the best described ligands for the selectins are derivatives of the fucosylated and sialylated tetrasaccharides sialyl Lewisα (sLeα, Neu5Aca2→3Galβ1→4(Fucα1→3) GlcNAc) and sialyl Lewisα (sLeα, Neu5Aca2→3Galβ1→4(Fucα1→3) GlcNAc). The context in which these glycans are presented alters their affinity for any given ligand. For instance, the primary ligand for P-selectin is P-selectin glycoprotein ligand 1 (PSGL1), a homodimeric mucin decorated with sLeα (REF. 12), but P-selectin also has high-affinity interactions with sulfatides, non-sialylated glycophospholipids and CD24 (REF. 13). P-selectin has a preference for binding to glycoproteins, such as CD34, glycosylation-dependent cell adhesion molecule 1 (GlyCAM1), mucosal addressin cell adhesion molecule 1 (MadCAM1) and PSGL1, which contain sulfated sLeα incorporated into N-linked and O-linked glycans (REF. 14). Interestingly, both L-selectin and P-selectin display affinity for the glycosphingolipid VIM2 epitope, which contains internally fucosylated sialyllactosamine (REF. 15). The variation within these ligand repertoires suggests that, although sLeα-related glycans are important, they are not the sole determinants of selectin binding. Rather, the selectins recognize a molecular surface comprising both glycan and scaffold.

In normal physiology, the selectins are critical mediators of leukocyte circulation through secondary lymphoid tissues and recruitment to sites of inflammation. Cells flowing through the vascular compartment that are captured by selectin–ligand interactions begin rolling on endothelial cells, which is the earliest stage of leukocyte extravasation (REF. 11). At secondary lymphoid tissues,
leukocytes expressing L-selectin engage glycoproteins displayed by endothelial cells in the high endothelial venule to promote extravasation. Leukocytes are recruited to sites of inflammation because endothelial cells there upregulate expression of E-selectin and display more P-selectin, which these cells store in Weibel–Palade bodies, and activated platelets display P-selectin, which they store in α-granules. The L-selectin expressed by the gathering immune cells themselves further contributes to leukocyte adhesion and recruitment. Therefore, upregulation of selectins or their ligands directs leukocytes to particular locations: through the combinatorial display of particular selectins and their ligands, leukocyte subsets can be recruited to specific sites in the body.

Fig. 1 | Selectins and their primary ligands. In humans, selectin and selectin ligands expressed on the endothelium, platelets and other cells (shown on the left) interact with selectin and selectin ligands on leukocytes or haematopoietic stem cells (HSCs) (shown on the right). The ligands for each selectin comprise glycoproteins bearing sialofucosylated glycans that are closely related to sialyl Lewis^x^ (sLex), and, in some cases, glycolipids such as VIM2 and glycosaminoglycans such as heparin. L-selectin is both constitutively expressed and shed from leukocyte cell surfaces. E-selectin and P-selectin are displayed on cells in response to inflammatory stimuli. Mucins refers to endothelial glycoproteins not otherwise depicted that function as L-selectin ligands, including mucosal addressin cell adhesion molecule 1 (MAdCAM1), podocalyxin-like protein, Sgp200, endoglycan and endomucin. E-selectin is constitutively expressed on the endothelium in the bone marrow and skin, but requires exposure to inflammatory cytokines to be expressed in other organs. The dotted arrow indicates that sLex^Le^ on L-selectin itself is a ligand for E-selectin. EGF, epidermal growth factor; (e)PSGL1, (endothelial) P-selectin glycoprotein ligand 1; GlyCAM1, glycosylation-dependent cell adhesion molecule 1; HCELL, haematopoietic cell E/L-selectin ligand; IL-1β, interleukin-1β; LPS, lipopolysaccharide; SCR, short consensus repeat (Sushi domain); TNF, tumour necrosis factor.
**Discovery of selectins and ligands.** Because selectin–glycan interactions provide the molecular basis for recruitment of leukocytes to inflamed tissues, targeting these interactions with a therapeutic holds promise for spatially controlling the immune system. However, early investigations into selectin biology highlighted two major hurdles: glycan heterogeneity and low receptor–ligand affinity. Pioneering work in the 1950s established that leukocyte extravasation through high endothelial venules in the lymph node and into inflamed sites in the periphery is a tightly regulated process, dependent on cell surface carbohydrates. The crucial realization that leukocyte–endothelium adhesion occurs when blood is flowing enabled the next several decades of selectin research by encouraging investigators to incorporate this shear stress into assays of selectin–ligand interactions. Aided by the development of monoclonal antibody technology, the identities of the selectins were finally established when P-selectin, E-selectin and L-selectin were all cloned and described as part of the same family in 1989.

Animal models were quickly generated to explore the contribution of selectins to various acute and chronic inflammatory pathologies, and the results generated interest among those hoping to translate these discoveries. Among the first models tested were ischemia–reperfusion injuries, dermal injuries, allergen-induced inflammation and cobra venom-induced lung injury. In these models, administration of selectin–immunoglobulin chimeric decoy receptors or blocking antibodies targeting the selectins or sLex could broadly decrease the damage caused by neutrophil entry into tissue. Models of ischemia–reperfusion injury, in particular, produced impressive results: a P-selectin blocking antibody reduced tissue necrosis by up to 90% after ischemia. These results hinted that selectin inhibition may be a promising therapeutic strategy for numerous pathologies.

Pharmaceutical development could not progress, however, without an improved understanding of the glycan ligands used by selectins in vivo. In 1990, sLex was identified as the primary binding determinant for E-selectin. The resulting glycomimetic drug, Cylexin (CY-1503), comprising sLex β-linked to a methylacetyllactosamine (LacNAc) of sLex, reduced neutrophil inflammation after myocardial ischemia in animal models. In humans, early results showed that Cylexin prevented reperfusion injury after pulmonary thromboendarterectomy. Ultimately, a phase II trial (NCT00226369) found that Cylexin was not effective in reducing myocardial infarctions in infants undergoing cardiac surgery and led to cancellation of the programme. Clearly, the simple infusion of sLex was insufficient for selectin inhibition.

Indeed, the in vitro equilibrium dissociation constant ($K_d$) values of the selectins for sLex lie in the low millimolar range. These measurements, made on free oligosaccharides, suggest weak interactions between glycan and selectin, and point towards the importance of other factors for binding in vivo. Additional information regarding selectin ligands would be necessary to enable the next generation of high-affinity inhibitors.

**Small-molecule selectin inhibitors.** A key insight came from more detailed studies of the interaction between P-selectin and its principal ligand, PSGL1. First, three sulfotyrosines in PSGL1 were found to be important binding determinants for P-selectin. Soon thereafter, crystal structures of P-selectin and E-selectin in complex with sLex and PSGL1 were solved. The published structures demonstrated why the interaction of P-selectin with PSGL1 is decidedly higher affinity than that with the glycan alone: the three sulfotyrosines in PSGL1 create an anionic pocket that increases its affinity for P-selectin. In essence, the glycan and protein portions of PSGL1 may be considered distinct pharmacophores that both make important contributions to the interaction with P-selectin. This finding was paradigm-shifting because it challenged the prevailing view that lectins interact only with glycans. These structures demonstrated that the combination of glycan and scaffold provide a single epitope that is recognized by lectins such as P-selectin.

Additional NMR studies of the solution structure of sLex bound to E-selectin highlighted the importance of contacts with the hydrogens of galactose and fucose. This information was incorporated into the design of GlycoMimetics’ pan-selectin inhibitor, rivipansel (WY-14643), which retained moieties analogous to the sLex tetrasaccharide, the carboxylic acid of sialic acid, and included a sulfated naphthalene group to mimic the sulfotyrosines in PSGL1. Rivipansel bound E-selectin, P-selectin and L-selectin with micromolar affinities, was capable of inhibiting E-selectin and P-selectin-mediated leukocyte rolling and reversed vascular occlusions in a mouse model of sickle cell disease.

A phase II trial (NCT01119833) in patients with acute sickling crises demonstrated that rivipansel could decrease the time to resolution of vaso-occlusion and reduce opioid use. The phase III trial (NCT02187003) of rivipansel in sickle cell disease failed to meet its primary end points in mid-2019, but a post hoc analysis found that patients who received the drug early after the start of pain from vaso-occlusion (within 24 h) did benefit.

Other small-molecule inhibitors that were developed to target the selectins suffered from a lack of drug-like properties, off-target effects and low binding affinities. Texas Biotechnology Corporation (later Encysive Pharmaceuticals) avoided oligosaccharide inhibitors by developing a small-molecule glycomimetic antagonist that replaced the N-acetyllactosamine (LacNAc) of sLex with a biphenyl unit, substituted a carboxylic acid moiety for sialic acid and utilized mannose as a proxy for fucose. Inspired by reports that branched sLex structures enhanced selectin affinity, the team added an additional mannose to their structure to afford bimosiamose (TBC-1269). Bimosiamose was effective in ischemia–reperfusion injury and asthma models, and later work demonstrated efficacy in psoriasis and chronic obstructive pulmonary disease. Despite these positive results, Revotar, who took over the programme, no longer appears to be developing bimosiamose.

Wyeth endeavoured to develop an orally available specific P-selectin inhibitor. Agnostic to the incorporation of carbohydrate pharmacophores in the therapeutic,
| Therapeutic type and name; manufacturer | Indication | Phase and status | Results | Trial identifiers (refs) |
|----------------------------------------|------------|-----------------|---------|-------------------------|
| **Pan-selectin antagonists**           |            |                 |         |                         |
| Small molecule                         |            |                 |         |                         |
| Cylexin (CY-1503); Cytel              | Ischaemia–reperfusion injury in infant heart surgery | Phase II/III completed 2001 | NR | NCT00226369 |
| Small molecule                         | Vaso-occlusive crisis in sickle cell disease | Phase I/II completed 2010 | Well tolerated, no adverse events | NCT00911495 (REF.\(^{275}\)) |
| Rivipansel (GMI-1070); GlycoMimetics   | Vaso-occlusive crisis in sickle cell disease and S-ß-thalassaemia | Phase II completed 2013 | Trend towards reduced time to vaso-occlusive crisis resolution | NCT01119833 |
| Vaso-occlusive crisis in sickle cell disease | Phase III completed 2019 | Post hoc analysis showed efficacy in a subset of patients | | NCT02187003 (REF.\(^{44}\)) |
| Small molecule                         | Psoriasis (as a cream) | Phase II completed 2009 | NR | NCT00823693 |
| Bimosiamose (TBC-1269); Texas Biotechnology Corporation | Ozone-induced sputum neutrophilia | Phase II completed 2010 | NR | NCT00962481 |
| Chronic obstructive pulmonary disease  | Phase II completed 2011 | Attenuates airway inflammation | | NCT01108913 (REF.\(^{51,276}\)) |
| Small molecule                         | Vaso-occlusive crisis in sickle cell disease | Phase II completed 2013 | No effect on saphenous vein graft failure, possibly because prior activation of P-selectin pathway not evaluated | NCT02515838 |
| **P-selectin antagonists**             |            |                 |         |                         |
| Small molecule                         | Scleritis   | Phase I terminated 2007 | NR (terminated) | NCT00367692 |
| Biologic (decoy ligand)                |            |                 |         |                         |
| YSPSL (rPSGL–Ig); Genetics Institute and Wyeth | Delayed graft function during kidney allograft | Phase I/IIa completed 2007 | Safe, no effect on renal function | NCT00298181 (REF.\(^{66}\)) |
| Delayed graft function during kidney allograft | Phase I/IIb completed 2007 | Attenuated biomarkers of inflammation | | NCT00298168 (REF.\(^{66,277}\)) |
| Ischaemia–reperfusion injury during liver allograft | Phase II completed 2009 | Safe, measures of graft function trended towards improvement, liver enzymes normalized | | NCT00876902 (REF.\(^{66}\)) |
| Delayed graft function during liver allograft | Phase II completed 2008 | NR | | NCT00450398 |
| **Monoclonal antibody**                |            |                 |         |                         |
| Inclacumab (anti-P-selectin; RO4905417); Hoffman–La Roche | Myocardial infarction | Phase II completed 2012 | Reduced myocardial damage (troponin I levels) in NSTEMI patients | NCT01327183 (REF.\(^{55,67}\)) |
| Coronary heart disease graft occlusion | Phase II completed 2013 | No effect on saphenous vein graft failure, possibly because prior activation of P-selectin pathway not evaluated | | NCT01245634 (REF.\(^{66}\)) |
| **Monoclonal antibody**                |            |                 |         |                         |
| Crizanlizumab (anti-P-selectin; SEG101 or SelG1); Selexys and Novartis | Vaso-occlusive crises in sickle cell disease | Phase II completed 2016 | Reduced rate of vaso-occlusive crises and time to first crisis | NCT01895361 (REF.\(^{70}\)) |
| Vaso-occlusive crises in sickle cell disease | Phase II ongoing | Estimated completion in 2021 | | NCT03264989 |
| PK in paediatric patients with sickle cell disease | Phase II ongoing | Estimated completion in 2023 | | NCT03474965 |
| Vaso-occlusive crises in sickle cell disease in adolescents and adults | Phase III ongoing | Estimated completion in 2027 | | NCT03814746 |
| Sickle cell disease related priapism | Phase II ongoing | Estimated completion in 2022 | | NCT03938454 |
| Chronic kidney disease in sickle cell disease patients | Phase II ongoing | Estimated completion in 2022 | | NCT04053764 |
| Myelofibrosis (combination treatment with ruxolitinib) | Phase I/II ongoing | Estimated completion in 2024 | | NCT04097821 |
| **E-selectin antagonists**             |            |                 |         |                         |
| Small molecule                         | Deep vein thrombosis | Phase I/II terminated 2016 | NR (terminated with grant expiration) | NCT02744833 |
| Uproleselan (GMI-1271); GlycoMimetics  | Multiple myeloma | Phase I completed 2019 | NR | NCT02811822 |
| Acute myeloid leukaemia                | Phase II/III and III ongoing | Estimated completion in 2023 | | NCT03616470, NCT03701308 |
| Therapeutic type and name; manufacturer | Indication | Phase and status | Results | Trial identifiers (refs) |
|----------------------------------------|------------|-----------------|---------|------------------------|
| Siglec antibody–drug conjugates        |            |                 |         |                        |
| Gemtuzumab ozogamicin (Mylotarg; anti-CD33–calicheamicin conjugate); Wyeth and Pfizer | Acute myeloid leukaemia | Phase II completed 2000 | Improved survival with reasonable safety profile; FDA approval granted | Trials 201, 202, 203 (REFs 125,126) |
| Gemtuzumab ozogamicin (Mylotarg; anti-CD33–calicheamicin conjugate); Wyeth and Pfizer | Acute myeloid leukaemia | Phase III completed 2014 | No survival benefit and higher rates of fatal toxicity; removed from US market in 2010 | NCT00085709 (REF 125) |
| Gemtuzumab ozogamicin (Mylotarg; anti-CD33–calicheamicin conjugate); Wyeth and Pfizer | Acute myeloid leukaemia | Phase III completed 2013 | Lower doses of drug on new dosing schedule improved outcomes without increasing death from toxicity; FDA approval in 2017 | NCT00927498 (REFs 125,126) |
| Inotuzumab ozogamicin (Besponsa; anti-CD22–calicheamicin conjugate); Pfizer | Acute lymphoblastic leukaemia | Phase III completed 2017 | Improved progression-free and overall survival | NCT01564784 (REF 103) |
| Pinatuzumab vedotin (anti-CD22–MMAE conjugate); Genentech and Hoffman–La Roche | Follicular lymphoma and diffuse large B cell lymphoma | Phase I/II completed 2019 | Achieved objective responses, but development shelved in favour of other more robust therapies | NCT01691898 (REF 103) |
| CD33 antagonists                        |            |                 |         |                        |
| Monoclonal antibody AL003; Alector     | Alzheimer disease | Phase I ongoing | Estimated completion in 2021 | NCT03822208 |
| Siglec-8 agonists                      |            |                 |         |                        |
| Monoclonal antibody Lirentelimab (AK002); Allakos | Keratoconjunctivitis, vernal conjunctivitis, allergic conjunctivitis | Phase I completed 2019 | NR | NCT03379311 |
| Monoclonal antibody Lirentelimab (AK002); Allakos | Eosinophilic gastritis, eosinophilic gastroenteritis | Phase II completed 2019 | Reduced gastrointestinal eosinophil count and symptoms in a majority of patients | NCT03496571 (REF 173) |
| Monoclonal antibody Lirentelimab (AK002); Allakos | Chronic urticaria | Phase II completed 2020 | Estimated completion in 2020 | NCT03436797 |
| Monoclonal antibody Lirentelimab (AK002); Allakos | Eosinophilic gastroenteritis | Phase II ongoing | Estimated completion in 2021 | NCT03664960 |
| Monoclonal antibody Lirentelimab (AK002); Allakos | Eosinophilic oesophagitis | Phase II/III ongoing | Estimated completion in 2022 | NCT04322708 |
| Monoclonal antibody Lirentelimab (AK002); Allakos | Eosinophilic gastritis, eosinophilic duodenitis | Phase III ongoing | Estimated completion in 2021 | NCT04322604 |
| Siglec-10 agonists                     |            |                 |         |                        |
| Recombinant ligand CD24Fc; Oncolimmune | Severe COVID-19 | Phase III ongoing | Estimated completion in 2020 | NCT04317040 |
| Recombinant ligand CD24Fc; Oncolimmune | Immune-related adverse events associated with checkpoint inhibitors | Phase I/II not yet recruiting | Estimated completion in 2023 | NCT04060407 |
| Recombinant ligand CD24Fc; Oncolimmune | Acute graft-versus-host disease | Phase III not yet recruiting | Estimated completion in 2024 | NCT04095858 |
| Siglec-15 antagonists                  |            |                 |         |                        |
| Monoclonal antibody NC318; NextCure    | Metastatic solid tumours, head and neck squamous cell carcinoma, NSCLC, ovarian cancer, triple-negative breast cancer | Phase I/II ongoing | Estimated completion in 2021; NSCLC and ovarian cancer cohorts will not advance after interim analysis | NCT03665285 (REF 174) |
| Mammalian glycan vaccines              |            |                 |         |                        |
| Carbohydrate vaccine Theratope (sTn–KLH vaccine); Biomira | Breast cancer | Phase III completed 2008 | No benefit to overall survival or time to progression; post hoc analysis showed benefit when combined with endocrine therapy | NCT00003638 (REFs 127,128) |
| Peptide vaccine MUC1 peptide plus poly-ICLC; University of Pittsburgh | Colorectal adenoma | Phase II ongoing | Patients produced anti-MUC1 IgG; adenoma recurrence data pending | NCT02134925 |
| Peptide vaccine MUC1 peptide plus poly-ICLC; University of Pittsburgh | Lung carcinoma | Phase I ongoing | Estimated completion in 2020 | NCT03300817 |
Table 1 (cont.) | Selected clinical trials of glycobiology-targeted therapeutics

| Therapeutic type and name; manufacturer | Indication | Phase and status | Results | Trial identifiers (refs) |
|----------------------------------------|------------|-----------------|---------|-------------------------|
| **Mammalian glycan vaccines (cont.)**   |            |                 |         |                         |
| Adenoviral vaccine ETBX-011 (Ad5 CEA vaccine); Etubics and NCI | Colorectal carcinoma | Phase I/II completed 2013 | Generated T cell response to CEA | NCT01147965 (REF. 236) |
| Adenoviral vaccine ETBX-011/ETBX-061/ETBX-051 (Ad5 CEA/MUC1/brachyury vaccine); Etubics and NCI | Colon, breast, lung and prostate cancers | Phase I ongoing | Generated T cell response to CEA, MUC1 and brachyury | NCT03384316 (REF. 222) |
| Carbohydrate vaccine BMS-248479 (GM2–KLH/OS-21 vaccine); Bristol-Myers-Squibb | Melanoma | Phase III terminated 2007 | Terminated for futility | NCT00005052 (REF. 211) |
| Carbohydrate vaccine Trivalent (GM2/GD2/GD3–KLH) vaccine with OPT-821; MabVax | Metastatic sarcoma | Phase II completed 2013 | No benefit, trend towards reduced progression-free survival | NCT01141491 |
| Carbohydrate vaccine Globo H–GM2–sTn–TF–Tn–KLH conjugate/QS-21 vaccine; Memorial Sloan Kettering Cancer Center | Fallopian tube, ovarian and peritoneal cancers | Phase I completed 2017 | Most patients developed serologic response to at least three antigens in vaccine | NCT01248273 (REF. 228) |
| Carbohydrate mimetic peptide vaccine P10s-PADRE vaccine; University of Arkansas | Breast cancer | Phase I completed 2019 | Serologic response to Lea and GD2 in all subjects | NCT01390064 (REF. 229) |
| Carbohydrate vaccine GD2/GD3 lactone–KLH/OPT-821 vaccine; Memorial Sloan Kettering Cancer Center | Neuroblastoma | Phase I/II ongoing | Estimated completion in 2020 | NCT00911560 (REF. 211) |
| Carbohydrate vaccine Adagloxad simolenin (OPT-822; Globo H–KLH/OS-21 vaccine); OBI Pharma and Memorial Sloan Kettering Cancer Center | Breast cancer | Phase II/III completed 2019 | No improvement in survival; progression-free and overall survival did improve in patients with serologic response | NCT01516307 (REF. 211) |
| Carbohydrate vaccine sLea–KLH conjugate/QS-21; Memorial Sloan Kettering Cancer Center | Triple-negative breast cancer | Phase III ongoing | Estimated completion in 2025 | NCT03562637 |
| Carbohydrate vaccine Oregovomab (anti-MUC16/CA125; B43.13); ViRexx Medical Corp | Ovarian cancer | Phase III terminated 2007 | No clinical benefit as a monotherapy following frontline carboplatin–paclitaxel treatment | NCT00050375 (REF. 238) |
| Carbohydrate vaccine BIW-8962 (anti-GM2); Kyowa Hakko Kirin Pharma, Inc. | Multiple myeloma | Phase I terminated 2011 | Terminated for lack of efficacy | NCT00775502 (REF. 237) |
| Carbohydrate vaccine Dinutuximab (ch14.18; anti-GD2); Scripps Research Institute | Neuroblastoma | Phase III completed 2012 | Improved event-free and overall survival | NCT00026312 (REF. 238) |
Table 1 (cont.) | Selected clinical trials of glycobiology-targeted therapeuticsa

| Therapeutic type and name; manufacturer | Indication | Phase and status | Results | Trial identifiers (refs) |
|----------------------------------------|------------|-----------------|---------|-------------------------|
| Anti-glycan antibodies (cont.)         |            |                 |         |                         |
| Monoclonal antibody                    |            |                 |         |                         |
| MORAb-028 (anti-GD2 IgM); Morphotek    | Melanoma   | Phase I terminated 2012 | Terminated for lack of drug availability | NCT01123304 |
| Monoconal antibody                     |            |                 |         |                         |
| Ecromeximab (KW2871; anti-GD3); Kyowa Hakko Kirin Pharma, Inc. | Metastatic melanoma | Phase II completed 2014 | Limited efficacy with high-dose interferon, possibly because tumour burdens in the population studied were already high | NCT00679289 (REF.241) |
| Monoconal antibody                     |            |                 |         |                         |
| BMS-986012 (anti-Fuc-GM1); Bristol-Myers-Squibb | Relapsed and refractory small cell lung cancer | Phase I/II ongoing | Estimated completion in 2020 | NCT02247349 |
| Monoconal antibody                     |            |                 |         |                         |
| OBI-888 (anti-globo H); OBI Pharma     | Solid tumours | Phase I/II ongoing | Estimated completion in 2021 | NCT03573544 |
| Antibody drug conjugate                |            |                 |         |                         |
| OBI-999 (anti-globo H–MMAE conjugate); OBI Pharma | Solid tumours | Phase I/II ongoing | Estimated completion in 2023 | NCT04084366 |
| Monoconal antibody                     |            |                 |         |                         |
| MVT-5873 (clone SB1 anti-CA19-9); MabVax Therapeutics | Pancreatic cancer | Phase I ongoing | Estimated completion in 2020 | NCT02672917 |
| Radiolabelled monoclonal antibody      |            |                 |         |                         |
| MVT-1075 (177Lu SB1 anti-CA19-9); MabVax Therapeutics | Pancreatic cancer, tumours expressing CA19-9 | Phase I ongoing | Estimated completion in 2020 | NCT03118349 |
| Radiolabelled monoclonal antibody      |            |                 |         |                         |
| MVT-2163 (175Zr-DFO-SB1 anti-CA19-9) with MVT-1075 for PET imaging; MabVax Therapeutics | Pancreatic cancer, tumours expressing CA19-9 | Phase I ongoing | Estimated completion in 2020 | NCT02687230 |
| Anti-idiotypic antibodies               |            |                 |         |                         |
| Monoclonal antibody                     |            |                 |         |                         |
| Abagovomab (anti-MUC16/CA125); Menarini Group | Ovarian cancer | Phase II/III terminated 2011 | Administered as maintenance therapy; no clinical benefit and no induction of CA125-specific T cells | NCT00418574 (REFs.292,293) |
| Monoclonal antibody                     |            |                 |         |                         |
| Racotumomab (anti-N-glycolyl-GM3); Recombio | NSCLC | Phase III completed 2014 | Increased progression-free and overall survival Serological response to N-glycolyl-GM3 | NCT01460472 (REF.294) |
| Neuroblastoma                           | Phase I completed 2014 | Well tolerated, serological response to N-glycolyl-GM3 in most patients | NCT01598454 (REF.295) |
| CAR cell therapies                      |            |                 |         |                         |
| Anti-GD2 CAR T; Baylor College of Medicine | Neuroblastoma | Phase I ongoing | 3 of 11 patients achieved completion remission; study completion estimated in 2021 | NCT00085930 (REF.296) |
| Anti-GD2 CAR T with iCaspase switch; Baylor College of Medicine | Neuroblastoma | Phase I ongoing | Estimated completion in 2030 | NCT01822652 |
| Anti-GD2 tri-virus CAR T; Baylor College of Medicine | Neuroblastoma after haematopoietic stem cell transplant | Phase I completed 2015 | Safe, partial response in 3 of 3 patients | NCT01460901 |
| Anti-GD2 CAR NKT; Baylor College of Medicine | Neuroblastoma | Phase I ongoing | Estimated completion in 2021 | NCT03294954 |

Ad5, adenovirus serotype 5; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; KLH, keyhole limpet haemocyanin; Le¹, Lewis¹; MMAE, monomethyl auristatin E; MUC1, mucin 1; NCI, National Cancer Institute; NR, not reported; NSCLC, non-small cell lung cancer; NSTEMI, non-ST elevation myocardial infarction; PADRE, pan-HLA DR binding-epitope; PET, positron emission tomography; PK, pharmacokinetics; poly-ICLC, polyinosinic–polycytidylic acid stabilized with polylsine and carboxymethylcellulose; rPSGL–Ig, recombinant PSGL1 fused to immunoglobulin; sLe¹, sialyl Lewis¹; sTn, sialyl-Tn; TF, Thomsen–Friedenreich antigen. *Only trials registered at ClinicalTrials.gov are included.
they used an enzyme-linked immunosorbent-based assay followed by extensive structure–activity relationship refinement to identify their lead compound, PSI-697, which was based on a tetrahydrobenzoquinoline salicylic acid scaffold. Although PSI-697 decreased thrombosis in mice, it was no better than placebo in preventing thromboembolic events in smokers. A team at Nippon Organon developed OJ-R9188, a higher affinity pan-selectin antagonist that showed efficacy in a reperfusion injury model but was not pursued further because it lacked drug-like properties. Modus Therapeutics developed a modified heparin, sevuparin, that maintained the interaction with P-selectin and L-selectin while avoiding binding to antithrombin III and, therefore, interference with the clotting cascade. However, sevuparin failed to show efficacy in a phase II trial for acute vaso-occlusive crises in sickle cell disease. Finally, the natural product efomycine M was believed to be a useful selectin inhibitor for treatment of inflammatory diseases, but was subsequently discovered to operate via selectin-independent mechanisms.

**Biologic selectin inhibitors.** Knowledge of selectin ligand identities also enabled antagonism with biologics. P-selectin inhibition using a recombinant P-selectin ligand decreased thrombosis in non-human primate animal models. These results encouraged Genetics Institute, Inc., which was acquired by Wyeth, to develop a chimeric P-selectin–immunoglobulin fusion protein, rPSGL–Ig, that was able to promote thrombolysis in porcine models of acute thrombosis and myocardial infarction. Y’s Therapeutics subsequently licensed rPSGL–Ig (YSPSL) from Wyeth/Pfizer to assess its use in improving organ allograft function because the mechanism of tissue damage in allografts involves ischaemia–reperfusion injury. Although a phase II study of liver allograft function showed that YSPSL improved liver function after transplant, these benefits were not replicated in a phase IIa trial and development was shelved. A variant of this idea — a recently generated PSGL1 mimetic peptide — demonstrated tight binding to P-selectin with an affinity in the low nanomolar range.
Selectin-blocking antibodies have shown even greater promise. Hoffmann–La Roche developed the monoclonal antibody inclaclumab as a P-selectin antagonist. In a phase II trial (NCT01327183), inclaclumab reduced myocardial damage in patients undergoing percutaneous coronary intervention for non-ST-segment elevation myocardial infarction. However, the programme was abandoned when another phase II trial (NCT01245634) of inclaclumab in patients undergoing coronary artery bypass graft surgery showed that the drug had no benefit. It was later discovered that both L-selectin and P-selectin are inhibited by heparin, which is the standard of care treatment for patients with myocardial infarctions. Selexys opted to develop their P-selectin blocking antibody crizanlizumab (SEG101, also known as SelG1) as a prophylactic agent for vaso-occlusive crises in patients with sickle cell disease. Notably, crizanlizumab reduced the frequency of pain crises and could be administered on a dosing schedule of once every 3–4 weeks. The strength of these results led to the acquisition of Selexys by Novartis and the FDA approval of crizanlizumab in late 2019.

Selectins in cancer. Publications in the early 1990s showing a role for selectins in tumour cell metastasis garnered interest in targeting selectins in cancer. All members of...
the selectin family have been implicated. Ligands for L-selectin on lymphoid tissues were found to promote leukaemia and lymphoma seeding. On colon cancer cells, the display of sialyl Lewis structures that can interact with E-selectin correlates with metastatic potential. P-selectin expression by platelets and endothelium in the microvasculature may arrest metastatic cells. Recently, data have corroborated and expanded these early observations: for instance, binding to P-selectin on mesothelial cells through CD24 is a major mechanism by which ovarian cancer cells metastasize.

The application of selectin inhibitors in cancer was catalysed by the identification of E-selectin as a bone marrow homing receptor. Early work on this topic was inspired by the idea that if homing receptors exist for immune cell infiltration into the periphery, homologous mechanisms must exist for the entry of haematopoietic stem cells (HSCs) into the marrow following bone marrow transplantation. Through careful application of sheer stress conditions, HSCs were found to be decorated with HCELL. Importantly, HCELL+/− HSCs failed to home to the bone marrow whereas their HCELL+/+ counterparts entered the bone marrow niche and proliferated. Soon thereafter, E-selectin was identified as a key regulator of HSC homeostasis.

In a landmark discovery, leukaemia cells were found to home to regions of the bone marrow microenvironment characterized by E-selectin expression. This discovery, along with evidence that numerous solid and blood cancers express the ligands for E-selectin, galvanized the notion that E-selectin–ligand interactions promote cancer metastasis to the bone. In acute myeloid leukaemia (AML) in particular, the interaction of malignant cells with E-selectin promotes regeneration and resistance to chemotherapy by activating pro-survival signalling pathways. Several pharmaceutical companies seized this opportunity to develop first in class therapies. GlycoMimetics, fresh off its rivipansel campaign, led the pack with the development of uproleselan (GMI-1271), an E-selectin-specific inhibitor. A phase I/II trial of uproleselan in combination with chemotherapy for AML showed promise, leading to the initiation of a phase III trial (NCT03616470) with results expected in late 2023. The finding that uproleselan also disrupts the tumour microenvironment in multiple myeloma launched a phase I trial (NCT02811822) that was ultimately halted due to low patient recruitment.

Recent data have shed mechanistic light on the contribution of E-selectin to bone marrow metastasis. E-selectin expression by the vasculature was found to regulate breast cancer cell entry into, but not retention by, the bone marrow niche. Interactions with E-selectin were also found to promote mesenchymal–epithelial transition and activate WNT signalling, implying that targeting these networks, such as with the WNT inhibitor LF3, may indirectly interfere with E-selectin interactions in cancer.

New aspects of PSGL1 biology are poised to enter the immuno-oncology arena. Reports that PSGL1 modulates antigen-presenting cell and T cell activity have existed in the literature for years without coalescing on a clear mechanism, although signalling cascades downstream of PSGL1 crosslinking appear to be involved. Along these lines, Verseau is developing a PSGL1 blocking antibody as a macrophage checkpoint inhibitor. V-type immunoglobulin domain-containing suppressor of T cell activation (VISTA), a B7 family protein expressed on activated lymphocytes and some myeloid populations, binds PSGL1 in an sLeα-independent but sulfotyrosine-dependent manner. Importantly, a VISTA blocking antibody reversed immune cell inhibition and promoted tumour rejection in a mouse model of MC38 colorectal adenocarcinoma.

**Prospects for selectin-targeted therapies.** Early efforts to harness selectin inhibition as a therapeutic modality focused on cardiovascular disease and travelled a rocky road to success. Positive outcomes from selectin inhibition in cancer, as showcased by trials of uproleselan in AML, have reinvigorated the field and encouraged other efforts to target selectins. These approaches include nanoparticles decorated with P-selectin ligands for targeted drug delivery, and chimeric antigen receptor (CAR) T cells with enforced expression of sLex to boost E-selectin ligand display and increase infiltration into the marrow.

Despite early failures, selectin inhibitors may still prove useful for vascular disease. Identifying an appropriate patient population will be central to demonstrating efficacy. Indeed, it is likely that patients were already selectin-inhibited by heparin in the phase II trial that showed no benefit of inclacumab administration during coronary artery bypass graft surgery. Likewise, the phase III trial of rivipansel for vaso-occlusive crises in sickle cell disease included patients who received the drug days after the onset of pain; the post hoc analysis of this trial suggests that early selectin inhibition is necessary for efficacy. Biologics such as crizanlizumab benefit from long half-lives, meaning that patients can receive doses less frequently, and that outcomes such as the incidence of vaso-occlusive crises, or the time to vaso-occlusive crisis resolution in a hospitalized setting, was as monitored for rivipansel, could be monitored. Potential catalysts for selectin inhibitors include GlycoMimetics’ new E-selectin antagonist, GMI-1687, which displays a Kd value in the low nanomolar range and can be administered subcutaneously, and uproleselan, trials of which will report in 2023.

Efforts to inhibit selectins with small molecules have certainly experienced turbulence. Nevertheless, these endeavours have laid the foundation for future drug development campaigns, including suggesting that fragment-based screening may be a particularly fruitful modality for discovering novel inhibitors of glycan binding proteins.

**Siglecs**

The Siglecs are a family of receptors expressed by most immune cell types. Siglec ligands comprise proteins and lipids adorning with sialic acid (‘sialylated’) (FIG. 4). Due to their homology to the immunoglobulin superfamily, the Siglecs are considered I-type lectins. Structurally, they comprise an amino-terminal V-set domain that binds sialylated glycans.
a Human Siglecs

Conserved Siglecs

CD22

MAG

Siglec-15

• Macrophage
• Monocyte
• Schwann cell
• B cell

CD33

Siglec-5

Siglec-6

Siglec-7

Siglec-8

Siglec-9

• MyPro
• Macrophage
• Granulocyte
• Neutrophil
• Megakaryocyte
• Mast cell

Siglec-10

Siglec-11

Siglec-14

Siglec-16

• Neutrophil
• Monocyte
• Macrophage
• Microglia

• B cell
• Platelet

• Eosinophil
• Mast cell

• T cell
• Platelet

CD33-related Siglecs

b Mouse Siglecs

Conserved Siglecs

Sialoadhesin

CD22

MAG

Siglec-15

• Macrophage
• Monocyte
• Schwann cell
• ODC

CD33

Siglec-E

Siglec-F

Siglec-G

Siglec-H

• Macrophage
• Granulocyte
• Neutrophil
• Microglia

• DC

• Microglia

• T cell
• Platelet

• B cell
• Platelet

• Neutrophil
• Macrophage
• Microglia

• T cell

CD33-related Siglecs

c Immunoreceptors for comparison

PD1

TREM2

SIRPα

• T cell
• B cell
• Monocyte
• DC

• Microglia
• Macrophage
• DC

• Macrophage
• DC

• Neutrophil
• Monocyte
• Eosinophil
• Basophil

d Signalling cascades

Siglec

Siglecs with basic residues in transmembrane region

PD1

TREM2

SIRPα

• T cell
• B cell
• Monocyte
• DC

• Microglia
• Macrophage
• DC

• Macrophage
• DC

• Neutrophil
• Monocyte
• Eosinophil
• Basophil
Siglecs and downstream signalling. (a) Protein domains of all members of the human (part a) and mouse (part b) Siglec families. The Siglecs can be broadly divided into the conserved Siglecs (sialoadhesin, CD22, myelin associated glycoprotein (MAG) and Siglec-15) and the CD33-related (CD33r) Siglecs that have diverged more recently on the evolutionary timescale. Cell types that express each Siglec are indicated. Double-headed arrows show functional orthologues among CD33r Siglecs. Murine Siglec-E is considered the functional orthologue of human Siglec-5, Siglec-7 and Siglec-9. Siglec-F is the functional paralogue of human Siglec-8, although it is an orthologue of human Siglec-5 and Siglec-6 (dashed line). Siglec-G is the functional orthologue of human Siglec-10. Human Siglec-XII lacks the arginine essential for sialic acid binding and is non-functional. Chimpanzee Siglec-13 was deleted in humans. Signalling domains in the cytoplasmic tails of each protein are depicted as coloured boxes. The immunoreceptor tyrosine-based inhibitory motif (ITIM) sequence is [I/L/V]xYxx[L/V], the ITIM-like sequence is [D/E]xYxx[V/I][R/K], the immunoreceptor tyrosine-based switch motif (ITSM) sequence is TxYxx[V/I], the growth factor receptor-bound protein 2 (GRB2) SH2 binding motif is YxNx and the FYN kinase site is RxxS. Other non-consensus motif tyrosines, such as in murine CD33 and Siglec-F, are not depicted. The GRB2-binding motif in Siglec-10 and Siglec-G is contained within an ITIM. Siglec expression patterns are indicated according to independent reports in the literature. Recent data also suggest that murine T cells express Siglec-E130 and Siglec-G297, and that murine platelets express Siglec-E137.

(b) Siglecs recruit their ligands to deliver ITIMs to the plasma membrane of the responding cell. The ligand, sialylated asparagine linked glycans, anchors to the cell membrane via an alpha-N-acetyl glucosaminyl linkage. The sialylated glycan is then recognized by the Siglec receptor on the cell surface, stimulating a signal cascade that leads to suppression of further immune responses. (c) Domain organization and signalling motifs of other immune cell receptors with known roles in immune modulation, illustrated for comparison. (d) Siglecs with ITIMs and ITIM-like signalling motifs may be phosphorylated by SRC family kinases, thereby enabling the recruitment of the protein phosphatases SRC homology region 2 domain-containing phosphatase 1 (SHP1) and SHP2. The ITIM domains in CD33 and Siglec-7 have also been shown to recruit suppressor of cytokine signalling 3 (SOCS3). Siglec-F contains a protein tyrosine kinase DAP12 binding sites, and is therefore likely to play less of a role in signalling than in adhesion. Siglec activity is associated with immune cell killing, pathogen clearance and cytokine production, and has thus been linked to numerous inflammatory diseases and phenotypes.

Siglecs mediate immune homeostasis. Functionally, the Siglecs are important mediators of immune homeostasis. This biology is enabled by the presence of either immunoreceptor tyrosine-based inhibitory motifs (ITIMs), related immunoreceptor tyrosine-based switch motifs or ITIM-like sequences in the cytoplasmic tails of most Siglecs. Alternatively, in a minority of Siglecs, a basic residue in the transmembrane region couples to the immunoreceptor tyrosine-based activation motif containing adaptor protein DAP12 to initiate signalling. Typically, Siglecs inhibit immune cell activation by recruiting SHP family phosphatases to their ITIM or ITIM-like domains, which suppresses other signalling pathways. Those Siglecs that engage DAP12 transmit activating signals. Sialoadhesin has neither ITIM sequences nor DAP12 binding sites, and is therefore likely to play less of a role in signalling than in adhesion. Siglec activity is associated with immune cell killing, pathogen clearance and cytokine production, and has thus been linked to numerous inflammatory diseases and phenotypes.

As the best studied Siglec, CD22, provides a useful model for understanding Siglec biology. The discovery that CD22 bound to sialylated ligands led to hypotheses that it played a role in cell adhesion. However, CD22 also negatively regulates the B cell receptor, raising the possibility that it suppresses B cells and tempers humoral immunity. Experiments intended to validate this concept produced conflicting results. As expected, CD22 knockout mice exhibited increased reactivity to self-antigens and reduced tolerance. However, knocking out ST6GAL1, the sialyltransferase responsible for manufacturing sialylated ligands for CD22, produced B cells with substantially less reactivity. The paradox was resolved with the finding that CD22 interacts not only with sialosides on target cells in trans but also in cis with sialosides on the membrane of the same B cell. Indeed, CD22 interacts with a2,6-linked sialosides on neighbouring CD22 molecules, thereby sequestering itself away from the B cell receptor in homo-oligosomers. According to this model, recently supported by crystal structures, interaction of a B cell with sialylated...
self-antigen provides contacts for CD22 in \textit{trans} that disperse nano-clusters and permit CD22 association with and inhibition of the B cell receptor. In addition, CD22 is an endocytic receptor that is continually internalized and recycled to the surface\textsuperscript{116}. Therefore, Siglec activity in general is governed by several parameters, including the availability of \textit{cis} and \textit{trans} interactors, the relative affinity for and density of any given ligand, and the rate of internalization and recycling. This remarkable complexity is a hurdle for pharmaceutical development.

\textbf{Siglecs engage tumour sialic acids.} The Siglecs are potentially attractive targets for cancer immunotherapy alongside established checkpoint proteins such as PD1, CTLA4 and SIRP\textsubscript{α}\textsuperscript{117}. Indeed, the presence of ITIM domains in the cytoplasmic tail of many Siglecs alongside their expression on many immune cell subtypes is reminiscent of members of the B7 family of regulatory immune receptors, such as PD1.

The first hints that sialic acids are important in tumorigenesis came from studies in the 1960s, in which increased sialic acid content was observed on malignant cells\textsuperscript{118} and desialylated tumours exhibited reduced engraftment in in vivo models\textsuperscript{119}. Negative results from subsequent human trials, in which sialidase-treated autologous tumour cells were administered as adjuvant immunotherapies, and a lack of mechanistic understanding dampened excitement for targeting tumour

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Modalities_for_Siglec-targeted_therapies.png}
\caption{Modalities for Siglec-targeted therapies. Antibody–drug conjugates target the Siglecs (CD22 and CD33) that are expressed by cancers, including acute myeloid leukaemia and B cell lymphomas. Anti-Siglec antibodies can either agonize or antagonize Siglec activity. Siglec-blocking antibodies can function as antagonists by preventing ligand binding, but many serve as mild agonists by promoting dimerization. Siglec agonist antibodies can dampen immune cell activity and promote apoptosis. Siglec ligand-blocking antibodies antagonize Siglec function via competitive binding to the Siglec ligand. Recombinant Siglec ligands, shown here as a recombinant glycoprotein fused to an antibody Fc domain, could theoretically act as either receptor agonists or antagonists, likely depending on their ability to cluster Siglecs. Siglec-based decoy receptors comprising a soluble Siglec protein bind to and block the set of ligands for any given Siglec. Therefore, they may antagonize Siglec activity when ligand identities are unclear or diverse. Antibody–enzyme conjugates comprise antibodies directed to target cell-specific antigens conjugated to a glycosylating enzyme such as a sialidase. In the case of an antibody–sialidase conjugate, removal of sialic acid destroys Siglec ligands, thereby antagonizing immune cell Siglecs. Glycosyltransferases in circulation or administered therapeutically may use nucleotide sugars released from platelets to alter the glycosylation of siglecs by creating or destroying Siglec binding sites. Nanoparticles such as liposomes bearing Siglec ligands may agonize Siglecs via aggregation or serve as a means for targeted payload delivery. Finally, small-molecule inhibitors, such as the fluorinated sialic acid analogue 3F\textsubscript{α}-Neu5Ac\textsuperscript{154}, of de novo sialic acid synthesis, or the sialyltransferases, the enzymes responsible for linking sialic acid to nascent glycoproteins and glycolipids, may reduce the sialic acid content of the glycoconjugate and destroy Siglec ligands. CMP–Neu5Ac, cytidine monophosphate-N-acetyl-neuraminic acid; HER2, human epidermal growth factor receptor 2 (also known as ERBB2); P, phosphate; UDP–GalNAc, uridine diphosphate-N-acetyl-galactosamine.
\end{figure}
sialic acids\textsuperscript{120}. The observation that desialylated fibrosarcoma cells proliferated slower than their fully sialylated counterparts only in immunocompetent, and not irradiated, mice\textsuperscript{121} reinvigorated the field and clearly established that tumour sialic acids play a role in immune evasion. The Siglecs have emerged as likely mediators of this effect\textsuperscript{1}. Although the association of hypersialylation with cancer was evident, the mechanistic details were opaque. Ligands for Siglec-7 and Siglec-9 were found on various human cancers, and removing sialic acids from cancer cells increased their susceptibility to cytotoxicity from natural killer cells\textsuperscript{122}. Because natural killer cells have a demonstrated role in the early stages of tumorigenesis, natural killer cell activity towards Siglec-7 and Siglec-9 may be an important determinant of tumour engulfment. As a complementary approach, our group synthesized glycopolymers displaying sialylated glycans as mucin mimetics. By decorating tumours with these polymers and observing their ability to protect cells from being killed by natural killer cells, we provided evidence that natural killer cells are directly inhibited by tumour sialosides and that blocking Siglec-7 on natural killer cells removes this inhibition\textsuperscript{123}.

The case for Siglec-mediated immune evasion mounted. Siglec-9, which is broadly expressed on neutrophils, natural killer cells, monocytes, dendritic cells, macrophages and subsets of T cells, also garnered attention. Siglec-9 ligands are upregulated on carcinomas of different histological subtypes and the \textit{rs16988910} SNP in Siglec-9 correlates with improved survival of non-small cell lung cancer patients, although only in the short term (<2 years)\textsuperscript{124}. This study also found that tumours bearing Siglec-9 ligands inhibit neutrophil activation and, surprisingly, prevent macrophage M2 polarization. The function of Siglecs on macrophages is not well defined, as stimulating macrophages with a Siglec-9 ligand comprising the mucin MUC1 decorated with truncated O-glycans polarized these cells towards an immunosuppressive M2 phenotype\textsuperscript{125}. M2 polarization in macrophages in this experimental set-up did not go through the SHP phosphatases, as occurs with most ITIM-containing Siglec signalling, but instead was directed by PI3K activation and calcium influx\textsuperscript{125}. Additional functions for Siglecs on innate immune cells were identified in mouse models of lung adenocarcinoma, in which tumours are infiltrated by neutrophils bearing Siglec-F (a murine homologue to human Siglec-5 and Siglec-8) that remodel the immune microenvironment to promote tumour growth\textsuperscript{126}.

Although Siglecs are not expressed on naive human T cells\textsuperscript{127}, recent evidence suggests that T cells do express, and are negatively regulated by, Siglec-5, Siglec-7, Siglec-9 and Siglec-10 in certain contexts\textsuperscript{128,129}. A key study found that tumour-infiltrating lymphocytes express Siglec-9 and exhibit increased cytotoxicity against MC38 cells that lack sialic acids (GNE-null) or following treatment with a Siglec-9 blocking antibody fragment\textsuperscript{130}. In the same study, mice with subcutaneous MC38 tumours demonstrated improved survival when they lacked Siglec-E, the mouse homologue of human Siglec-9, or when their Siglec-E locus was engineered to replace the inhibitory cytoplasmic domain of Siglec-E with the activating motifs of Siglec-16 [REF.\textsuperscript{130}]. Regulation of T cell activity by Siglec-9 was also found in melanoma\textsuperscript{131}.

Microbiology has also provided evidence of Siglec-mediated immune evasion. Neutrophils are inhibited by capsular sialic acids on group B \textit{Streptococcus} (GBS) strains via Siglec-5 and Siglec-9 [REFS\textsuperscript{125,126}]. GBS also inhibits natural killer cell sentinel activity by engaging Siglec-7 through sialic acid-independent contacts with the β-protein of GBS\textsuperscript{134}. Hyaluronan in the capsule of group A \textit{Streptococcus}, as well as host hyaluronan, inhibits neutrophils via Siglec-9 [REF.\textsuperscript{135}]. Intriguingly, hyaluronan also has a recognized role in shaping the tumour microenvironment in breast, prostate, ovarian and lung cancers\textsuperscript{136}. Even the Siglecs on platelets are exploited by pathogens. α2,3-Linked capsular sialic acids on GBS promote virulence by binding platelet Siglec-9 and inhibiting degranulation\textsuperscript{137}. Given the intense selective pressure exerted by the immune system, it seems likely that pathogens and cancers might converge on similar strategies to avoid detection and clearance.

Our understanding of the mechanisms behind Siglec-mediated immune evasion is rapidly improving. Siglec-10, known previously only as a promotor of B-cell homeostasis, recently garnered attention as a negative regulator of macrophage-mediated phagocytosis. Siglec-10 functions in parallel to the SIRPα–CD47 axis by binding to its ligand, CD24, on breast and ovarian cancer cells\textsuperscript{138}. The CD24 blocking antibody developed in that study enhanced phagocytosis of CD24-positive tumour cells and could be developed into a new cancer immunotherapy.

The guiding hypothesis on Siglecs in cancer has been that immune cells bearing Siglecs are inhibited upon binding to their sialoside ligands on target cells. This proposition was recently upended by the discovery that Siglec-15, which contains a DAP12 binding site and is considered a conserved Siglec, is present on both tumour-infiltrating myeloid cells and tumour cells. In human cancers, Siglec-15 is abundant and mutually exclusive with PDL1 across various tumour types; mouse models show that high Siglec-15 levels increase tumour growth and decrease T cell infiltration\textsuperscript{139}. The identity of the Siglec-15 ligand has not been elucidated, and it is not clear whether Siglec-15 on the tumour or infiltrating antigen-presenting cells are responsible for these effects. Nevertheless, evidence that treatment with a Siglec-15 blocking antibody decreased tumour burdens launched a phase I/II trial (NCT03665285) of the anti-Siglec-15 antibody, NC318, that is currently ongoing.

\textbf{Identification of Siglec ligands.} If Siglecs are analogous to checkpoint proteins, then what are their tumour cell ligands — the functional analogues of PDL1 and CD47? Likewise, what transcriptional and metabolic programmes are active in tumour cells that lead to Siglec ligand expression? This information may provide clues for how to interfere with the sialoside–Siglec axis of immune evasion, and inform potential therapy options for patients. The Siglec ligands used by healthy cells to support normal physiology and homeostasis may also be different from those observed in pathological states.
such as cancer. Moreover, any given Siglec may bind various ligands with high affinity. For instance, Siglec-7 has high affinity for both the sialylated ganglioside GD3 (ref.143) and N-linked disialyl Lewisα in the normal colonic epithelium. The heavily glycosylated protein galectin-3-binding protein (LGALS3BP) is one Siglec-9 ligand on cancer, but several other physiological ligands such as MUC1 (ref.125), disialyl Lewisα (ref.140) and gangliosides have been described. Analogous to the manner in which both glycan and protein portions of PSGL1 form a molecular surface recognized by P-selectin, Siglec ligands are likely defined by particular glycans and the context in which they are presented. In support of this notion, paired immunoglobulin-like type 2 receptor-a (PILRa) and PILRβ are receptors that modulate immune cell function, interact with sialic acid and have Siglec-like folds. A crystal structure of PILRa in complex with a mucin glycopeptide revealed critical contacts to both the O-glycan and peptide backbone.

Glycan array data generated by flowing recombinant lectins over collections of glycans printed onto glass slides provide a useful starting point for understanding these interactions, but do not adequately recapitulate the presentation of glycans in the context of particular protein and lipid scaffolds or capture the complexity of Siglec–ligand interactions on the native cell surface. Multivalent Siglec–Fc constructs have recently improved the detection of Siglec ligands on cells and tissues. Other approaches to identify Siglec ligands include proximity labelling and glycan library screening against Siglec reporter cells. Strategies to manipulate Siglec–ligand interactions based on metabolic glyco-engineering are useful tools to study Siglec function. Additional approaches to elucidate natural Siglec ligands and the mechanisms leading to their production are nevertheless needed.

### Siglecs-targeted therapies for cancer

Therapies can either target the Siglec or inhibit the Siglec ligand (Fig. 5). The most straightforward approach is to create a Siglec blocking antibody, and several companies are pursuing this strategy (see below). One obstacle is the challenge of identifying purely blocking antibodies that do not agonize the Siglec via dimerization. A second strategy is to remove the Siglec ligand. When the ligand is known, it may be blocked: for example, anti-CD24 antibodies were used to prevent interactions with Siglec-10 and promote tumour phagocytosis. For Siglecs with poorly characterized ligands, blockade is not an option. Instead, Siglec interactions can be inhibited by complete desialylation. For example, treating B16F10 melanoma with peracetyl-3Fα-hepialyl Neu5Acα inhibited sialylation, reduced growth and migratory capacity in vivo.

Our group adopted the complementary strategy of targeted degradation. We engineered antibody–enzyme conjugates comprising an antibody directed against a tumour antigen such as HER2 and a broad-acting sialidase capable of complete target cell desialylation. The first-generation antibody enzyme conjugate, consisting of an anti-HER2 antibody (trastuzumab) conjugated to a bacterial sialidase, removed Siglec ligands on breast cancer cells, boosting natural killer cell antibody-dependent cellular cytotoxicity against resistant breast cancer lines in vitro. Second-generation antibody enzyme conjugates were designed to improve biocompatibility and stability in vivo by utilizing a human neuraminidase and transitioning from acid-labile amine oxide linker chemistry to a more secure linker synthesized via Pictet–Spengler ligation. Palleon Pharmaceuticals was formed on the back of these results, and has further developed reagents to analyze and edit the cancer cell glycocalyx.

Whereas sialidases are an obvious choice to remove Siglec ligands, other glycocalyx-modifying enzymes may present therapeutic opportunities. For instance, bacterial mucinases can cleave the cancer-associated mucins MUC1 and MUC16 (ref.158). One major advantage of using broad-acting hydrolases to remodel the glycocalyx is that the ligands for several Siglecs may all be targeted simultaneously using a single therapeutic. Infusion of soluble glycosyltransferases could also theoretically take advantage of activated nucleotide sugars released by platelets at sites of inflammation to remodel the glycocalyx in situ.

### Siglecs in autoimmunity and inflammation

The Siglecs control inflammation in various pathologies ranging from autoimmunity and allergy to sepsis. Siglec agonists and improved survival in mouse models of sepsis and acute respiratory distress syndrome. Recombinant Siglec ligands also show potential as Siglec agonists. The best example of this approach comes from the growing recognition of Siglec-10 as an important regulator of innate immune responses. In particular, dendritic cell Siglec-10 reduced responses to damage-associated molecular patterns by interacting with CD24 (ref.169). Subsequent research found that recombinant CD24 could engage Siglec-G to prevent deleterious inflammation in a mouse model of...
Siglecs in neuroimmune homeostasis. Sialic acids are critical regulators of phagocyte homeostasis in the brain, where they are abundantly incorporated into gangliosides and α2,8-linked polysialic acids, which are linked to glycoproteins such as neural cell adhesion molecule and α2,8-linked polysialic acids, which are linked where they are abundantly incorporated into gangliosides and α2,8-linked polysialic acids, which are linked to glycoproteins such as neural cell adhesion molecule sides and α2,8-linked polysialic acids, which are linked.

Antibody-based Siglec agonists have developed alongside the recognition that Siglecs comprise an important node for eosinophil and mast cell regulation. An agonist antibody targeting murine Siglec-F reduced eosinophilic inflammation and tissue remodelling. In humans, eosinophil apoptosis is promoted by Siglec-8 agonism and mast cells are suppressed by Siglec-7 and Siglec-8 activity. These findings led Allakos to develop lirulentimab (AK002), a monoclonal antibody recently shown to be effective in treating eosinophilic gastritis and duodenitis, that is currently being tested in several additional phase II and III clinical trials for other allergic conditions such as chronic urticaria (NCT03436797) and eosinophilic gastroenteritis (NCT03664960).

Outlook for Siglec-directed therapies. Currently, the only FDA-approved Siglec-targeted therapies are ADCs against CD22 and CD33, which use the Siglec as a tumour-specific antigen to identify target cells. Moving forward, it is likely that the importance of the Siglecs in regulating inflammation in normal physiology and disease will be increasingly recognized as our understanding of Siglec biology improves. Clustering is a key principle to consider when developing therapies that modulate Siglec activity. Dimerization is generally agonistic, as crosslinking antibodies that target ITIM domain-containing Siglecs inhibit immune cell activity. Likewise, glycomimetic Siglec ligands displayed on a multivalent scaffold, such as a nanoparticle, liposome or polymer, can aggregate Siglecs and act as receptor agonists.

The apparent requirement for Siglec aggregation to promote agonism must be balanced against induced internalization, which is the mechanism of action for intracellular delivery of cargo-bearing liposomes decorated with Siglec ligands, and an antagonistic antibody targeting Siglec-15 on osteoclasts.

Perhaps the greatest need in this area is a catalogue of biologically relevant ligands for each Siglec and the glycoproteomics and glycolipidomics tools to detect them on tissues of interest. Several therapies directed at Siglec function are nevertheless in advanced development by biotechnology and pharmaceutical companies, many of which will move towards clinical trials. Based on patent literature, in addition to those already mentioned, Innate Pharma’s Siglec neutralizing antibodies for cancer, Medimmune’s anti-Siglec-15 antibody for leukaemia, Alethia’s anti-Siglec-15 antibody for osteogenesis imperfecta, Octapharma AG’s Siglec-modulating...
glycopeptides, Cellectis’ Siglec null CAR T cells and Onkimmune’s Siglec null natural killer cell therapies are all worth watching.

Glycan-targeted antibodies, vaccines
Glycans can be neoantigens that are useful for diagnosis and therapy of cancer and other inflammatory diseases. Indeed, glycans can be convenient targets, and many common vaccines target the polysaccharides displayed by pathogens, such as Pneumovax and Prevnar for *Streptococcus pneumoniae*, Menactra and Meningitis B for *Neisseria meningitidis* and Synflorix for *Haemophilus influenzae*. Nevertheless, most therapeutic antibodies target proteins. The reason for this is not the lack of suitable glycan neoantigens but, rather, practicality. Three main challenges confront the use of mammalian glycans as therapeutic targets. First, tolerance must be broken because many of these glycans are expressed during embryonic development or are present on normal adult tissue at low levels. Second, stimulation of an efficient response to a glycan immunogen requires a functional population of marginal zone B cells or conjugation to protein because the glycan alone is considered a T cell-independent antigen and does not stimulate a robust T cell response. Last, producing complex glycans is a synthetic challenge.

These hurdles can be, and have been, overcome. As antibodies targeting glycans are developed, we must keep in mind that antibodies are themselves glycylated. The glycoforms decorating the Fc domains of IgG regulate binding to Fcγ receptors, thereby modulating antibody effector functions and contributing to inflammatory pathologies. Glycosylation is an important consideration when developing a new therapeutic antibody, and antibodies can be glycoengineered (reviewed elsewhere).

In this section, we review glycan neoantigens in cancer and strategies deployed to target them, including vaccines, monoclonal antibodies and CAR T cells.

Glycosylation is a hallmark of disease. Altered glycosylation is a hallmark of disease. In numerous inflammatory conditions, patients develop antibodies to carbohydrate epitopes, and these antibodies are potential biomarkers. For instance, anti-glycan antibodies have been detected that are characteristic of multiple sclerosis or Crohn’s disease. Patients with systemic sclerosis have antibodies directed against sulfated LacNAc. In this population, sulfation in particular was found to be critical for immunogenicity, suggesting more broadly that the glycans against which these diagnostic antibodies are directed may contribute to disease pathogenesis. Dysregulated glycosylation is particularly characteristic of cancer. Indeed, tumour-associated glycoproteins have utility as biomarkers to monitor cancer progression and response to therapy. For instance, the ovarian tumour marker CA125 is a mucin (MUC16) decorated with high mannosetype N-glycans, the colon cancer marker carcinoembryonic antigen (CEA) is a sialofucosylated GPI-anchored protein and the pancreatic cancer biomarker CA19-9 is sLeα conjugated to highly glycosylated bottlebrush-shaped proteins known as mucins.

In all cases, pathological glycosylation is a direct result of dysregulated metabolic pathways. Common motifs are reversion to an embryonic state, glycan truncation and extended branching. Although most mammalian cells display O-linked glycans that have been elaborated from their simple GalNAc base to longer and more complex architectures, mucinous carcinomas frequently display short, truncated glycans. In many of these cases, glycan truncation has been linked to mutations in COSMC, a chaperone necessary for folding of glycoprotein-N-acetylgalactosamine 3-β-galactosyltransferase 1 (C1GALT1). Cancers also frequently display more high mannosetype N-linked glycans produced from increased branching. We are only beginning to fully understand the functional consequences of altered glycosylation in health and disease, but these phenotypes have been linked to immune evasion, metastasis, cell survival and growth factor receptor signalling.

Tumour vaccines. The rationale for a tumour vaccine is to harness the power of the immune system to identify and eliminate malignant cells bearing certain antigens. To target mammalian glycans in practice, strategies for breaking self-tolerance and encouraging a T cell response have ranged from administration of multivalent antigens, conjugation of glycans to immunogenic carrier proteins such as keyhole limpet haemocyanin (KLH) and addition of vaccine adjuvants such as QS-21. Development of vaccines against tumour-associated carbohydrates stems from the observation that the gangliosides GM2 and GD2 were among the most commonly recognized antigens in patients’ sera following administration of a whole-cell melanoma vaccine as adjuvant immunotherapy. A GM2-specific vaccine was prepared by isolating the ganglioside from natural sources. This GM2 was poorly immunogenic in isolation, although a combination with Bacillus Calmette–Guérin stimulated antibody production in melanoma patients and improved disease-free survival. The duration of response and antibody titres were nevertheless low, thus spurring the replacement of Bacillus Calmette–Guérin with KLH and the addition of the adjuvant QS-21 in an attempt to augment IgG titres. Unfortunately, in a phase III trial (NCT00005052) of GM2–KLH with QS-21 in patients with stage II melanoma, the vaccine lacked efficacy and the trial was stopped. Although the study’s authors surmise that the vaccination schedule was ineffective, it is possible that the monovalent GM2 used in the trial was unable to stimulate a vigorous
immune response. One simple contributor to the failure was the low percentage of patients who developed high antibody titres against GM2.

Vaccines against other carbohydrate antigens were propelled by improved methods for organic synthesis of glycans. Low abundance of some tumour-associated carbohydrate antigens in natural sources coupled with the challenges of purifying a glycan of interest from heterogeneous mixtures hampered isolation of material from natural sources. These new synthetic methodologies, namely glycal assembly, enabled the creation of KLH conjugate vaccines against the glycolipid globo H in breast cancer212, fucosyl-GM1 (Fuc-GM1) in small cell lung cancer213 and Lewis’ (Le*) in ovarian cancer214. The efficacy of these vaccines was mixed. In order for a carbohydrate-based vaccine to be an effective therapy, it must consistently generate high titres of high-affinity anti-glycan antibodies in patients, and these monomeric vaccines tended to produce only moderate IgM and poor IgG titres in immunized patients. For instance, a phase II/III trial (NCT01516307) of the globo H vaccine in breast cancer patients did not improve progression-free survival but did offer a benefit to patients who developed high IgG titres215. A phase III trial (NCT03562637) of the same vaccine in triple-negative breast cancer is currently underway.

Even vaccines capable of inducing high IgG titres are not guaranteed success. The mucin MUC1 is differentially glycosylated in cancer, which makes it an attractive target216. In preparing their MUC1 peptide–KLH conjugate for use in breast cancer, one group was inspired by the notion that truncating the glycans on cancer-associated MUC1 [REF.217] might lead to greater exposure of the peptide backbone, and therefore left all of the glycans off their immunogen. Although this strategy generated high IgM and IgG titres, the ability of these antibodies to bind tumour MUC1 in vivo was limited218. Another MUC1 peptide, administered in conjunction with the TLR3 agonist poly-ICLC219, is in clinical trials for the prevention of colorectal adenoma and lung cancer. Although preliminary results (NCT02134925) suggest that the vaccine generates anti-MUC1 antibodies, the capacity for this immune memory to prevent disease progression is unknown220. Euthics is pursuing a complementary strategy in which colon and breast cancer patients are administered modified adenovirus coding for tumour-associated antigens, including MUC1 and CEA221. The vaccine appears to generate T cells that are reactive to MUC1 and CEA (NCT03384316), although in vitro analysis suggests that the MUC1 produced by the virus is not glycosylated and its efficacy in reducing disease remains to be seen222.

MUC1 decorated with sialyl-Tn (sTn), a truncated O-glycan comprising Neu5Acα2–6GalNAc, could predict prognosis in ovarian cancer, which inspired an sTn–KLH conjugate vaccine (Theratope)223. In breast cancer patients, Theratope promoted the generation of anti-sTn antibodies224, and the presence of these antibodies correlated with improved survival225. Ultimately, a phase III trial (NCT00003638) of Theratope revealed no benefit in metastatic breast cancer patients despite high IgG titres in patients receiving the vaccine226. One possible explanation for failure is that patient eligibility was not determined by tumour sTn expression.

Glycan multivalency may be the most promising path forward. A heptavalent vaccine comprising a mixture of carbohydrate–KLH conjugates induced a moderate humoral response227, and a second-generation pentavalent vaccine comprising the glycan portions of globo H, GM2, Tn, and sTn antigens (Galβ1–3GalNAc) linked together on a single scaffold increased IgG titres to these antigens in a recent phase I trial (NCT01248273)228. Taking an alternative approach, a vaccine comprising a carbohydrate mimetic peptide conjugated to the pan-HLA DR binding epitope (PADRE) peptide T cell agonist generated antibodies against both Le* antigen and GD2, and had a potential benefit for breast cancer patients in a phase I trial (NCT01390064)229,230. A bivalent vaccine comprising GD2 and GD3, stabilized as lactones, conjugated to KLH and injected in combination with the immunostimulants OPT-821 (a QS-21 equivalent) and β-glucan (a Mac-1 ligand), is the subject of an ongoing phase II trial (NCT00911560) for high-risk neuroblastoma231. Going full circle, whole-cell vaccines, the ultimate in multivalency, were again recently shown to generate antibodies against carbohydrate antigens, although they are likely only effective when the glycan neoantigen is expressed at very high levels232.

Glycan-directed passive immunotherapy. Therapies based on anti-glycan antibodies avoid many of the challenges facing active immunotherapy. Among the first anti-glycan therapeutics to be successfully translated to the clinic was the anti-GD2 antibody dinutuximab, currently FDA-approved for treatment of high-risk paediatric neuroblastoma233. The lead molecule that eventually produced dinutuxumab was not created with a glycan in mind, however; the primary goal was to develop any antibody specific to neuroblastoma. Two independent groups immunized mice with human whole-cell neuroblastomas, isolated B cell clones and screened for reactivity against neuroblastoma antigens234–236. Remarkably, the most selective and highest-affinity antibodies isolated by each group targeted the disialoganglioside GD2, highlighting the prevalence of gangliosides on cancer and the utility of targeting tumour glycans. Humanization of one of these clones yielded the ch14.18 antibody, which became dinutuximab237. A landmark phase III trial (NCT02641782) trial of ch14.18 in paediatric neuroblastoma revealed that GD2-targeted immunotherapy improved overall survival238.

Likewise, other promising anti-glycan antibodies have emerged to complement the carbohydrate vaccine programmes described above. The common ganglioside GM1 has only been detected in its fucosylated form in small cell lung cancer, and this has garnered interest in using Fuc-GM1 monoclonal antibodies in this disease. However, Fuc-GM1–KLH conjugate vaccines generated only a low-affinity IgM response in patients239. Nevertheless, data suggesting that Fuc-GM1 antibodies could inhibit tumour engraftment in mice and promote complement dependent cytotoxicity240 led Bristol–Myers Squibb to develop a fully human anti-Fuc-GM1 antibody
(BMS-986012) for use in small cell lung cancer\textsuperscript{241}. Results from the clinical trial (NCT02247349) were expected in late 2020. Similarly, antibodies directed against globo H inhibit angiogenesis and promote antibody-dependent cellular cytotoxicity against globo H-positive tumours of various backgrounds\textsuperscript{242,243}. These results encouraged a phase I/II trial (NCT03573544) of the globo H antibody OBI-888 and a phase I/II trial (NCT04084366) of OBI-999, an ADC based on the same antibody\textsuperscript{244}, in patients with solid tumours. Both trials are underway.

Among glycoproteins, the mucin MUC1 has perhaps received the most attention as a target for cancer immunotherapy. In mucinous adenocarcinomas, MUC1 is abundantly expressed and aberrantly O-glycosylated with short glycans including sTn, Tn and T antigen. These MUC1 glycoforms are not displayed on healthy tissue\textsuperscript{245}, and their expression correlates with adverse outcomes in numerous tumours\textsuperscript{246,247}. A potent and specific monoclonal antibody, 5E5, was isolated from mice immunized with MUC1 Tn glycopeptide–KLH conjugates\textsuperscript{248}. Intriguingly, the MUC1 epitopes recognized by 5E5 and other B cell clones isolated from these mice map to the same tandem repeat region, suggesting the existence of immunodominant glycopeptides\textsuperscript{249}. The 5E5 anti–Tn-MUC1 antibody bound to human breast cancer lines and induced antibody-dependent cellular cytotoxicity.

The broad and selective expression of Tn-MUC1 on cancer made it an attractive target in attempts to generate a universal CAR T therapy with utility in various tumour types. Using the 5E5 antibody as a starting point, one group generated a Tn-MUC1 CAR T cell that demonstrated efficacy in mouse models of leukaemia and disseminated pancreatic cancer\textsuperscript{250}. CAR T cells against the disialoganglioside GD2 have also advanced to human trials in neuroblastoma\textsuperscript{251}. Indeed, GD2-targeted CAR T cells can cross the blood–brain barrier, thereby permitting the treatment of aggressive cancers such as midline gliomas\textsuperscript{252}.

**Theranostics.** As additional glycan neo-epitopes are identified, one of the most promising avenues for translation of these findings comes from the field of theranostics, in which a diagnostic tool is paired to a therapy. Pancreatic ductal adenocarcinoma is an aggressive tumour that is exceptionally lethal, partially because it is diagnosed late. CA19-9 (mucin decorated with sLe\(\alpha\)) is the most highly expressed tumour antigen on pancreatic ductal adenocarcinoma and its display on breast and colon cancer correlates with more aggressive tumours\textsuperscript{253,254}. sLe\(\alpha\) was packaged into a KLH conjugate vaccine and administered to metastatic breast cancer patients (NCT00470574), with results expected in 2021 \textsuperscript{[REF 255]}. Anti-glycan antibodies were isolated from one of the patients receiving the experimental vaccine and validated for binding to CA19-9 \textsuperscript{[REF 256]}. A phase I trial (NCT02672917) of one of these antibodies (5B1; MVT-5873) in CA19-9-positive malignancies is ongoing. To facilitate monitoring of CA19-9\(^\text{+}\) tumours in vivo, Lewis and colleagues adapted the 5B1 antibody into a probe for positron emission tomography\textsuperscript{257} and near-infrared fluorescent optical imaging (NCT02687230)\textsuperscript{258}. Recently, they used a tetrazine ligation between a \textsuperscript{225}Ac radioligand and a trans-cyclooctene-labelled anti-CA19-9 antibody to transform the diagnostic antibody into a potent α-particle-emitting radiotherapeutic\textsuperscript{259}. Results from a phase I trial (NCT03118349) on a matching β-particle-emitting \textsuperscript{177}Lu-labelled version of the antibody (MVT-1075)\textsuperscript{260} are expected soon. As additional highly specific anti-glycan antibodies are generated, theranostics may be a rapid way of translating discoveries to the clinic and validating targets by ensuring that only patients most likely to benefit from a therapy are treated.

**Outlook.** The human glycome is dynamic and the functions encoded by its structures are poorly understood. The glycome contains a wealth of information that correlates with cell and organism status, and, in many cases, particular glycans also perform essential functions. Uncovering this information, integrating it with existing data and collating it into useful models is a formidable task. Nevertheless, a cadre of persevering glycobiologists has already produced several drug candidates and shown that the field is ripe to produce more (FIG. 7). Although outside the scope of this Review, one exciting area to watch is galectin biology. The galectins are a family of β-galactoside binding proteins involved in diverse aspects of cell physiology, and several galectin-targeted therapies have entered clinical trials for indications including cancer, cardiovascular disease and fibrotic disease (reviewed elsewhere\textsuperscript{261,262}).

Major challenges still remain. Most importantly, we do not yet have a full portrait of the glycome in health and disease. Improvements in our ability to detect altered glycosylation will catalyse the development of glycan-targeted therapeutics and increase the number of useful glycan biomarkers. A necessary first step already being taken is to develop a greater appreciation for the ‘dark matter’ of biology, including everything outside DNA, RNA and proteins\textsuperscript{263}. Indeed, awareness that glycans decorate many medically relevant biomolecules is burgeoning, as is recognition that even those molecules that have already been the subject of hundreds of clinical trials, such as PDL1, are glycosylated\textsuperscript{264}. GlyGen, GlyCosmos and glycan profiling and array data from the Consortium for Functional Glycomics are extremely valuable resources, and are not yet complete. We should strive to generate comprehensive information on glycosylation of proteins and lipids across various tissues, cell types and disease states that can be put at the fingertips of all biologists so that answering a question about glycosylation is no more difficult than looking up a gene expression data set.

How can we work towards this goal? A robust method for sequencing glycans and simultaneously identifying their point of attachment, whether to a particular site on a protein or a specific lipid, would advance the field substantially. In the absence of a technology for glycan sequencing, the next-best option for glycan and glycosite identification is mass spectrometry-based glycoproteomics. Glycoproteomics can provide detailed portraits of the glycans on a few predefined proteins, but cannot yet be used to reliably survey and quantify
The entire glycoproteome. Advances in glycomics and glycoproteomics have made analyses more robust, reproducible and feasible on small sample quantities, but single-cell analysis is currently out of reach. Indeed, radical simplification of the glycome with SimpleCell technology, which requires engineering cells to express only a single glycan, has been the only way to map glycosites on a proteome-wide scale.265,266.
Increased availability of reagents for glycan detection will also accelerate biological discovery. In addition to the mammalian vaccination strategies discussed above, high-affinity anti-glycan antibodies have been generated in lamprey262 and avian263 hosts. Glycan processing enzymes can be converted into binding reagents, as has been done by Lectenz264. Plant or microbial lectins could also be evolved for high specificity to a particular glycan of interest.

The most obvious application of the resulting increased understanding of glycobiology will be biomarker discovery. We are also likely to discover novel biology as we develop richer, more detailed data sets. For example, we are sure to find more examples of glycans that are important modulators of protein function and drivers of disease265. Ligand discovery for glycan-binding proteins such as the Siglecs and galectins is a particularly fertile area for translational medicine. Chip-based glycan arrays have enabled the screening of lectins against large libraries of synthetic glycans. Laser-based array synthesis technology is an important advance in this area as it enables on-chip glycopeptide synthesis at defined densities265. Cell-based glycan arrays go a step further, as lectin binding is observed on a native lipid bilayer, at appropriate glycan densities and in the context of other proteins266,267. Cell-based arrays also hold promise for unravelling the non-templated nature of glycan synthesis and glycosyltransferase function when paired with glycoproteomics analyses. As genome-wide CRISPR screens become increasingly common for identification of novel biology, we are certain to see ‘glycogenes’ implicated in more areas.

Among the most promising therapeutic agents in the pipeline are biologics targeting glycan binding proteins, such as the P-selectin blocking antibody crizanlizumab and Next Cure’s Siglec-15 blocking antibody. These agents benefit from the ease of manufacturing and favourable pharmacokinetics of monoclonal antibodies, while avoiding the challenges of targeting glycans directly. For glycan or lectin blocking reagents, the most effective designs will recognize that glycan and protein together make a continuous molecular surface that is read by glycan binding proteins (as occurs in the P-selectin–PSGL1 interaction). Finally, the development of glycomimetics with drug-like properties will be advanced by structure-guided design incorporating multiple fragments and binding sites, an approach that was recently showcased268. The groundwork has been laid for an exciting expansion of therapeutic opportunities in glycobiology.

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Author contributions

The authors contributed to all aspects of the article.

Competing interests

C.R.B. is a co-founder of Redwood Bioscience, Enable Biosciences, Palloen Pharmaceuticals, InterVenn Bio, Lycia Therapeutics and OliLux Biosciences, and a member of the Board of Directors of Eli Lilly. B.A.H.S. is a shareholder of GlycoMimetics.

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