OPINION

The tumour glyco-code as a novel immune checkpoint for immunotherapy

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Abstract | Tumour growth is accompanied by tumour evasion of the immune system, a process that is facilitated by immune checkpoint molecules such as programmed cell death protein 1 (PD1). However, the role of tumour glycosylation in immune evasion has mostly been overlooked, despite the fact that aberrant tumour glycosylation alters how the immune system perceives the tumour and can also induce immunosuppressive signalling through glycan-binding receptors. As such, specific glycan signatures found on tumour cells can be considered as a novel type of immune checkpoint. In parallel, glycosylation of tumour proteins generates neo-antigens that can serve as targets for tumour-specific T cells. In this Opinion article, we highlight how the tumour 'glyco-code' modifies immunity and suggest that targeting glycans could offer new therapeutic opportunities.

The immune system provides selective pressure on malignant cells and imprints increased fitness on traits that favour immune evasion; this phenomenon is known as immunoediting and leads to the emergence of more aggressive tumours¹. Combined with the high mutational rate, which enables high genetic variation among individual cells in the tumour, evolved tumours are inherently immunosuppressive². Therefore, it is not surprising that tumours frequently express molecules that inhibit effective antitumour immune responses, with examples including anti-inflammatory cytokines and chemokines that promote the infiltration and activity of suppressive immune cell populations^{3,4}. Furthermore, tumours express immune checkpoint molecules (such as programmed cell death 1 ligand 1 (PDL1) and CD155) that engage inhibitory receptors on lymphocytes (in this case, programmed cell death protein 1 (PD1) and T cell immunoreceptor with Ig and ITIM domains (TIGIT), respectively), and blocking these interactions has become an effective paradigm of cancer immunotherapy5.

It is well documented that glycans on cell surface glycoproteins and glycolipids are fundamentally altered in tumour cells and that tumour cells consequently have a different 'glycan coat' than healthy cells. Because immune cells express a large variety of glycan-binding receptors called lectins, they can sense and respond to changes in the glycan signature of their environment; this often leads to the induction of inhibitory immune processes. This induction can take place by overexpressing self-glycan structures to limit self-reactive responses by the immune system or by newly expressing glycan structures that can dampen effector T cell functions. Glycans such as sialic acids have been referred to as 'self-associated molecular patterns' (SAMPs) that are recognized by intrinsic inhibitory receptors, and they maintain the baseline non-activated state of the innate immune system and dampen its reactivation following an immune response; as such, these molecules counteract pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs)⁶(FIG. 1). Thus, tumour cell transformation causes aberrant glycosylation in tumour cells, which, in turn,

allows for new connections with immune cells that can actively suppress anti-tumour immunity. Glycan-binding receptors have been widely studied in the context of innate pathogen recognition and immune evasion and are selectively expressed by different subsets of both lymphoid and myeloid immune cells⁷. Cancer cells exploit glycans in a similar manner to pathogens. For example, by using host-like glycans or by expressing glycans that play a crucial role in the resolution phase of immunity, pathogens and cancer cells disguise themselves, hijacking the immune system for their own benefit. Furthermore, hijacking of glycan responses can promote immune evasion by modifying antigen-presenting cell functions, by driving the differentiation of tumour-associated or anti-inflammatory M2 macrophages and by altering T cell differentiation and natural killer (NK) cell activity7. For this reason, deciphering the specific glycan signature of tumour cells, which has been referred to as the 'glyco-code', is important to understand how glycan-lectin circuits drive immune suppression in the tumour microenvironment⁸. In this Opinion article, we review our current knowledge and understanding of how the cancer glyco-code dictates immune-suppressive circuits in the tumour microenvironment and discuss the potential use of the glyco-code in the development of new diagnostic and therapeutic strategies.

The glyco-code of cancer

The glycobiology of cancer has been studied in detail in relation to tumour growth and metastasis9. However, how tumour glycosylation affects immune cell activity within the tumour microenvironment is an emerging research topic. Glycan structures are the product of the cooperative action of multiple enzymes that are capable of catalysing the addition or removal of specific glycans covalently bound to proteins or lipids (BOX 1). Tumour-associated glycans, such as sialylated structures, Tn antigen (a single residue of N-acetylgalactosamine linked to the serine or threonine of a protein by a glycosidic bond) and Lewis antigen (BOX 1), are frequently part of membrane-bound or secreted tumour proteins, for example,

mucins (such as mucin 1 (MUC1)), carcinoembryonic antigen (CEA; also known as CEACAM5) and CD43. Additionally, tumour-associated glycans can also be attached to membrane lipids, as is the case for the gangliosides disialoganglioside 1 (GD1), monosialic ganglioside 2 (GM2) and GM3⁹. Malignant transformation of cells is accompanied by changes in the expression of genes involved in glycan synthesis, such as glycosyltransferases and glycosidases, which are often driven by transcription factors, genetic and epigenetic changes, altered metabolism or environmental cues. For example, hypoxia-inducible factor 1 α (HIF1 α) has been shown to induce the expression of glycosyltransferases and sugar transporters in tumour cells, creating a unique hypoxia-driven glyco-code¹⁰. In addition, epigenetic silencing during cancer development can lead to hypermethylation of core 1 β 3-galactosyltransferasespecific molecular chaperone (COSMC; also known as C1GALT1C1), which is key for the elongation of *O*-glycans, and this directly induces oncogenic features, such as increased tumour



Figure 1 | **Glycosylation changes in cancer that connect to immune recognition.** During malignant transformation, cancer cells can display multiple glycan structures or express different lectin receptors (such as galectins) that are absent in normal tissues. The figure depicts several glycan structures commonly found in tumours that can be attached to a glycoprotein or a glycolipid. These glycan structures can consist of one or more carbohydrates as sialic acid end-standing glycans, *N*-acetylgalactosamine (GalNAc (for example, Tn antigen)) glycans or Lewis X and/or Y glycans that contain fucose (left part of figure; see also BOX 1). These tumour-associated glycans can bind the sialic acid-binding immunoglobulin-like lectins (SIGLECs), macrophage galactose-specific lectin (MGL) or dendritic cell (DC)-specific ICAM3-grabbing non-integrin 1 (DC-SIGN) expressed on immune cells, including monocytes, macrophages, DCs, tumour-associated macrophages (TAMs) and natural killer (NK) cells. This binding can result in increased production of anti-inflammatory cytokines, decreased production of Inflammatory cytokines, decreased NK cell activity or the induction of T helper 2 (T_H2) and regulatory T (T_{reg}) cells. The malignant transformation of cancer cells may also result in the secretion of galectins (right part of figure). These galectins bind *N*-acetyl-D-lactosamine (LacNAc) structures on immune cells such as DCs, T cells or NK cells, promote T_H cell differentiation and induction of tolerogenic DCs, TAMs and myeloid-derived suppressor cells (MDSCs), and reduce NK cell antitumour activity. GD, disialoganglioside; GM3, monosialic ganglioside 3; tDC, tolerogenic dendritic cell; T_{FH}, T follicular helper cell; TNF, tumour necrosis factor; T_R1, type 1 regulatory T cell.

cell proliferation and invasiveness^{11,12}. The intracellular localization of these enzymes also determines the expression of the specific glycans displayed by the cancer cell. For example, polypeptide N-acetylgalactosaminyltransferase 1 (GALNT1), a glycosyltransferase important for the initiation of the mucin-type O-glycosylation that is normally restricted to the Golgi apparatus, can re-localize to the endoplasmic reticulum and induce an increase in truncated O-glycan structures, thereby favouring tumour growth^{13,14}. Because of the genetic heterogeneity within the tumour, all these processes can coexist while being spatially separated within the same tumour, supporting immunosuppressive niche formation.

Glycosylation and cancer immunity

Several studies have shown that lectin receptors (for example, sialic acid-binding immunoglobulin-like lectins (SIGLECs), macrophage galactose-specific lectin (MGL) and dendritic cell (DC)-specific ICAM-3-grabbing non-integrin 1 (DC-SIGN; also known as CD209)) expressed by immune cells mediate immune suppression by responding to the tumour glyco-code (FIG. 1). The carbohydrate Lewis antigens (BOX 1) that are attached to CEA, which is commonly detected on colon cancer cells, bind to the C-type lectin DC-SIGN, which is expressed by macrophages and immature DCs¹⁵. DC-SIGN triggering by fucose-containing structures results in upregulation of the anti-inflammatory cytokines IL-10 and IL-27 and in the induction of T helper 2 (T_H 2), T follicular helper (T_{FH}) or regulatory T (T_{reg}) cells^{16,17}. Hence, enrichment of Lewis structures in the tumour microenvironment could drive innate immune suppression. Similarly, Tn-enriched MUC1, CD43 and CD45 or the glycolipids GM2 and GD2, which carry an end-standing N-acetylgalactosamine, all interact with MGL on macrophages, driving an immune-inhibitory programme in these cells that is characterized by increased IL-10 production and induction of effector T cell apoptosis^{18,19}. Poor survival of patients with stage III colon cancer is correlated with BRAF mutation and increased presence of the carbohydrate Tn antigen²⁰. Furthermore, enhanced sialylation of tumour cells leads to increased expression of ligands for SIGLECs, a family of lectin receptors, most of which have immune-inhibitory functions²¹ (FIG 1). For example, the presence of sialylated structures on melanoma cells correlated with increased

Box 1 | Glycosylation and tumour-associated glycans

Glycosylation is a complex enzymatic process that leads to the generation of carbohydrate structures that are covalently bound to proteins or lipids. It mainly occurs in the endoplasmic reticulum and Golgi apparatus, although some glycosylation reactions can also happen within the cytoplasm⁶⁸.

Glycoproteins and glycolipids

In glycoproteins, structures can be classified on the basis of which atom provides the link in the protein backbone. There are two main types: *N*-glycans, which are attached via the terminal amine present in the side chain of asparagine; or *O*-glycans, which are linked to a hydroxyl group of serine, threonine or tyrosine.

Glycolipids are molecules in which the glycan structures are covalently linked to lipids and can be grouped depending on their lipid portion. The main group corresponds to the glycosphingolipids, in which the carbohydrates are linked to a ceramide.

Glycan synthesis

In humans, over 300 genes participate in glycan biosynthesis, with two types of enzymes being the main protagonists. Glycosyltransferases build up the glycan structures by transferring single carbohydrates from activated donors to an acceptor, which could be proteins, lipids or a carbohydrate structure, to then be elongated. Glycosidases are enzymes that catalyse the hydrolysis of oligosaccharides. The expression of these genes is controlled by different mechanisms during malignant transformation, and analysis of this expression may serve as a diagnostic and therapeutic tool.

Tumour-associated glycans

Some of the most common tumour-associated glycans include:

Sialylated glycans. These glycan structures display the negatively charged monosaccharide sialic acid as the most external sugar. Sialic acid is a family of sugars with nine carbons, which include N-acetylneuraminic acid, the predominant structure found in humans. The expression of sialylated glycans is often augmented in cancer.

Tn antigen. This glycan structure represents a single residue of *N*-acetylgalactosamine, linked to the serine or threonine of a protein. This structure is generated in the first step in the synthesis of *O*-glycans by the action of a family of enzymes called polypeptide

N-acetylgalactosaminyltransferases and is normally extended by subsequent enzymes. However, this does not happen in cancer, thereby giving rise to a phenomenon called 'truncated *O*-glycosylation'. Other structures in that category are the disaccharide T antigen (product of an addition of galactose over the Tn antigen) and sialyl Tn antigen (when the addition is sialic acid).

Lewis antigens. These structures represent a family of glycans characterized by the presence of one or two fucose moieties linked to a disaccharide of *N*-acetylglucosamine and galactose, and they can also be sialylated. Members of this family are usually upregulated in cancer.

tumour growth in vivo; this was associated with increased accumulation of T_{reg} cells, a low influx of effector T cells and reduced NK cell activity²². The binding of sialylated antigens to SIGLECE on DCs promoted DCs to generate increased levels of antigenspecific T_{reg} cells and reduced numbers of antigen-specific effector T cells23. In a similar fashion, in vivo injection of sialylated antigen promoted DC-mediated induction of antigen-specific T_{reg} cells and reduced their ability to promote the differentiation of antigen-specific CD4⁺ and CD8⁺ effector T cells²³. This finding may illustrate how tumour sialylation impedes T cell-mediated antitumour immune responses while promoting tumour-associated T_{reg} cells. The sialylated Tn antigen (sTn), which is widely expressed in carcinomas, is also associated with immune tolerance²⁴. sTn-positive mucins secreted by cancer cells impair the maturation of DCs and lead to DC-mediated induction of T cells that express high levels

of the T_{reg} cell-associated transcription factor forkhead box protein P3 (FOXP3) and low levels of IFN $\gamma^{24,25}$. Sialylation of the T antigen in MUC1 on breast cancer cells creates a ligand, MUC1-sT, that interacts with SIGLEC9 on tumour-infiltrating macrophages and initiates inhibitory immune signalling through the activation of the mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK) pathway²⁶. Moreover, sialylated tumour ligands can directly reduce NK cell activity by interacting with SIGLEC7 and SIGLEC9 on these immune cells²⁷. Consequently, the sialylation signature of tumour antigens has a profound impact on tumour-infiltrating immune cells and drives an immune-inhibitory circuit.

Clearly, sialylated structures, Tn and Lewis antigens all contribute to shape unique glyco-codes with distinct mechanisms of immune suppression (FIG. 2). Therefore, defining the glyco-code present in different

tumours is essential for understanding its immune evasion potential. Interestingly, glycosylation can also affect the structure and function of well-known immune checkpoint molecules. For example, *N*-glycans stabilize PDL1 by reducing its proteasomal degradation and, consequently, enhance its immunosuppressive activity²⁸.

Lectin expression by tumour cells

In addition to aberrant glycan expression, cancer cells may also display altered expression of glycan-binding lectins. Galectins, a family of soluble lectins, can be secreted by a wide range of tumours and are able to impair T cell effector function, instruct the differentiation of suppressive myeloid cells and modulate NK cell activity by binding to specific glycans expressed on these immune cell populations²⁹ (FIG. 1). Galectin 1 (Gal1) contributes to immune evasion through several mechanisms, including differentiation of tolerogenic DCs and the induction of apoptosis in $T_{H}1$ and $T_{H}17$ cells^{30,31}. Gal1 expression positively correlates with the aggressiveness and metastatic phenotype of tumours. Its blockade in the tumour microenvironment augments the effector functions of CD4+ and CD8⁺ T cells^{29,32}. Other galectins, such as Gal3, drive anergy of tumour-specific T cells. Gal3 also dampens NK cell activity by interfering with the glycosylationdependent interaction between the NK cell receptor D (NKG2D; also known as KLRK1) and MHC class I polypeptide-related sequence A (MICA), its stress-induced ligand^{29,33}. T cell immunoglobulin and mucin receptor 3 (TIM3; also known as HAVCR2), an immune checkpoint molecule expressed by T_H1 and CD8⁺ T cells, is bound by Gal9 in a glycan-dependent manner and induces a suppressive programme in T cells. Moreover, Gal9 increases the frequency of granulocytic

myeloid-derived suppressor cells, which can induce exhaustion in CD8⁺ T cells³⁴. Thus, the secretion of galectins by tumours is a predictor of a profound immune-suppressive state in the tumour microenvironment that affects many immune cells (FIG. 2).

Neo-antigens and glycosylation

Antigens presented to T cells by MHC class I and class II molecules can retain certain post-translational modifications^{35,36}. Aberrant post-translational modifications, including phosphorylation and glycosylation, can add a new layer of neo-antigenicity to tumour-specific peptides presented by MHC class I complexes. Indeed, T cells with a T cell receptor (TCR) specificity for glycopeptides originating from processed post-translationally modified antigen have been identified and shown to specifically recognize the glycosylated form of the peptide and not the unglycosylated



Figure 2 | **The glyco-code in the tumour analysis of patients with cancer.** The analysis of the glyco-code using glyco-tools such as antibodies or lectins reveals the presence of Tn antigen (a single residue of *N*-acetylgalactosamine linked to the serine or threonine of a protein), sialyl Lewis X and Y, sialic acids or galectin in the tumour microenvironment. The types of glyco-code, Tn antigen, sialyl Lewis, sialic acid or galectin that are expressed reveal the immune-suppressive signature of the tumour microenvironment, as distinct immune cell populations are associated with particular glycan-binding receptors. The glyco-code is based on the recognition with human lectins of

tumour tissue that detect the presence of the glycans that alter the immune function of cells. Immunohistochemistry images are illustrative and were downloaded from colorectal cancer data in the Human Protein Atlas Portal (version 18; http://v18.proteinatlas.org/) using the following gene names: *MUC1* for Tn score; *FUT3* for Lewis score; *ST3GAL1* for sialic score; and *LGALS1* for galectin 1 score⁷⁴. DC, dendritic cell; DC-SIGN, DC-specific ICAM-3-grabbing non-integrin 1; LacNAc, *N*-acetyl-D-lactosamine; MGL, macrophage galactose-specific lectin; NK, natural killer; SIGLEC, sialic acid-binding immunoglobulin-like lectin.

peptide, indicating that the post-translational modification results in a different antigen and cognate TCR. For example, post-translational modification of MUC1, a highly glycosylated protein with a great abundance of O-glycosylation sites, has been demonstrated to yield an altered glycosylated antigen. This neo-antigen was presented by MHC class I complexes and could be recognized only by a glycoform-specific TCR³⁶. Additionally, missense mutations can provide additional N-glycosylation sites, directly affecting protein function³⁷. Whether such 'gain-of-glycosylation' mutations exist in rapidly mutating tumours is still unknown and remains to be investigated.

Clearly, aberrant glycosylation adds another layer of complexity to tumour neo-antigenicity and the nature of the tumour-infiltrating T cell repertoire requires further investigation. Future experiments aimed at characterizing the glycopeptide– MHC class I complexes expressed by tumour cells may identify novel tumour-specific epitopes that could serve as targets for glycopeptide-specific T cells³⁸.

Another interesting example of gain of glycosylation is seen commonly in follicular lymphoma and chronic lymphocytic leukaemia. In this case, new *N*-glycosylation sites in the immunoglobulin variable domain lead to the presence of high-mannose oligosaccharides on the B cell receptor of the leukaemia cells that are capable of interacting with the DC-SIGN on macrophages and DCs and initiating antigen-independent signalling events in the tumour microenvironment that drive tumour growth and survival^{39,40}.

The glyco-code in cancer diagnostics

Altered glycosylation of tumour cells often occurs in the early stages of tumour development, and certain tumourassociated glycans have been shown to be expressed in precursor lesions of different types of cancer, making them powerful early diagnostic markers^{9,41,42}. Given the structural complexity of glycan structures and the heterogeneity in glycosylation sites, a complete characterization of tumour glycomics and glycoproteomics represents a challenge.

Cancer glycomics is currently performed on total cell preparations using liquid chromatography and mass spectrometry (LC–MS) analysis, which enables the detailed analysis of all the structural glycans of cancer cells⁴³. Imaging mass spectrometry (IMS) is a new approach that enables the detection and spatial distribution of N-linked glycan distributions in fresh and/or frozen tissues and formalin-fixed paraffin-embedded tumour tissues44. In situ detection of native-occurring bioactive glycan fragments in formalin-fixed tissues from patients with gastric cancer has shown the potential of these glycans to influence patient outcomes⁴⁵. Recently, Stadlmann et al. developed a novel high-throughput approach that allowed these authors to characterize the structure of complex glycans and localize their specific glycosylation site within their carrier protein by enriching, sequencing and identifying glycopeptides within complex mixtures^{46,47}. However, single-cell detection is limited to antibodies and lectins, which can be conjugated using fluorescent dyes or heavy-metal isotopes and detected in single-cell suspensions (using flow and mass cytometry), as well as in tissue sections (using microscopy and imaging mass cytometry).

Tumour-associated glycans. Changes in the cancer-associated glyco-code can be characterized using monoclonal antibodies against specific glycan structures (FIG 2). Many of these antibodies serve as cancer biomarkers (CA15-3, CA125 and CA19-9) in the clinic and are specific for circulating O-glycoproteins expressing specific glycans, such as sTn or sialyl Lewis A. CA19-9 is the most widely used serum tumour marker in pancreatic cancer and is the glycan structure sialyl Lewis A. Assays that detect CA19-9 in patient serum have shown promising results for the early detection of pancreatic cancer⁴⁸. Other glycans and glycoproteins currently used in cancer diagnostics include prostatespecific antigen (PSA), CEA, mucins and CA72-4 (REFS 9,49-51). Antibodies recognizing these carbohydrate antigens are used for biomarker analysis of serum but are also used for detection of glycan structures in tissue. However, because single glycans per se may not identify aberrant glycoforms of a protein in cancerous tissue and because their multivalent presentation on a protein backbone may determine the strength of the immune-inhibitory signalling through lectins, there is a need to identify the glycan as well as the underlying protein in patient material. Proximity ligation assays have shown the potential for simultaneously detecting a glycan and glycoprotein that are present in cancerous tissue but absent in benign tissues52.

Lectins and the tumour glyco-code. Lectins also represent interesting tools to assess the tumour glyco-code (FIG. 2). To date,

hundreds of lectins from different species have been characterized, each of which recognizes a discrete glycan structure. Using lectins in a microarray format allows for high-throughput analysis of glycoconjugates, and their application in clinical samples (such as liquid biopsies) could serve as a novel diagnostic tool in cancer⁵³. Lectins can be used not only to analyse cancer-associated markers in the serum of patients but also to analyse changes in the glycosylation of cells in blood or to perform histochemical staining in tissue biopsies. In addition to the many animal and plant lectins that uncover a fairly 'overall' glycan landscape, human immune cell lectin receptors have been conjugated to the Fc fraction of an immunoglobulin, generating lectin-Fc chimaeras that can be used to uncover those glycan-binding epitopes present on proteins, lipids, cells and tissues that may interfere with immune modulation in the tumour microenvironment. Harnessing these tools for immunohistochemistry and cytometry will potentiate systematic analysis of the glyco-code in patients with cancer and reveal the immune status of the tumour microenvironment.

Genes involved in glycan synthesis. Changes in glycan structures often reflect changes in the expression of the genes that encode enzymes that synthesize specific glycans or their substrates (such as their sugar donors) or that determine their localization in the cell⁵⁴ (BOX 1). With increasingly accessible technologies to determine gene expression (such as RNA-sequencing and microarray), the expression status of glycosylation-related genes could serve as a novel diagnostic tool. Indeed, the expression of specific glycosyltransferase profiles correlates with tumour mutational status and the metastasis and survival of patients^{55,56}. A deep analysis of the expression and sequencing data currently available and their integration with structural and clinical data could make major contributions to deciphering the glyco-code and its impact on a patient's immune system.

The glyco-code in cancer

In the preceding sections, we have discussed how tumour-specific glycosylation patterns determine the immune-inhibitory properties of the tumour. Accordingly, we suggest that these inhibitory glycan–lectin interactions should be considered as novel immune checkpoints that can be targeted for tumour immunotherapy (FIG. 3). Below, we discuss how the tumour glyco-code can be harnessed for cancer therapy.

Anti-glycan vaccines. Classically, glycantargeting strategies have focused on the development of vaccines that induce specific anti-glycan immune responses⁵⁷ (FIG. 3a). For example, the Theratope (Biomira) vaccine, targeting the sTn antigen, induces strong sTn-specific immunity with a significant increase in survival of patients with metastatic breast cancer in phase II clinical trials58. A phase III clinical trial failed to reproduce these findings, probably owing to heterogeneity in the expression of sTn which was not evaluated before patient selection⁵⁹. A proper evaluation of the glyco-code of cancer patients may therefore serve as a tool not only for immune diagnostic purposes but also for the rational selection of anti-glycan vaccination strategies.

Blocking tumour-associated glycan-lectin interactions. Strategies that prevent the interaction of tumour-associated glycans with inhibitory immune receptors could serve as antitumour therapies. Indeed, a broad spectrum of inhibitors capable of blocking glycan-lectin interactions have been developed⁸. Therapeutic modification of the glyco-code, such as sialic acid blockade using metabolic mimetics or glycosidases attached to tumour-targeting antibodies, suppresses tumour growth^{60,61}. This suppression is due to enhanced T cell-mediated antitumour responses and enhanced NK cell activity, presumably as a result of decreased triggering of SIGLECs on these cells60,61. Blocking antibodies with specificity for lectin receptors or for tumour-specific glycans that can selectively inhibit glycan-lectin interactions can be locally applied and could serve as novel immunotherapies³² (FIG. 3b). Monoclonal antibodies could be further genetically engineered and glycomodified to enhance effector functions (such as





antibody-dependent cellular cytotoxicity and complement activation) or used to generate chimeric therapeutic molecules, such as through antibody conjugation to immunotoxins or glycosylation-modifying enzymes (such as glycosidase)⁶² (FIG. 3c).

The value of glycan–lectin interference on tumour growth and immune modulation has been studied in both human *in vitro* studies and translational mouse *in vivo* studies. However, one must take into account that the glycosylation machinery and the lectin expression on immune cells are not identical and that fundamental differences in the physiology of humans and mice complicate a one-to-one translation (BOX 2).

Cellular immunotherapy. Identification of TCR reactivity to tumour-specific glycopeptides may be the future for effective tumour-specific chimeric antigen receptor (CAR) T cells to be used in the clinic (FIG. 3d). Recently, the group of Carl June demonstrated that cloning of the single-chain fragment variable regions of anti-Tn antibodies to generate Tn– MUC-specific CAR T cells is an effective strategy to eradicate leukaemia and pancreatic cancer in mice⁶³. The generation of new tumour glyco-specific antibodies may tremendously boost the field of CAR T cell development.

Dendritic cell targeting. In recent years, the targeting of DCs has emerged as an interesting approach for the induction of antitumour immunity⁶⁴. Current strategies include using glycans to target the DC-specific lectin DC-SIGN. Several glycans show the potential when coupled to antigen to target DC-SIGN to facilitate internalization of antigen, favour antigen cross-presentation and stimulate tumour-specific CD4+ and CD8⁺ T cell responses (FIG 3e). The targeting of DCs by glycan-modified tumour antigens has improved tumour-specific T cell responses and long-term tumour regression when combined with transient T_{reg} cell inhibition, illustrating the power of cancer vaccines when combined with immune checkpoint blockade65. In the future, the personalized glyco-code of the tumour may serve to dismantle the immune status of the tumour and be targeted for glycan checkpoint interference by modifying tumour glycosylation. Alternatively, these novel checkpoint blockades may be combined with DC-targeting vaccination strategies for the optimal success of future immunotherapy regimens.

The immune system harbours an intrinsic capacity to eradicate cancer. However, despite clinical success, only a selection of patients benefit from current immunotherapies. The immunological representation of a patient with cancer has recently been proposed as a cancer–immune set point. It includes many immunological and oncological parameters aimed at predicting the responses to immunotherapy⁶⁶. A personalized glyco-code of the tumour can contribute to the cancer–immune set point and aid combination therapy tailored to the patient (FIG 3).

Conclusions and outstanding questions

In this Opinion article, we have discussed why tumour glycosylation should be investigated as a new variable for diagnostic and prognostic value in cancer that could be linked to immune infiltration scores. Immune cells are programmed through their lectin receptors to decode and interpret the 'glycan language', and tumour cells exploit this language to programme immune suppression and facilitate immune evasion. The extent to which glycans interfere with these immunosuppressive programmes is enormous, as one type of glyco-code affects a variety of immune cells. Therefore, it is crucial to include the glyco-code in the analysis of tumour specimens from patients with cancer and to foster development

Box 3 | Outstanding questions and future directions for the field

Below, we discuss how future diagnostics and therapeutics might enable a more accurate prediction of how glycans, glycoproteins and glycolipids are expressed by tumours and have an impact on the immune system.

Bioinformatics. In recent years, next-generation sequencing techniques have allowed the creation of large databases with a detailed characterization of the genomic and transcriptomic characteristics of tumour biopsies. A glycobiology-focused bioinformatic analysis of these data that examines not only the expression of specific enzymes but also the presence of gain-of-glycosylation mutations and splicing variants of glycoproteins and enzymes could help to predict the tumour glyco-code and potentially open new lines of research in the field.

Analytical methods. Currently, the use of lectins and antibodies is the most common way to analyse the glycosylation of cells, with the chemical methods left aside in routine testing owing to their complexity. The development of new and clinic-friendly techniques for the determination of the glyco-code may help to extend the use of this type of analysis.

Spatial description of tumour and immune system glycan interactions. The combination of the spatial identification of glycan structures (for example, by using mass spectrometry imaging with multiparametric immune phenotyping) could provide clues on the cellular positioning of suppressive and effector immune cell populations within the tumour that communicate through glycan interactions and help in the identification of potential glycan-driven niches.

Development of novel tools for targeting the tumour glyco-code. Because of the important role of glycans in cancer biology and immune escape, the development of new antibodies or chimeric antigen receptor (CAR) T cells that specifically target tumour glycans or glyco-peptide MHC complexes could serve to provide novel therapies or boost current immunotherapies.

of new tools and methods to implement outstanding fundamental questions and translate these to clinical application (BOX 3). The access to biodatabases for glycomics and glycoproteomics is essential for current glycoimmunological research. Genomic databases can be explored

Box 2 | Glycosylation and the translational value of mice models

In cancer research, mice models represent essential tools for the study of the tumour's biology and its interaction with the immune system. However, fundamental differences in the physiology of humans and mice may complicate and limit the translational value of findings made in mice^{69,70}. Although the general glycosylation pathways are conserved between the species, some differences can be found in genes encoding specific enzymes and lectin receptors.

One example is the SIGLECs, a family of sialic acid-binding immunoglobulin-like lectins composed of 14 members in humans but only 9 in mice. Some of the SIGLECs are conserved across mammals, and functional homologues have been well characterized (such as SIGLEC9 in humans and SIGLECE in mice); however, this is not the case for all SIGLECs²¹. Moreover, the mouse SIGLEC3 (also known as CD33) lacks the intracellular immunoreceptor tyrosine-based inhibition motif (ITIM) found in its human counterpart²¹.

By contrast, the DC-SIGN family comprises only two receptors in humans (dendritic cell-specific ICAM-3-grabbing non-integrin 1 (DC-SIGN) and liver/lymph node-specific ICAM3-grabbing non-integrin (L-SIGN; also known as CLEC4M)), whereas eight different homologues have been identified in mice, with no clear functional homologues between the species⁷¹. Some of the ligands for these receptors are fucosylated antigens (in particular, the Lewis antigens). Interestingly, some enzymes involved in the synthesis of these structures in humans are absent in mice, as is the case for the fucosyltransferases FUT3, FUT5 and FUT6 (REF. 72).

The sialic acid that is mainly present in human cells is N-acetylneuraminic acid (Neu5Ac), whereas in most other mammals, N-glycolylneuraminic acid (Neu5Gc) can also be found, which differs from Neu5Ac in only one oxygen atom. Humans are not able to synthesize Neu5Gc owing to an exon deletion in the enzyme cytidine monophosphate-N-acetylneuraminic acid hydroxylase gene (CMAH). It has been proposed that this difference shaped the ligands and the function of human SIGLECs during their evolution⁷³.

The display of a different array of receptors with diverse ligands and structures could compromise the direct translation from mouse models to human patients. Despite the fundamental value of mouse models, it is important to know, understand and take into account their glycobiology differences with humans.

for expression of glycosylation-related genes to reveal glycosylation signatures related to cancer development, epithelialmesenchymal transformation and metastasis. Glycobioinformatics is indispensable to unveil the glyco-code. A better understanding of the nature of the glycan-lectin interactions that occur between the tumour and the immune system could lead to the design of improved antitumour immunotherapies. For example, this information could be used to develop novel tumour-glycan-specific antibodies or to improve future combination therapies. The development of new strategies targeting the tumour glyco-code could ultimately be of great benefit to those patients who do not respond to current immunotherapy regimes⁶⁷.

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Author contributions

Y.v.K. and E.R. researched data for the article, made substantial contributions to the discussion of content and wrote, reviewed and edited the manuscript before submission. S.T.T.S. researched data for the article and made substantial contributions to the discussion of content and the writing of the manuscript before submission.

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