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Review article

Targeting galectin-driven regulatory circuits in cancer and fibrosis

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Abstract

Galectins are a family of endogenous glycan-binding proteins that have crucial roles in a broad range of physiological and pathological processes. As a group, these proteins use both extracellular and intracellular mechanisms as well as glycan-dependent and independent pathways to reprogramme the fate and function of numerous cell types. Given their multifunctional roles in both tissue fibrosis and cancer, galectins have been identified as potential therapeutic targets for these disorders. Here, we focus on the therapeutic relevance of galectins, particularly galectin 1 (GAL1), GAL3 and GAL9 to tumour progression and fibrotic diseases. We consider an array of galectin-targeted strategies, including small-molecule carbohydrate inhibitors, natural polysaccharides and their derivatives, peptides, peptidomimetics and biological agents (notably, neutralizing monoclonal antibodies and truncated galectins) and discuss their mechanisms of action, selectivity and therapeutic potential in preclinical models of fibrosis and cancer. We also review the results of clinical trials that aim to evaluate the efficacy of galectin inhibitors in patients with idiopathic pulmonary fibrosis, nonalcoholic steatohepatitis and cancer. The rapid pace of glycobiology research, combined with the acute need for drugs to alleviate fibrotic inflammation and overcome resistance to anticancer therapies, will accelerate the translation of anti-galectin therapeutics into clinical practice.

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Sections

Introduction

The complex repertoire of glycan structures present in cells and tissues (that is, the glycome) stores crucial biological information that contributes to the reprogramming of cellular fate and function and thus has a profound influence on the delicate balance between health and disease[1–4]. The diversity and spatiotemporal regulation of glycans within glycoconjugates rely on the synchronized action of glycan-modifying enzymes, including glycosyltransferases and glycosylhydrolases. Glycan remodelling is regulated by intracellular and environmental signals, such as metabolic stress, oxygen and nutrient availability, growth factors and cytokines[2,5]. Dysregulation of many cellular processes, including cellular communication, proliferation, differentiation and survival has been linked to aberrant glycosylation. This finding implicates the glycome in the pathophysiology of nearly every major disease, with notable impact on cancer, inflammation and fibrosis[5,6]. Changes in the glycosylation signature of tumour, immune and endothelial cells (ECs) are among the common hallmarks of the tumorigenic process, as these signatures can influence cell adhesion, epithelial-to-mesenchymal transition (EMT), angiogenesis, immunoediting and metastasis[1,5,7–12]. Moreover, selective glycan profiles may also help to control the initiation, persistence and resolution of inflammatory and fibrotic processes[1,13]. Thus, an aberrant glycome might alter cellular functions by regulating the exposure or masking of specific glycoepitopes. These aberrations can ultimately lead to the development of pathological responses.

Converting glycan-encoded information into biological programmes relies, at least in part, on the contributions of endogenous glycan-binding proteins or lectins[6]. The three major lectin families that have decisive roles in shaping both inflammatory and tumour microenvironments are sialic acid-binding immunoglobulin-like lectins (Siglecs), C-type lectin receptors (including selectins[14]) and galectins. Galectins are a family of soluble lectins that share affinity for β-galactoside-containing saccharides[15]. Examples of some natural ligands for mammalian galectins are summarized in Box 1. Galectins might be involved in the transition from healthy to neoplastic or inflamed tissues and contribute to the persistence of these pathological conditions via both intracellular and extracellular mechanisms[16,17]. Galectins also influence most hallmarks of tumour progression[17] and modulate resistance to numerous anticancer treatments, including immunotherapy, chemotherapy, radiotherapy, targeted therapies and anti-angiogenic therapy[18]. Furthermore, galectins might contribute to regulatory circuits that amplify, sustain or alleviate tissue fibrosis and inflammation by selectively targeting different cell types and their microenvironments[16,19–21]. Accordingly, these glycan-binding proteins have been proposed as therapeutic targets in a broad range of pathological conditions and are currently under clinical evaluation.

In this Review, we consider the pathophysiological relevance of specific galectin–glycan interactions from a translational perspective and discuss the design, mechanisms of action, selectivity and therapeutic relevance of galectin-targeted agents. We focus on the results of preclinical studies and clinical trials and highlight lessons learned from targeting galectins in a diverse group of disease states, including nonalcoholic steatohepatitis (NASH)[19], idiopathic pulmonary fibrosis (IPF)[22–24], as well as various malignancies[11,17]. These examples underscore the numerous potential opportunities to capitalize on galectin–glycan interactions for therapeutic purposes.

We focus in particular on agents designed to target galectin–glycan interactions, including small-molecule inhibitors, natural polysaccharides and their synthetic derivatives, as well as peptides and peptidomimetics. We also discuss the relevance of biological agents such as neutralizing monoclonal antibodies (mAbs), aptamers and truncated galectins, which have emerged as potential therapeutic modalities to target galectin-regulated circuits. Finally, we discuss various galectin-targeted strategies, highlight new translational applications and consider the current challenges and prospects for using these agents to modulate treatments for cancer, IPF and NASH. However, we do not discuss other targets of the translational potential of galectins, which include allergic inflammation[25,26], autoimmune inflammation[16,27–29], neuroinflammation[30,31], cardiovascular disorders and atherosclerosis[32–34], sepsis[35] and failing pregnancies[36].

Cellular roles of galectins

Galectins regulate cell signalling and recalibrate the nature and magnitude of cellular responses via their impact on both intracellular and extracellular mechanisms[16,37–41]. Galectins were originally defined as β-galactoside-binding proteins, as they recognize epitopes containing Galβ(1–4)GlcNAc — also known as *N*-acetyllactosamine, LacNAc — present in various glycoconjugates. However, recent biochemical studies on mammalian galectins have revealed subtle differences in their carbohydrate recognition domains (CRDs) that can explain some of their divergent biological activities[15,42–44] (Box 1). Members of the galectin family have been categorized into three subfamilies (Fig. 1), which includes prototype galectins (GAL1, GAL2, GAL5, GAL7, GAL10, GAL11, GAL13, GAL14 and GAL15) that function as monomers or non-covalent homodimers with identical CRDs; chimera-type galectins (GAL3), which contain only one CRD and can oligomerize via a non-lectin N-terminal domain; and tandem repeat-type galectins (GAL4, GAL6, GAL8, GAL9 and GAL12), which contain two CRDs each with a distinct specificity[15,43,44] joined by a flexible linker peptide.

The expression and subcellular localization of galectins differ considerably in individual cell types. These properties are dynamically regulated by cell activation, differentiation and anatomical distribution and can undergo dramatic modulation in response to specific pathological conditions[8,15]. Galectins are synthesized as cytosolic proteins and in response to environmental signals can shuttle between the cytosol and the nucleus. They control intracellular processes via specific protein–protein or protein–glycan interactions[45,46]. For example, GAL1 and GAL3 interact with RAS GTPases in the cytoplasm, whereas through binding to Gemin4 (ref. [47]) they have a role in spliceosome assembly in the nucleus[48]. Moreover, intracellular partners of GAL3 include β-catenin[45] and hnRNPA2B1 (ref. [49]) in the nucleus, and Alix, a component of the endosomal sorting complex required for intracellular transport[50,51]. Interestingly, cytosolic galectins might act as 'danger signal sensors' that detect abnormal exposure of glycan moieties within the cytosolic compartment[52,53].

Galectins are also secreted from cells via a non-canonical pathway that is independent of the endoplasmic reticulum and Golgi[35,37]. In the extracellular space, galectins can cross-link glycosylated receptors on the cell surface (Fig. 1), thereby establishing multivalent lattices that convert glycan-based information into diverse signalling programmes[40,54,55]. The relatively low affinity of galectins for most, but not all, small oligosaccharidic structures[43,56,57] can be compensated by the cluster glycoside effect (Box 2), which explains the apparent increase in affinity due to multivalent interactions between a ligand and its target. Segregation of glycoconjugates into membrane microdomains and the assembly of galectin–glycan complexes can control the retention of cell surface glycosylated receptors, thereby modulating the threshold for signalling and amplifying or attenuating cellular responses[9,58,59].

Box 1

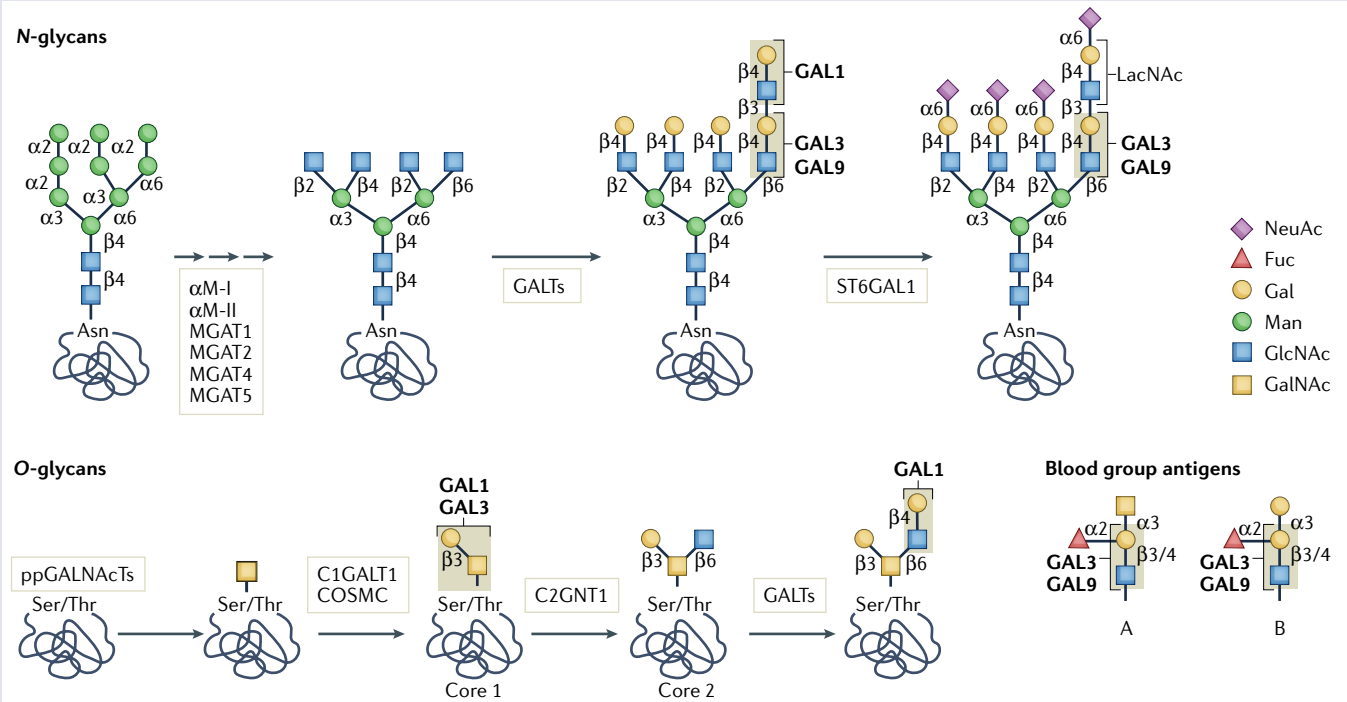
The cellular glycome dictates galectin binding and function

The enzymes α(1–2)-mannosidase I and II (αM-I and αM-II) trim the *N*-glycan precursors of complex *N*-glycans. *N*-acetylglucosaminyltransferases (MGAT1, MGAT2, MGAT4 and MGAT5) generate the antennae, and galactosyltransferases (GALTs) generate *N*-acetyllactosamine (LacNAc, Galβ(1–4)GlcNAc) structures, essential for galectin binding. Galectin 1 (GAL1), GAL3 and GAL9 show subtle binding differences; although the three galectins display an increasing affinity for branched *N*-glycans, GAL1 recognizes terminal LacNAc residues only. GAL3 can also interact with oligosaccharides bearing 2- or 3-O-substituents on the galactose residue, including α(2–3)-sialyllactosamine or blood group A (GalNAcα(1–3)[Fucα1–2]Galβ(1–3/4)GlcNAc)[53] whereas GAL9 has a preference for glycosphingolipids and poly-LacNAc repeats[43]. Incorporation of α(2–6)-linked sialic acid via the actions of β-galactoside α(2–6)-sialyltransferase 1 (ST6GAL1) can block GAL1 binding, although this terminal modification is less restrictive with respect to other galectins (that is, GAL3, GAL9)[306], which are able to interact with internal LacNAc repeats[43].

Mucin-type O-glycosylation is initiated by the actions of polypeptide *N*-acetylgalactosamine transferases (ppGalNAcTs). Synthesis of core 1 *O*-glycan structures (Galβ(1–3)GalNAc) is initiated by C1GALT1 (core 1 β(1–3)-galactosyltransferase 1), assisted by the chaperone protein, COSMC. Although both GAL1 and GAL3 can recognize core 1 structures, GAL3 binds with higher affinity. Core 1 can be sialylated by β-galactoside-α(2–3) sialyltransferase 1 (ST3GAL1), elongated or branched. Branching by *N*-acetylglucosaminyltransferases such as C2GNT1 results in a core 2 *O*-glycan structure, which can be further elongated by GALTs, generating LacNAc residues. Functional roles of GAL1 have been associated with binding to both core 1 and extended core 2 *O*-glycans.

Additionally, GAL3 and GAL9 exhibit affinity towards blood group-related glycans present in various glycoconjugates, including glycolipids. GM1 (Gal-β(1–3)GalNAc-β(1–4)-[NeuNAc-α(2–3)] Gal-β(1–4)Glc) has been described as a potential ligand for GAL1, GAL3 and GAL9 (refs. [43,307,308]).

The altered glycosylation profile observed in tumour cells led to the definition of tumour-associated carbohydrate antigens (TACAs). Depending on the cell type, *N*-glycans can be altered by increasing synthesis of poly-LacNAc chains or β(1,6) branching, which are permissive modifications for galectin binding. In turn, mucin-type *O*-glycans can be truncated and consequently, tumour cells express higher levels of structures such as the Thomsen–Friedenreich antigen (Tf antigen), which may serve as galectin ligands[5,11,61].



The responses generated by galectin–glycan interactions — typically resulting from intracellular and extracellular activities — modulate a broad range of cellular responses, including proliferation, differentiation and survival[60].

Dysregulated galectin expression occurs in a range of malignancies and is frequently associated with poor prognosis and limited responses to anticancer therapies[17,18,61,62]. Moreover, galectin levels are frequently elevated at various stages of the fibrotic cascade[21,63]. These

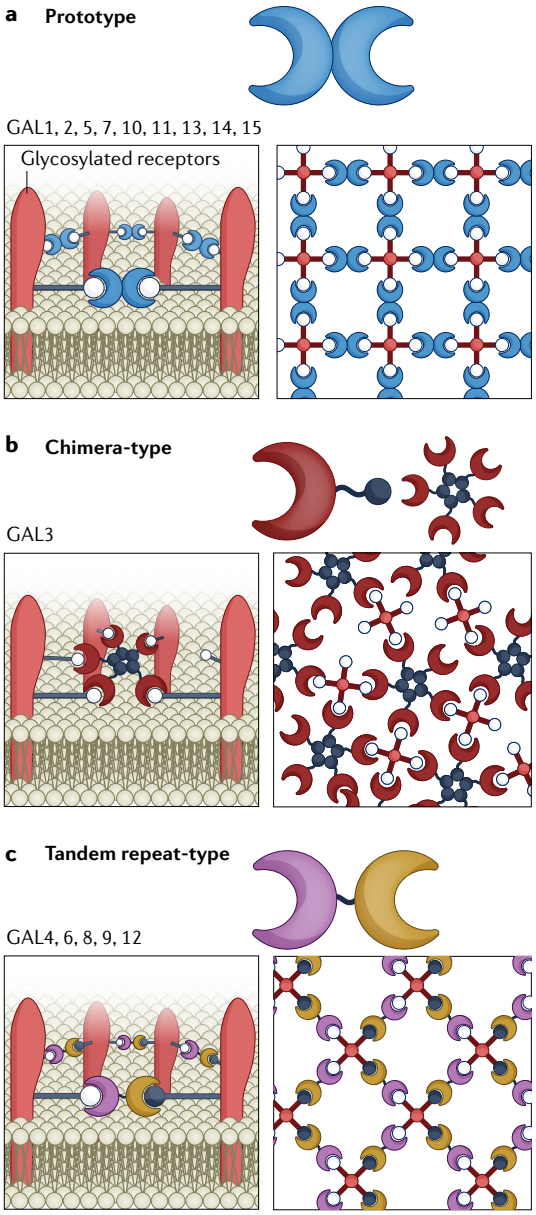


Fig. 1 | Structural classification of galectins. Galectins are classified into three different subfamilies on the basis of their structure: **a**, prototype galectins (GAL1, GAL2, GAL5, GAL7, GAL10, GAL11, GAL13, GAL14 and GAL15) that function as monomers or non-covalent homodimers with identical carbohydrate recognition domains (CRDs); **b**, chimera-type galectins (GAL3) that contain only one CRD and have the capacity to oligomerize via a non-lectin N-terminal domain; and **c**, tandem repeat-type galectins (GAL4, GAL6, GAL8, GAL9 and GAL12) that contain two CRDs each with a distinct specificity joined by a flexible linker peptide. Depending on distinct carbohydrate-binding specificities and oligomerization status, these proteins can form lattices by interacting with multiple glycosylated ligands on the cell surface and/or with extracellular matrix glycoproteins. In doing so, they elicit different biological responses via mechanisms that include assembly of multivalent glycan–galectin complexes and modulation of transmembrane signalling events by selective receptor segregation, retention and endocytosis.

data highlight the emerging roles of galectins as possible biomarkers and therapeutic targets in these pathological conditions. In the next sections we discuss the role of galectins in cancer and fibrosis, with a focus on GAL1, GAL3 and GAL9.

Galectins in cancer

Galectins have a broad influence on tumour progression via glycosylation-dependent or independent mechanisms that modulate proliferation, evasion of growth suppressors and immune responses, resistance to cell death, induction of angiogenesis, inflammation and metastasis[17,64]. These galectin-influenced circuits can shape tumour, endothelial and immune compartments in individual tumour microenvironments (Fig. 2). These actions have both local and systemic impact and might contribute to processes that influence clinical outcomes in patients with cancer. To substantiate the therapeutic relevance of these glycan-binding proteins, we provide several examples that highlight the impact of galectins, most notably GAL1, GAL3 and GAL9, on the reprogramming of the tumour, vascular and immune landscapes and their contributions to tumour growth and progression (Fig. 2).

Galectins reprogramme tumour cell fate

GAL1 is upregulated in most tumour tissues and their associated stroma and has been proposed as a biomarker of poor prognosis in breast, colon, lung, prostate adenocarcinoma and melanoma[65]. Tumours of the digestive tract and urinary system, as well as thyroid cancer and melanomas, express high levels of GAL3; by contrast, GAL3 expression is downregulated in tumours of the reproductive tract. Subcellular compartmentalization of GAL3 is a crucial factor contributing to its pro- or antitumoural roles. For example, increased GAL3 within the nuclear compartment has been associated with a better outcome among patients with neuroblastoma[66]. On the other hand, GAL9 expression can be up- or downregulated in association with neoplastic transformation depending on the specific tumour type[65,67]. As the expression of these lectins can change during tumour progression, they have been proposed as biomarkers for diagnosis, prognosis and/or intervention in various cancer stages[65].

Extracellular GAL1 and GAL3 directly modulate tumour cell fitness, migration, EMT and stemness through their interactions with glycosylated tumour-associated receptors, including epidermal growth factor (EGF), transforming growth factor β (TGFβ) receptors and cell surface integrins[68–74], that contribute to cancer progression and metastasis. GAL1 can trigger EMT in gastric cancer[75] and hepatocellular carcinoma[72,76] via non-canonical activation of the Hedgehog pathway[75], downregulation of E-cadherins[72] and induction of $\alpha_v\beta_3$ integrin-dependent AKT signalling[76]. Collectively, these actions have an impact on clinical outcomes and therapeutic responses to both sorafenib and doxorubicin[77,78]. GAL1 also regulates events that promote the growth of pancreatic adenocarcinoma, including tumour cell proliferation, invasion and metastasis[78], and GAL1 silencing inhibits migration and invasion of metastatic castration-resistant prostate cancer cells via suppression of androgen receptor and AKT-mediated signalling[79]. In turn, interactions between GAL3 and the glycosylated transmembrane protein mucin 1 (MUC1) enhance EGF receptor dimerization and activation (Fig. 2), and thus promote proliferation and motility of epithelial cancer cells by activating ERK1/2 and AKT signalling pathways[80,81]. Notably, complex *N*-glycans linked to EGF receptors on breast carcinoma cells control EMT, cell motility and tumour metastasis by facilitating the exposure of GAL3-specific glycoepitopes[68]

(Fig. 2). Tumour-derived GAL3 also promotes invasion via its capacity to impair interactions between adhesion molecules expressed on the surface of malignant cells and N-glycosylated proteins within the extracellular matrix, such as laminin and fibronectin[74]. Furthermore, GAL3 promotes the establishment of metastatic niches by binding to the Thomsen–Friedenreich antigen (Tf antigen) expressed by metastatic lung tumour cells[70] and facilitating homotypic and heterotypic aggregation and emboli formation[82]. Similarly, overexpression of GAL3 promotes proliferation, migration and invasion of oral squamous cell carcinoma (OSCC) cells via enhanced WNT–β-catenin signalling and EMT[83]. The interaction of GAL3 with $\alpha_v\beta_3$ integrin promotes KRAS addiction[84]; these findings identified GAL3 as a potential druggable target for 'KRAS-addicted' lung and pancreatic cancers (Fig. 2).

GAL1 and GAL3 also promote oncogenic signalling, apoptosis resistance and cell cycle progression when present in the intracellular compartment[85,86]. GAL1 associates with oncogenic HRAS (Fig. 2) and enhances HRAS membrane anchorage and oncogenesis by increasing signalling to the kinase RAF1 (refs. [87,88]). Likewise, GAL3 promotes cancer cell proliferation and survival via its interactions with activated KRAS and enhances colorectal tumour cell invasion via constitutive activation of RAF–MEK–ERK signalling[88,89] (Fig. 2). Interestingly, in vitro studies revealed that a combination of the KRAS inhibitor salirasib and the GAL3 inhibitor modified citrus pectin (MCP) induced cell cycle arrest and apoptosis in anaplastic thyroid cells[90]. Other studies showed that GAL3 expression was suppressed by p53. This response was largely eliminated in cells with *TP53* mutations, thus explaining the elevated levels of GAL3 expression in *TP53*-mutant tumours[91]. Remarkably, galectin-mediated mechanisms also promote tumour resistance to most anticancer therapies[18], including chemotherapy[77], immunotherapy[92], anti-angiogenic therapy[9], radiotherapy[93] and targeted therapies[94].

Galectins induce aberrant angiogenesis

Abnormal angiogenesis and diversion of pre-existing blood vessels are crucial in neoplastic as well as inflammatory and fibrotic conditions, as these processes provide the nutrients and oxygen needed to sustain otherwise hypoxic environments[95]. Interestingly, the glycome of ECs varies considerably in response to immunosuppressive or hypoxic stimuli, which favour exposure of galectin-specific glycan epitopes[9]. Several galectin family members have pro-angiogenic activity as a result of their capacity to bind distinct glycosylated receptors on the EC surface[96]. For example, GAL1 interacts with complex *N*-glycans on vascular endothelial growth factor (VEGF) receptor 2 (VEGFR2) and promotes receptor phosphorylation followed by activation of the AKT and ERK1/2 signalling pathways, thus preserving vascularization in tumours that are resistant to anti-VEGF treatment[9] (Fig. 2). These actions link tumour hypoxia to aberrant angiogenesis pathways identified in melanoma as well as lung, prostate and pancreatic adenocarcinoma[9,78,97–101]. Likewise, interactions with the transmembrane glycoprotein, neuropilin 1 (NRP1) are essential for GAL1-driven angiogenesis and induction of vascular permeability[102,103] (Fig. 2). Recently, an alternative mechanism was proposed whereby GAL1 regulates angiogenesis through binding to the mRNAs of angiogenesis-related factors, including VEGF-A, early growth response 1 protein (EGR1) and α_5 laminin[104].

GAL3 promotes vascularization via interactions with complex branched *N*-glycans present on $\alpha_v\beta_3$ integrin and VEGFR2 (Fig. 2) that promote retention of these receptors on the EC surface[105,106]. Interestingly, this pro-angiogenic effect involves cleavage of the N terminus of GAL3 by matrix metalloproteinase 2 (MMP2) and MMP9 (ref. [107]). By contrast, GAL9 mediates angiogenesis through different mechanisms[108]. In vitro studies with individual GAL9 CRDs revealed opposing anti- and pro-angiogenic effects, suggesting a dual role for this lectin in regulating vascular networks[109]. Finally, in addition to their role in promoting angiogenesis, interactions of GAL3 with ECs stimulate the secretion of metastasis-promoting cytokines[110] and the expression of cell adhesion molecules[111]. Thus, agents that target galectins and/or their glycosylated receptors may prevent aberrant vascularization, interrupt the metastasis cascade and circumvent resistance to anti-angiogenic therapies.

Galectins shape immune landscapes

Galectins dampen antitumour immune responses by targeting both lymphoid and myeloid cells[38] (Fig. 2). Notably, galectin–glycan interactions alter the immune landscape in several cancer types[112–118]. Moreover, galectins modulate cancer-associated fibroblasts and can influence their pro-tumorigenic and pro-metastatic activities[119].

The role of galectins in suppressing antitumour immunity involves multiple pathways linked to innate and adaptive immune

Box 2

Multivalency and ligand density in galectin recognition

The weak binding interaction between lectins and carbohydrate moieties can be compensated by a multivalent display of residues at the cell surface. This is known as the 'cluster glycoside effect', which refers to the fact that multivalent (as opposed to monovalent) interactions will result in an overall increase in binding affinity on a per mole or valence-corrected basis. This is one of the key factors regulating the biological activities of galectins at the cell surface. For example, galectin-permissive glycoepitopes can be exposed differentially, largely depending on the nature of the parent glycoconjugate, the target cell type, the cellular activation status and the underlying physiological or pathological conditions. Likewise, extracellular galectins can form multimers that will cross-link specific cell surface glycoconjugates, thereby generating galectin–glycan complexes or lattices that can regulate one or more intracellular signalling pathways. However, not every glycoconjugate with a permissive glycosylation profile can function in this role. A functional glycoconjugate must be presented in the proper orientation and with appropriate density. Therefore, receptor expression and glycosylation, ligand density and glycan geometry are crucial factors that control multivalent galectin binding, signalling and ultimately biological responses. A more thorough understanding of galectin–glycan lattices will be needed to support the design of multivalent glycosylated architectures, which is currently a highly active field of research in medicinal chemistry. High-affinity ligands for lectins, including those that mimic multimeric interactions identified in natural systems have been designed, synthesized and tested. Synthetic multivalent ligands typically include a central scaffold or core structure, a spacer or functionalized linker and lectin-binding glycoepitopes. Scaffolds with diverse properties, structure, flexibility and valency have been used to prepare multivalent ligands for biomedical applications.

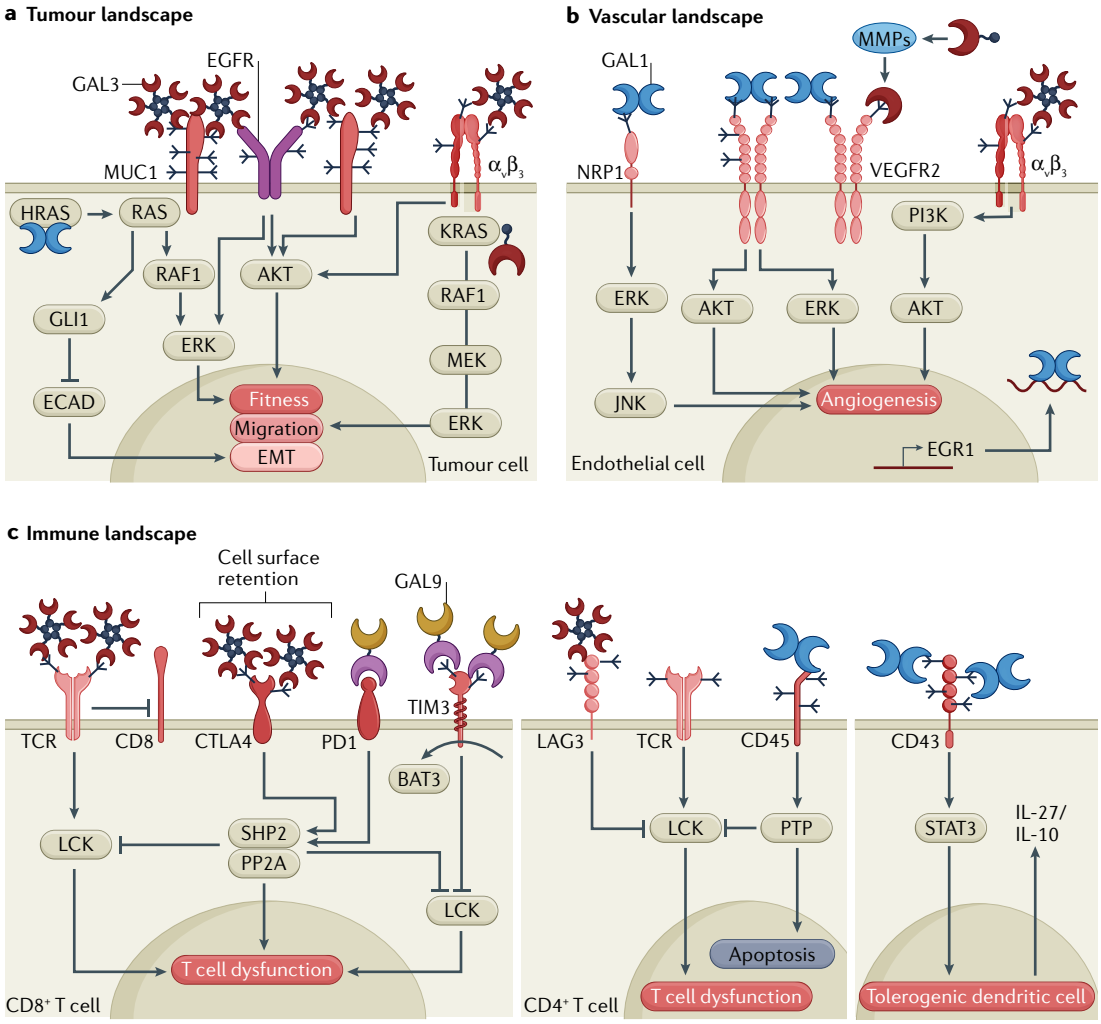


Fig. 2 | Galectins reprogramme tumour, endothelial and immune landscapes in the tumour microenvironment. a, Galectins (for example, GAL1, GAL3 and GAL9) use both intracellular and extracellular mechanisms to reprogramme the fate of tumour cells. They interact with receptors such as mucin 1 (MUC1), epidermal growth factor receptor (EGFR) and $\alpha_v\beta_3$ integrin and with intracellular proteins such as RAS to modulate signalling pathways that promote epithelial–mesenchymal transition (EMT), migration and invasion. **b**, Galectins control endothelial cell responses and promote aberrant angiogenesis by engaging various glycosylated cell surface receptors including neuropilin 1 (NRP1; ligand for GAL1), vascular endothelial growth factor receptor 2 (VEGFR2; ligand for GAL1 and GAL3) and $\alpha_v\beta_3$ integrin (ligand for GAL3), thereby modulating cell surface retention, endocytosis and signalling. GAL1 also modulates angiogenesis by binding to the mRNA of angiogenesis-related factors such as early growth response protein 1 (EGR1). **c**, Galectins shape the immune landscape of the tumour microenvironment by acting on both lymphoid and myeloid cells. Additionally, they suppress antitumour responses by engaging immune checkpoint or co-inhibitory molecules including CD45 and CD43 (GAL1), CTLA4 and LAG3 (GAL3) and PD1 and TIM3 (GAL9) leading to inhibition of lymphocyte cell-specific protein-tyrosine kinase (LCK)-mediated T cell activation, or promotion of STAT3-mediated myeloid cell reprogramming. Therefore, galectins facilitate tumour immune escape and mediate resistance to immunotherapeutic strategies. BAT3, HLA-B-associated transcript 3; ECAD, E-cadherin; GLI1, glioma-associated oncogene homologue 1; MMP, matrix metalloproteinase; PP2A, protein phosphatase 2 phosphatase activator; PTP, protein tyrosine phosphatase; SHP2, protein tyrosine phosphatase non-receptor type 11.

responses. For example, the induction of tolerogenic dendritic cells is driven by GAL1 (refs. [120,121]), and the recruitment and differentiation of immunosuppressive (M2-type) macrophages is triggered by GAL1 (ref. [31]), GAL3 (refs. [122,123]) or GAL9 (ref. [124]). Differentiation and expansion of $CD4^+$ and $CD8^+$ regulatory T cells (T_{reg} cells) is induced by GAL1 (refs. [103,125,126]) and GAL9 (ref. [127]); whereas apoptosis of effector T helper 1 (T_H1), T_H17 and $CD8^+$ T cells is mediated by GAL1 (refs. [128–132]) and GAL9 (ref. [133]); synthesis of anti-inflammatory cytokines such as IL-10 and IL-27 is elicited by GAL1 (refs. [126,134]); inhibition of natural killer (NK) cell-mediated cytotoxicity[135] and expansion of monocytic myeloid-derived suppressor cells (MDSCs) is induced by GAL3 (ref. [136]); T cell exclusion is orchestrated by GAL1 (ref. [92]), and the inhibition of chemokine gradients for T cell infiltration occurs in response to GAL3 (ref. [137]).

To add further complexity, galectins can also impair T cell activation by forming multivalent lattices that restrict the mobility of relevant immune receptors[50,138]. For example, GAL3 dampens antitumour

responses by decreasing T cell receptor (TCR) mobility through formation of a glycoprotein lattice that limits interactions between the TCR and CD8 coreceptor, thus promoting dysfunction and anergy of tumour-infiltrating lymphocytes[138] (Fig. 2). Interestingly, galectins may also support transmission of immune inhibitory signals by acting as ligands of co-inhibitory and immune checkpoint molecules, illustrated by the association of GAL1 with CD45 and CD43 (ref. [131]), GAL3 with LAG3 and CTLA4 (refs. [139,140]) and GAL9 with TIM3 and PD1 (refs. [133,141]) (Fig. 2).

By interrupting immune-activating mechanisms or triggering immune inhibitory pathways, galectins thwart various immunotherapeutic modalities. For example, GAL1 can reprogramme the tumour endothelium to upregulate both PDL1 and GAL9; blockade of this pathway increased T cell infiltration into the tumour and improved the response to anti-PD1 therapy[92]. Moreover, GAL9-mediated cross-talk between PD1 and TIM3 controls T cell exhaustion programmes and sensitivity to anti-PD1 therapy[133]. Thus, targeting galectins and/or their glycosylated ligands might counteract both local and systemic immunosuppressive circuits, dismantle tumour immune escape mechanisms and recalibrate responses to immunotherapeutic modalities.

Galectins in fibrosis

Pathological fibrosis is the consequence of abnormal mechanisms of tissue repair that are typically associated with cellular stress, chronic inflammation and/or severe tissue damage. It can lead to organ failure. Inadequate tissue regeneration has been described in several chronic fibroproliferative diseases such as IPF[24] and chronic inflammatory diseases such as NASH[19]. The abnormal wound-healing process results in scar formation with excessive collagen deposition via mechanisms that involve fibroblasts, epithelial and endothelial cells, and immune cells, notably macrophages[19]. Given the high prevalence of fibrotic diseases and the limitations of current drugs, which mainly limit the rate of organ dysfunction, the quest for effective therapies is clearly important.

GAL3 expression is upregulated in fibrotic lesions in human subjects[63], and the severity of hepatic and lung fibrosis is reduced in GAL3-deficient mice[33,142–145]. Thus, GAL3 has emerged as a promising therapeutic target for fibrotic diseases. Mechanistically, GAL3 induces a pro-fibrotic macrophage phenotype by interacting with the neutral amino acid transporter CD98 (ref. [123]). Likewise, GAL3-secreting macrophages drive myofibroblast differentiation, which ultimately results in scar formation[146]. Moreover, ECs and myofibroblasts upregulate GAL3 expression upon their activation[142,147,148]; this facilitates EMT, apoptosis, myofibroblast proliferation and enhanced production of fibronectin and other proteins found in the extracellular matrix[149–151].

Similarly to GAL3, GAL1 and GAL9 are also thought to contribute to the fibrotic process, largely on the basis of studies in galectin-deficient mice[152,153]. Increased levels of *GAL1* mRNA were detected in the lungs of patients with IPF[154], and GAL1 and GAL9 were prominently expressed in fibrotic liver[155,156]. The pro-fibrotic role of GAL1 is associated with TGFβ-driven fibroblast differentiation[157]. Accordingly, silencing GAL1 prevented hypoxia-induced pulmonary fibrosis and blocked the anticipated decline in lung function[154]. The role of GAL9 in fibrotic diseases of the lung remains under debate. Matsumoto et al.[158] proposed that GAL9 may have a protective role on the basis of its ability to suppress growth and induce dose-dependent apoptosis of human lung fibroblasts. However, GAL9 has also been highlighted as a mediator of lung fibrosis that acts via mechanisms that involve TGFβ signalling[153]. Thus, modulation of GAL1, GAL3 and/or GAL9 might control fibrotic processes by targeting fibroblasts, macrophages and ECs in several prominent pathological conditions, including IPF and NASH.

Galectin-targeted strategies

As our understanding of the role of galectins in cancer and fibrosis has improved, various chemical and biological agents that target GAL1, GAL3 and GAL9 have been developed. These agents include small-molecule chemical inhibitors, natural polysaccharides and their synthetic derivatives, peptides and peptidomimetics, and biological agents such as small interfering RNA (siRNA), aptamers, truncated galectins and neutralizing mAbs. In the next section, we describe both the advances and the difficulties in the development and clinical evaluation of current galectin-targeted agents.

Small-molecule carbohydrate inhibitors

The binding of galectins to β-galactosides such as LacNAc (**1**, Fig. 3) is determined by several distinct non-covalent interactions, including a hydrophilic hydrogen-bond network between the sugar hydroxyl groups and the conserved amino acids Arg, His, Asn and Glu in the galectin. Other interactions include hydrophobic CH–π bonds between the galactoside and a Trp-containing side chain in the galectin CRD[159] (Fig. 3a). The first rational approach to design galectin-antagonizing agents focused on small β-galactoside derivatives[160]. However, single carbohydrate–lectin interactions are typically low affinity, selectivity is not easily achieved, and carbohydrate molecules are cleared rapidly from the systemic circulation and are susceptible to degradation by glycosidases. Modifications at the C1 and C3 positions of monosaccharide β-galactose-based antagonists resulted in new molecules with improved interaction profiles within the galectin ligand-binding groove. These modified β-galactoside-based antagonists had enhanced affinity for galectins as determined by NMR and X-ray crystallography[161–163], but remained susceptible to hydrolytic degradation in vivo because of their labile O-glycosidic bonds.

In this context, N-, C- and S-glycosides were developed and evaluated as potentially hydrolytically stable galectin ligands[163–166], as well as novel α-thiogalactosides, which have an unexpectedly high affinity for GAL3. Zetterberg et al.[167] reported that C1 and C3 substitution with chlorophenylthiols and fluoroaryl triazoles, respectively, resulted in an α-thiogalactoside derivative (**2**) (3,4-dichlorophenyl 3-deoxy-3-[4-(3,4,5-trifluorophenyl)-1*H*-1,2,3-triazol-1-yl]-1-thio-α-D-galactopyranoside) with a dissociation constant (K_d) of 37 nM for GAL3 and 100-fold selectivity compared with GAL1 (Fig. 3b). Oral administration of compound (**2**), named GB1107 by Galecto (formerly Galecto Biotech), reduced the rate of human (A549) and mouse (LLC1) lung adenocarcinoma growth and blocked metastasis in experimental models[168]. Furthermore, treatment with GB1107 resulted in increased M1 polarization of intratumoural macrophages and favoured $CD8^+$ T cell infiltration, thereby enhancing the immunostimulatory impact of an anti-PDL1 mAb and inducing higher levels of expression of interferon-γ (IFNγ), granzyme B, perforin, Fas ligand and caspase 3 (ref. [168]). GB1107 also inhibited tumour growth in orthotopic mouse models of gastric cancer, thus substantiating the therapeutic relevance of this inhibitor under relevant neoplastic conditions[169].

Studies also documented the design of aminopyrimidinyl α-thiogalactoside (**3**), which, despite its comparatively low affinity for GAL3 (K_d = 1.7 μM) compared with GB1107 (**2**), proved to be a more selective GAL3 inhibitor. Specifically, it showed >500-fold selectivity compared with GAL1 and a range of 34- to >500-fold selectivity compared with other galectins tested as determined by fluorescence polarization assays[170] (Fig. 3b). Furthermore, a patent assigned to Bristol Myers Squibb reporting α-thiogalactosides derived from GB1107 bearing additional chemical modifications, such as alkylation of HO-2 and

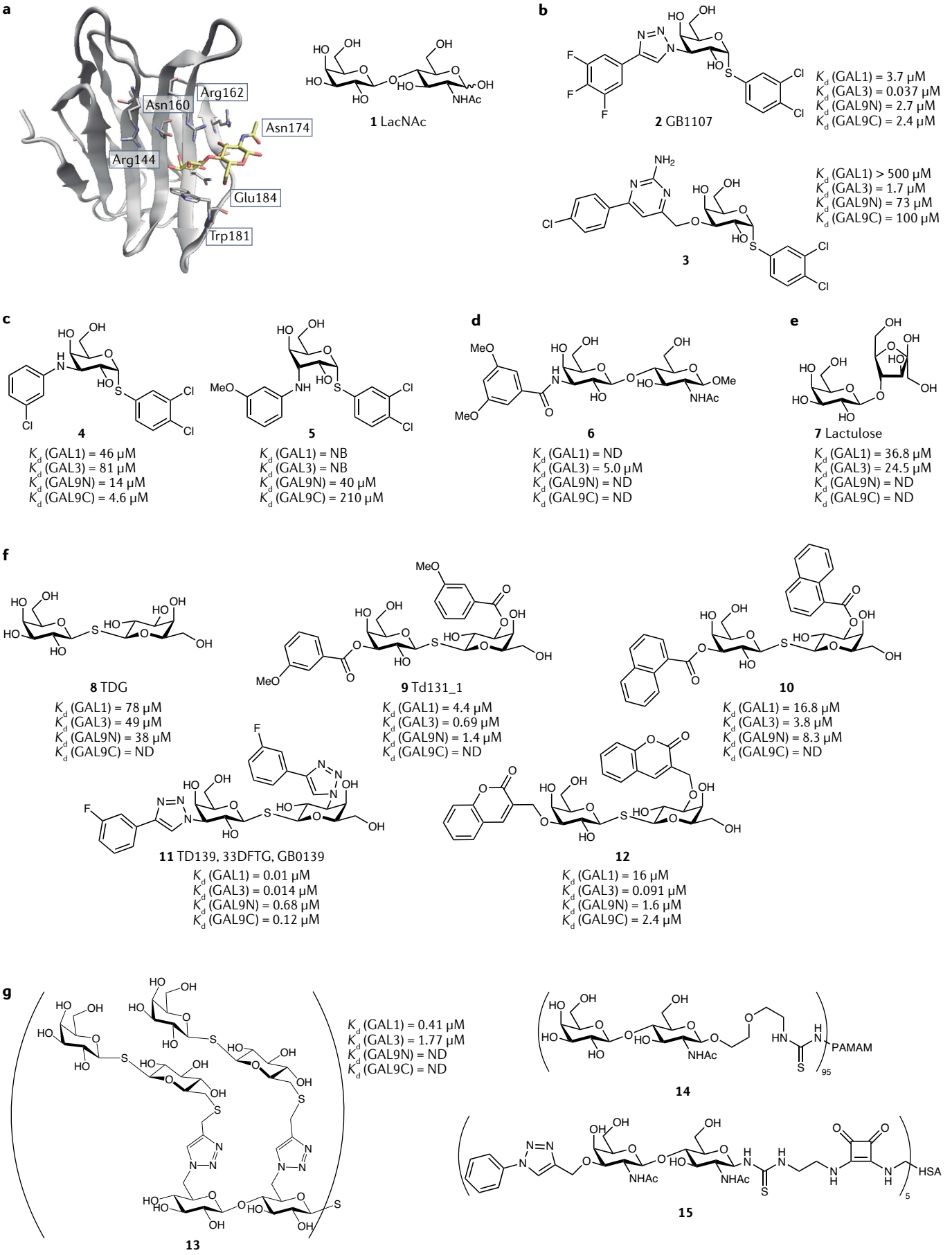

a
Arg162
Asn160
Asn174
Arg144
Glu184
Trp181
1 LacNAc
b
2 GB1107
K_d (GAL1) = 3.7 μM
K_d (GAL3) = 0.037 μM
K_d (GAL9N) = 2.7 μM
K_d (GAL9C) = 2.4 μM
3
K_d (GAL1) > 500 μM
K_d (GAL3) = 1.7 μM
K_d (GAL9N) = 73 μM
K_d (GAL9C) = 100 μM
c
4
K_d (GAL1) = 46 μM
K_d (GAL3) = 81 μM
K_d (GAL9N) = 14 μM
K_d (GAL9C) = 4.6 μM
5
K_d (GAL1) = NB
K_d (GAL3) = NB
K_d (GAL9N) = 40 μM
K_d (GAL9C) = 210 μM
d
6
K_d (GAL1) = ND
K_d (GAL3) = 5.0 μM
K_d (GAL9N) = ND
K_d (GAL9C) = ND
e
7 Lactulose
K_d (GAL1) = 36.8 μM
K_d (GAL3) = 24.5 μM
K_d (GAL9N) = ND
K_d (GAL9C) = ND
f
8 TDG
K_d (GAL1) = 78 μM
K_d (GAL3) = 49 μM
K_d (GAL9N) = 38 μM
K_d (GAL9C) = ND
9 Td131_1
K_d (GAL1) = 4.4 μM
K_d (GAL3) = 0.69 μM
K_d (GAL9N) = 1.4 μM
K_d (GAL9C) = ND
10
K_d (GAL1) = 16.8 μM
K_d (GAL3) = 3.8 μM
K_d (GAL9N) = 8.3 μM
K_d (GAL9C) = ND
11 TD139, 33DFTG, GB0139
K_d (GAL1) = 0.01 μM
K_d (GAL3) = 0.014 μM
K_d (GAL9N) = 0.68 μM
K_d (GAL9C) = 0.12 μM
12
K_d (GAL1) = 16 μM
K_d (GAL3) = 0.091 μM
K_d (GAL9N) = 1.6 μM
K_d (GAL9C) = 2.4 μM
g
13
K_d (GAL1) = 0.41 μM
K_d (GAL3) = 1.77 μM
K_d (GAL9N) = ND
K_d (GAL9C) = ND
14
15

Fig. 3 | Small-molecule carbohydrate inhibitors. a, Representation of human GAL3 in complex with LacNAc (**1**). Details of GAL3–LacNAc interactions, with GAL3 shown in silver (representation in new cartoon) and the key residues for LacNAc recognition in the same colour (licorice representation). LacNAc dissacharide is shown in yellow, also in licorice representation. On the right, the chemical structure of LacNAc disaccharide is also shown. **b**, GAL3 selective inhibitors, α-thiogalactosides **2** (also named GB1107) and **3**. **c**, GAL9C and GAL9N selective inhibitors, α-thiogalactoside **4** and α-thioguloside **5**. **d**, LacNAc-derived GAL3 inhibitor (**6**). **e**, Lactulose (**7**). **f**, TDG (**8**) and TDG derivatives (**9**, **10**, **11** and **12**). **g**, Multivalent inhibitors, including glycoclusters (**13**), glycodendrimers (**14**) and neoglycoproteins (**15**). K_d, dissociation constant; NB, no binding observed; ND, not determined.

oxidation of the anomeric thiol group, was recently published[171]. Moreover, Idorsia Pharmaceuticals patented α-C-galactopyranosides bearing modifications on HO-2, HO-3 and the anomeric group[172]. Galecto developed GB1211, an α-thiogalactoside that is closely related to GB1107 with excellent affinity for GAL3 (K_d = 23 nM)[173]. GB1211 was characterized as a highly selective, orally available GAL3 inhibitor, and its therapeutic potential was demonstrated in mouse models of CCl_4-induced liver fibrosis and bleomycin-induced lung fibrosis[174]. Orally administered GB1211 was well tolerated in phase I clinical studies in healthy subjects (NCT03809052, Table 1). A phase I/II study of this compound in patients with confirmed NASH and liver fibrosis was withdrawn (NCT04607655, Table 1). However, a phase I/II trial of GB1211 in participants with hepatic impairment (Child–Pugh class B and C) is ongoing (NCT05009680, Table 1). Galecto recently presented initial results from this phase I/II trial indicating that GB1211 is safe and well tolerated[175], and early signs of clinical effect in patients with moderate and severe hepatic impairment were noted[176]. A phase I/II trial to assess the combination of GB1211 with the anti-PDL1 mAb atezolizumab in patients with non-small-cell lung cancer (NSCLC) has started recruitment (NCT05240131, Table 1).

To achieve higher selectivity towards GAL3, interactions between galectins and alternative monosaccharides, including D-mannose, D-talose and D-gulose derivatives have been evaluated, although these compounds exhibited no significant functional activity in in vitro or in vivo assays[177–182]. However, a recent study reported that GAL9C and GAL9N CRDs demonstrated good affinity towards synthetic 3-deoxy-3-*N*-arylated-α-D-galactoside (**4**) and α-guloside (**5**) derivatives, respectively, compared with other galectins, as determined by fluorescence anisotropy assays[183] (Fig. 3c).

Lactose (Lac) and LacNAc disaccharides, the natural galectin ligands in glycoconjugates, have been evaluated as galectin inhibitors[37,184]. Substitution of LacNAc at O3′ with aromatic moieties has been explored extensively as a means to generate new and favourable π–arene interactions with the conserved Arg144 residue; this effort has resulted in the development of several effective GAL3 ligands, including a LacNAc derivative[163,185,186] (**6**, Fig. 3d). Alternatively, synthetic lactulose amines evaluated as galectin modulators were found to induce tumour cell apoptosis and inhibit homotypic cell aggregation and EC morphogenesis[187]. More recently, Kishor and colleagues[188] explored interactions of GAL1 and GAL3 with lactulose (**7**, Fig. 3e) by crystallography and proposed this disaccharide as another scaffold for the design of novel galectin inhibitors.

S-, C- and N-disaccharides have been investigated as stable galectin inhibitors. The synthetic non-reducing disaccharide thiodigalactoside (TDG) (**8**), a compound that binds to galectins with similar affinity to LacNAc but lacks labile glycosidic bonds, emerged as a valuable building block for the preparation of stable galectin ligands[189,190] (Fig. 3f). Similarly, previous work on galactose and lactose derivatives led to the symmetrical substitution of TDG at O3 and O3′ with aromatic esters, amides or triazole groups, which ultimately resulted in several galectin inhibitors with nanomolar affinities[164,189–191] (Fig. 3f). Bis-[3-*O*-(3-methoxybenzoyl)-β-D-galactopyranosyl] sulfane (named Td131_1) (**9**) and bis-(3-*O*-1-naphthoyl-β-D-galactopyranosyl) sulfane (**10**) (Fig. 3f) inhibited the migration of human prostate and NSCLC cell lines[189]. Td131_1 (**9**) was also tested in papillary thyroid cancer cells in vitro, and, despite the high concentrations required, it promoted cancer cell apoptosis and synergized with doxorubicin[192]. These successful results led to the evaluation of 3,3′-disubstituted TDG derivatives as GAL3 inhibitors in chronic inflammation and fibrosis[193]. For example, bis-(3-deoxy-3-(4-(3-fluorophenyl)-1*H*-1,2,3-triazol-1-yl)-β-D-galactopyranosyl) sulfane (**11**, Fig. 3f; also named TD139, 33DFTG, and later renamed GB0139 by Galecto), is a selective potent GAL1 and GAL3 inhibitor[145] with low affinity for GAL2, GAL4N, GAL4C, GAL8N and GAL9N[145] and low or no significant affinity for GAL7 (refs. [144,145]). Inhibition of GAL3 with GB0139 (**11**) in a model of bleomycin-induced lung fibrosis resulted in decreased TGFβ receptor at the cell surface, together with suppressed β-catenin activation and attenuated severity of lung fibrosis[145]. This result was obtained when GB0139 was administered at much lower concentrations than pirfenidone, an anti-inflammatory drug approved for the treatment of IPF[194]. Bratteby et al.[195] recently developed surrogate positron emission tomography (PET) radiotracers for GB0139 and GB1107 (**2**) and described their pharmacokinetics. The ^{18}F-radiolabelled GB0139 surrogate was cleared much more rapidly than the GB1107-derived compound, suggesting that although systemic administration may be favourable for monosaccharide-based galectin inhibitors, the rapid excretion of disaccharide derivatives could limit their activity. A phase I/II clinical trial initiated in 2014 to assess the safety, pharmacokinetics and tolerability of GB0139 via a dry-powder inhaler in human volunteers and patients with IPF (NCT02257177) showed that this compound was safe and well tolerated. Furthermore, administration via this route inhibited GAL3 expression by bronchoalveolar lavage macrophages and decreased the concentration of plasma biomarkers associated with IPF progression[23]. A phase IIb clinical trial to investigate the safety and efficacy of GB0139 (**11**) in patients with IPF has completed enrolment (NCT03832946). As TDG-based ligands progressed towards clinical trials, further studies to improve selectivity highlighted that their substitution with bulky aromatic groups favours interactions with GAL3 over GAL1 (ref. [196]). Consistent with these results, a coumarin-derived TDG (**12**) showed a 175-fold increase in selectivity over GAL1 with similar efficacy to GB0139 in the bleomycin-induced model of lung fibrosis[197] (Fig. 3f). Recently, tetrahydropyran-based thiodisaccharide mimics with a reduced number of H-bond donors to improve permeability and bioavailability were also described[198].

Natural multimeric ligands such as multi-antennary *N*-glycans have inspired the design of multivalent glycosylated architectures to achieve tighter binding and overcome the naturally weak lectin–glycan interactions based on the cluster glycoside effect[199]. Research over the past decade has identified a wide variety of galectin-targeted multivalent ligands of distinctive nature and valency, including glycoclusters, glycodendrimers, glyconanoparticles, glycopolymers and neoglycoproteins. Multivalent glycoclusters and low-valency multivalent ligands have been designed with strict control of valency, geometry and conformation, and with scaffolds ranging in size from oligosaccharides and cyclodextrins (**13**, Fig. 3g) to calixarenes and macrocycles[165,200–202]. Poly(amidoamine) lactose or LacNAc-bearing glycodendrimers with valencies up to 95 have also been evaluated as potential GAL3 inhibitors and shown to inhibit (when small dendrimers) or enhance GAL3-mediated cell clustering in vitro[203] (**14**, Fig. 3g). Moreover, conjugation of LacdiNAc (GalNAcβ(1,4)GlcNAc) disaccharides or TDG derivatives to bovine serum albumin generated neoglycoproteins with good affinity and moderate selectivity for GAL3 over GAL1 (refs. [204,205]). Notably,

Table 1 | Clinical trials of galectin-modulating agents

Galectin inhibitor type	Drug	Clinical trial (phase)	Primary outcome	Status
Malignancies				
Small-molecule carbohydrate inhibitor	GB1211	NCT05240131	NSCLC (with atezolizumab)	2022, recruiting
		GALLANT-1 (I/II)		
Polysaccharide	Belapectin GR-MD-02	NCT04987996 (II)	Metastatic melanoma and HNSCC (in combination with pembrolizumab versus pembrolizumab monotherapy)	2021, suspended
		NCT02575404 (I)	Melanoma, NSCLC and HNSCC (in combination with pembrolizumab)	2016–present; active, not recruiting
		NCT02117362 (I)	Metastatic melanoma (in combination with ipilimumab)	2014–2018, completed
	PectaSol-C	NCT01681823 (II)	Prostate cancer	2013–2020, completed
	Davanat GM-CT-01	NCT00054977 (I)	Solid tumours (with or without 5-FU)	2003–2006, completed
		NCT00110721 (II)	Metastatic CRC (with 5-FU as third- or fourth-line therapy)	2005–2008, terminated (study protocol amended to a new treatment regimen: study DAVFU-006)
		NCT00388700	CRC (with 5-FU, bevacizumab and leucovorin)	2006–2009, withdrawn
		DAVFU-006 (II)		
	GCS-100	NCT00609817 (I)	Multiple myeloma (in combination with bortezomib/dexamethasone)	2008–2009, terminated
		NCT00514696 (II)	CLL	2007–2009, completed
Peptide	OTX008	NCT01724320 (I)	Advanced solid tumours	2012, unknown
mAb	LYT200 (anti-GAL9 mAb)	NCT04666688 (I/II)	Metastatic solid tumours, alone or with chemotherapy (gemcitabine/nab-paclitaxel) or anti-PD1	2020–present, recruiting
Fibrotic diseases				
Small-molecule carbohydrate inhibitor	GB1211	NCT05009680	Hepatic impairment, Child–Pugh B,C	2021–present; active, not recruiting
		GULLIVER-2 (I/II)		
		NCT04607655	NASH and liver fibrosis	2021, withdrawn (owing to COVID-19 pandemic and change in the clinical development strategy)
		GULLIVER-1 (I/II)		
		NCT03809052 (I)	HS and NASH/liver fibrosis	2019, completed
	GB0139	NCT03832946	IPF	2019–present; active, not recruiting
		GALACTIC-1 (II)		
		NCT02257177 (I/II)	HS and patients with IPF	2014–2016, completed
Polysaccharide	Belapectin GR-MD-02	NCT04365868	Prevention of oesophageal varices in NASH	2020–present, recruiting
		NAVIGATE (IIb/III)		
		NCT02421094	Liver fibrosis in NASH	2015–2016, completed
		NASH-FX (II)		
		NCT02462967	Liver fibrosis and portal hypertension in NASH cirrhosis	2015–2017, completed
		NASH-CX (II)		
		NCT01899859 (I)	NASH and advanced fibrosis	2013–2015, completed

5-FU, 5-fluorouracil; CLL, chronic lymphocytic leukaemia; COVID-19, coronavirus disease 2019; CRC, colorectal cancer; HNSCC, head and neck squamous cell carcinoma; HS, healthy subjects; IPF, idiopathic pulmonary fibrosis; mAb, monoclonal antibody; NASH, nonalcoholic steatohepatitis; NSCLC, non-small-cell lung cancer.

a natural glycopeptide rich in Galβ(1,3)GalNAc structures isolated from the cod antifreeze glycoprotein binds to GAL3 with high affinity (K_d 97 pM) and inhibits adhesion of PC-3 prostate cancer cells to ECs as well as GAL3-mediated T cell apoptosis[206]. Moreover, complex type *N*-glycans prepared from chicken eggs and coupled by click chemistry to human serum albumin (HSA) as scaffold led to multivalent GAL3 ligands[207]. More recently, the chemoenzymatic synthesis of neoglycoproteins based on coupling of C3-substituted LacdiNAc glycomimetics

to HSA (**15**, Fig. 3g) has been reported; these derivatives protected T lymphocytes against GAL3-induced apoptosis in vitro[208].

Given that the functions of galectins vary between the intracellular and extracellular compartments[16,46], Stegmayr et al.[209] investigated the intracellular activity and membrane permeability of selected small-molecule GAL3 inhibitors including compounds GB1107 (**2**), GB0139 (**11**) and a 1*H*-1,2,3-triazol-1-yl TDG derivative[210]. Inhibitor uptake and intracellular potency correlated with the polar surface area of the compounds, which is a determinant for passive membrane permeability. Of the compounds investigated, GB1107 was the most potent, although GB0139 had increased intracellular activity following pre-incubation, suggesting that it can reach the cytosolic compartment given sufficient time to cross the plasma membrane[209]. Thus, small-molecule galectin inhibitors that can cross the cell membrane, such as GB0139, are important tools to discern extracellular and intracellular functions of galectins, and could potentially be used to target intracellular galectins.

Polysaccharides and derivatives

Complex polysaccharides derived from natural sources such as pectins and galactomannans, pH- and heat-modified pectins (for example, MCPs), and galactomannan-derived compounds have shown antitumoural, antimetastatic and anti-inflammatory activities[138,211–214].

Pectins are a class of complex polysaccharides found in the primary cell walls of plants and are composed of an anionic galacturonan backbone (a linear chain of α(1–4)-linked D-galacturonic acids) and neutral sugar side chains that contain primarily D-galactose, L-rhamnose and L-arabinose[215,216]. Underivatized pectins have shown beneficial health effects, primarily when introduced as dietary fibre to prevent colon cancer[217]; however, the most promising anticancer effects were observed in response to MCPs. Treatment of pectins to obtain MCPs eliminates galacturonic acid esters from the homogalacturonan regions and exposes galactose-containing side chains, which have been proposed as major determinants for galectin binding. However, the affinity and selectivity of these compounds is still under debate, as little or no inhibitory activity was detected by fluorescence anisotropy[218]. Nevertheless, MCPs have been extensively studied for potential antitumour and antimetastatic activities in several cancer types[211,212,219,220]. For example, PectaSol and PectaSol-C (**16**) (Fig. 4) are commercial MCPs developed by EcoNugenics that have cytotoxic activity against murine and human prostate cancer cell lines via suppression of MAPK signalling and induction of caspase 3 cleavage[213]. PectaSol-C functions synergistically with paclitaxel to promote cytotoxicity in ovarian cancer cells by modulating GAL3-driven activation of signal transducer and activator of transcription 3 (STAT3)[221]. In 2003, a phase II pilot study[222] evaluated tolerability and efficacy of PectaSol-C in patients diagnosed with prostate cancer. Oral administration of PectaSol-C resulted in a statistically significant increase in the prostate-specific antigen (PSA) doubling time, potentially indicating a slowing of tumour progression. In a subsequent phase II clinical trial in patients with biochemical relapse of prostate cancer, oral administration of PectaSol-C was evaluated by measuring PSA kinetics as a potential marker for cancer progression (NCT01681823, Table 1). Results showed that patients treated with PectaSol-C exhibited no PSA progression or lengthening of PSA doubling time compared with historical data, potentially indicating slower disease progression[223].

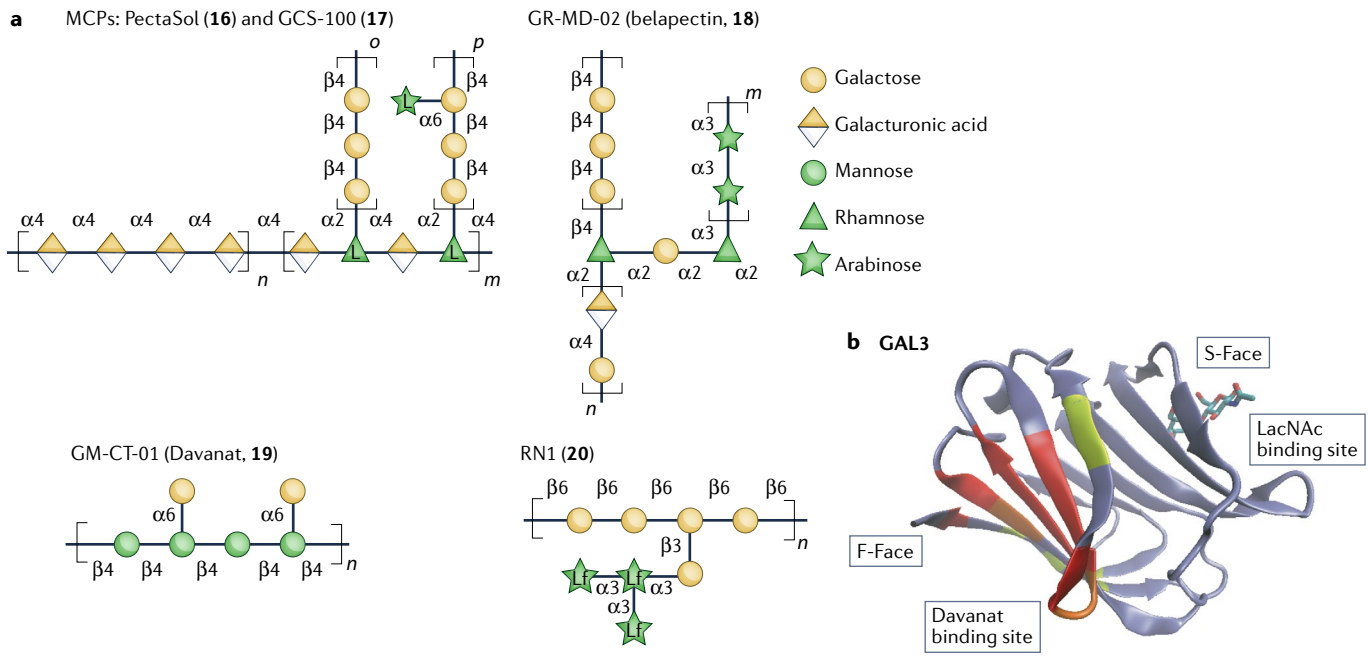


Fig. 4 | Structure of natural polysaccharides and their derivatives as galectin inhibitors. a, Structures of modified citrus pectin (MCP) polysaccharides PectaSol (**16**) and GCS-100 (**17**), galactoarabino-rhamnogalacturonate GR-MD-02 (belapectin, **18**), galactomannan Davanat (**19**) and arabinogalactan RN1 (**20**). *n*, *m*, *o* and *p* denote the number of repetitive residues in the polysaccharide structure. Dissociation constant (K_d) values were not measured for these compounds. **b**, Non-canonical binding site of Davanat on GAL3. The S- and F-faces of the GAL3 carbohydrate-recognition domain (CRD) are shown. Amino acids that interact with Davanat are shown in greenish yellow (low interaction), orange (moderate interaction) or red (high interaction). K_d values were not measured for these compounds. Glycans are depicted following guidelines from the Symbol Nomenclature for Glycans Group[305].

GCS-100 (**17**, Fig. 4), developed by La Jolla Pharmaceutical Company, was initially developed as a potential inhibitor of GAL3. GCS-100 has broad antitumour activity, including inducing apoptosis of multiple myeloma cells[212,224–226]. Moreover, GCS-100 alone or in combination with BCL-2 homology domain 3 (BH3) mimetics induced apoptosis in acute myeloid leukaemia cells[227]. A phase II clinical trial (NCT00514696) evaluated intravenous administration of GCS-100 in patients with chronic lymphocytic leukaemia. The inhibitor showed excellent tolerability and led to partial remission in 25% of patients and >50% shrinkage of lymph node lesions in 16% of patients.

GR-MD-02 (**18**) (renamed belapectin by Galectin Therapeutics) (Fig. 4), a pectin-derived galactoarabino-rhamnogalacturonate substituted with predominantly β(1–4)-D-galactose and α(1–5)-L-arabinose side chains, has been proposed as a GAL3 inhibitor. The antitumour effects of belapectin were explored in immunocompetent cancer models in combination with an agonist anti-OX40 antibody. This drug combination promoted tumour regression and increased survival in mouse models by mitigating MDSC-driven immunosuppression, increasing $CD8^+$ T cell recruitment and reducing the frequency of $CD4^+FOXP3^+$ T_{reg} cells[122]. This compound was also tested in two phase I clinical trials in combination with mAbs that inhibit the immune checkpoint proteins CTLA4 (ipilimumab) or PD1 (pembrolizumab) in patients diagnosed with melanoma (NCT02117362 and NCT02575404). A combination of belapectin and pembrolizumab administered intravenously to patients with advanced metastatic melanoma or head and neck squamous cell carcinoma (HNSCC) (NCT02575404) was well tolerated and generated fewer immune-mediated adverse events than anticipated, based on findings from pembrolizumab monotherapy. Moreover, belapectin and pembrolizumab treatment enhanced the activation of effector memory T cells and decreased the frequency of MDSCs in patients responding to this regimen (50% of patients with metastasic melanoma and 33% of patients with HNSCC)[228].

GM-CT-01 (**19**), also known as Davanat (Galectin Therapeutics; Fig. 4), is a chemically modified galactomannan from the plant *Cyamopsis tetragonoloba* (guar gum) that has been shown to enhance the efficacy of the chemotherapeutic agent 5-fluorouracil (5-FU) in models of colon and breast cancer[229]. These results led to phase I (NCT00054977) and phase II (NCT00110721) clinical trials that demonstrated a lack of toxicity and a 46% increase in survival of patients with colorectal cancer who received this drug combination[214,230]. A comparison of GCS-100 (**17**) and Davanat (**19**) suggests that these two polysaccharides might have different mechanisms of action. Although the binding site for PectaSol or GCS-100 is still not clear[218], Davanat binds to the F-face of the GAL3 β-sandwich and not to the canonical site for β/α-galactosides[231] (Fig. 4b).

Plant-derived polysaccharides have also been proposed as therapeutics for liver, kidney and lung fibrosis, mainly via mechanisms that involve inhibition of GAL3 (refs. [142,146]). Inhibition of GAL3 with belapectin (**18**, Fig. 4) and Davanat (**19**, Fig. 4) was first evaluated in a toxin-induced model of liver fibrosis[232]. Intraperitoneal administration of these polysaccharides resulted in decreased collagen content, attenuated liver fibrosis, diminished cirrhosis and a reduced percentage of GAL3-expressing macrophages[232]. These two inhibitors were also tested in a murine model of NASH[233]. Intravenous administration of belapectin resulted in a substantial reduction in collagen deposition, hepatocellular damage, NASH activity and fibrosis — features that were associated with reduced markers of inflammation that included inducible nitric oxide synthase (iNOS) and $CD36^+$ pro-inflammatory macrophages. By contrast, administration of Davanat had no effect[233].

A phase I clinical trial of belapectin (**18**) in patients with NASH with advanced hepatic fibrosis revealed no toxicity and good tolerability[234] (NCT01899859, Table 1). Two phase II clinical trials evaluated the efficacy of this compound in liver fibrosis. In patients with NASH with advanced fibrosis (NCT02421094, Table 1), belapectin had no significant effects on levels of non-invasive biomarkers of liver inflammation or fibrosis over a 4-month period[234]. In liver fibrosis and resultant portal hypertension in patients with NASH cirrhosis[235] (NCT02462967, Table 1), belapectin had no impact on fibrosis or nonalcoholic fatty liver disease activity score. However, in a patient subgroup it showed a significant effect on portal pressure and prevented the development of oesophageal varices, which is an early sign of serious complications in patients with cirrhosis. This led to the development of a phase IIb/III trial designed to evaluate its safety and efficacy specifically in patients with NASH-associated cirrhosis for the prevention of oesophageal varices (NCT04365868, Table 1).

Other polysaccharides have also emerged as potential galectin inhibitors. For example, RN1 (**20**, Fig. 4), an arabinogalactan polysaccharide isolated from the flowers of the Chinese ginseng plant (*Panax notoginseng*), was proposed as a GAL3 inhibitor. This compound showed antitumoural activity in pancreatic ductal adenocarcinoma both in vitro and in vivo[236]. Other ginseng-derived pectins prevented GAL3-driven T cell apoptosis and reduced tumour growth in mouse models of sarcoma[237].

Peptides and peptidomimetics

A peptide known as Anginex (βpep-25, **21**, Fig. 5) that was designed based on the structure of anti-angiogenic proteins was later found to be a potential anti-galectin therapeutic[238,239]. Anginex is a 33-mer with an amphipathic β-sheet that initially lacked a specific molecular target. However, several studies revealed that this compound had potent anti-angiogenic activity via its capacity to block EC adhesion and migration, ultimately leading to inhibition of angiogenesis and tumour growth[240–242]. GAL1 was subsequently identified as the molecular target of Anginex using fluorescence microscopy, NMR and surface plasmon resonance (SPR) analysis, with a strong binding constant (K_d 90 nM) (ref. [243]). However, despite these initially promising results, Anginex was beset by several drawbacks that hampered its translation to clinical settings; these include its complex chemical synthesis, short half-life and poor stability. Aiming at a more affordable alternative than peptide synthesis, an artificial gene encoding Anginex was developed, offering a recombinant peptide with the same structure and slightly decreased activity[244]. To overcome its rapid clearance and sensitivity to endogenous endopeptidases, an adeno-associated virus vehicle delivery system was evaluated[245]. Using this delivery system, Anginex suppressed proliferation, migration and invasion of human umbilical vein endothelial cells (HUVECs) and inhibited angiogenesis and tumour growth in an ovarian cancer xenograft model. Finally, the Anginex derivative 6DBF7 (**22**, Fig. 5), a dibenzofuran (DBF)-based partial peptidomimetic, demonstrated improved stability[246]. 6DBF7 retained anti-angiogenic and tumour growth inhibitory activity in ovarian cancer mouse models and was even more effective in vivo than Anginex[246]. Dings et al.[247] designed and tested new optimized analogues of 6DBF7 (**22**) and introduced DB21 (**23**, Fig. 5), a derivative capable of increased inhibition of angiogenesis and tumour growth in melanoma, lung adenocarcinoma and ovarian cancer models. Both 6DBF7 and DB21 were identified as GAL1 inhibitors, although no data on their interactions with other members of the galectin family are available.

More recently, OTX008 (or 0118, also called PTX008; **24**, Fig. 5) was developed as a non-peptidic calixarene-based Anginex topomimetic with GAL1 binding capacity[248]. OTX008 inhibited angiogenesis in both in vitro and mouse model systems[99,249]. It also synergized with the tyrosine kinase inhibitor sunitinib in ovarian carcinoma and glioblastoma xenograft models[250]. A phase I clinical trial sponsored by Oncoethix assessed the impact of OTX008 administered subcutaneously to patients with advanced solid tumours and documented its rapid absorption and urinary excretion (NCT01724320)[251]. More recently, Leung et al.[252] demonstrated that OTX008 combined with sorafenib reduced tumour growth and enhanced the therapeutic effects of sorafenib alone in experimental models of hepatocellular carcinoma. OTX008 was also effective against thyroid cancer in in vitro and in vivo studies[253]. Other calixarene derivatives include the polycationic calixarene-based compound PTX013 (**25**), which displayed 50-fold enhanced activity over that of OTX008 in an experimental melanoma model[254]. Furthermore, new analogues showed increased cell growth inhibitory activity in HUVECs and MA148 ovarian cancer cells, when compared with the parent calixarene compound **24** (ref. [255]). However, the quest for improved activity led to 'off-target' side effects: PTX013 was toxic in mice, and considering that, in vitro, PTX013 could be considered a cytotoxic drug more than a cytostatic agent (like parental PTX008), the authors postulated that GAL1 might not be the only target of this compound[254,256]. In this sense, PTX013 has recently been

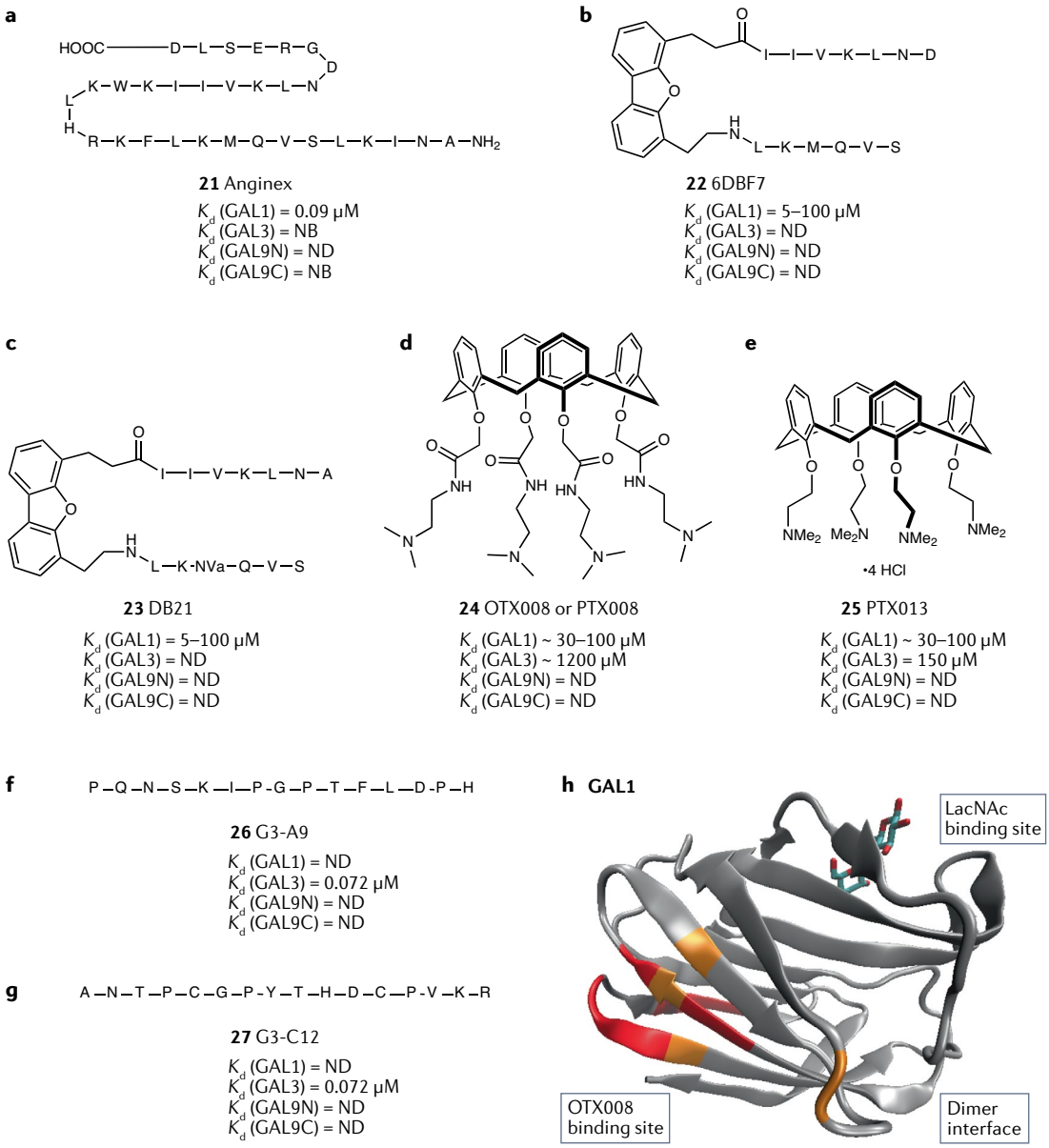


Fig. 5 | Peptides and peptidomimetics as galectin inhibitors. Structures of: **a**, Anginex (**21**); **b**, 6DBF7 (**22**); **c**, DB21 (**23**); **d**, OTX008 (**24**); **e**, PTX013 (**25**); **f**, G3-A9 (**26**); and **g**, G3-C12 (**27**). **h**, GAL1 binding site for OTX008 (**24**). Amino acid residues that interact with OTX008 are highlighted in orange (moderate interaction) or red (high interaction). NB, no binding observed; ND, not determined.

shown to bind to both GAL3 and GAL1; this was suggested as a potential mechanism underlying its increased activity[257]. The development of PTX013 is an important example of how structural modifications aimed at improving biological activity can result in increased toxicity via potentially different mechanisms of action[254].

An alternative approach to designing galectin-targeting peptides was developed by the Deutscher group[258], which generated two small synthetic 15-mer peptides, G3-A9 (**26**, Fig. 5) and G3-C12 (**27**, Fig. 5), which were identified by combinatorial bacteriophage display. These peptides were selective for GAL3, but not for GAL1 or GAL4. They blocked the interaction of GAL3 with the Tf antigen and inhibited the adhesion of MDA-MD-435 human breast carcinoma cells to ECs in vitro. Peptide G3-C12 (**27**) is antimetastatic via its capacity to modulate GAL3 activity[258]; its administration resulted in a 72% reduction in MDA-MB-231 cell adhesion to vasculature in nude mice. However, despite its therapeutic potential, in vivo studies showed that G3-C12 accumulated in the kidneys[259].

To overcome this limitation, control its renal clearance and improve its therapeutic efficacy, G3-C12 (**27**, Fig. 5) was conjugated to an *N*-(2-hydroxypropyl)methacrylamide polymeric carrier. This substitution increased its molecular size and generated a larger GAL3-targeted copolymer that was proposed as an anticancer agent[260,261]. Conjugation of HMPA–G3-C12 copolymer to 5-FU led to increased cytotoxicity, tumour cell apoptosis and inhibition of migration in vitro compared with 5-FU, as well as greater reductions in tumour volume in a PC-3 prostate cancer mouse model[262]. Micellar nanoparticles assembled by poly(oligo(ethylene glycol)) monomethyl ether methacrylate (POEGMA) and poly(ε-caprolactone) (PCL) copolymers conjugated to G3-C12 peptide were designed to deliver G3-C12 (**27**, Fig. 5) to the tumour microenvironment. This copolymer formed nanoparticles that were loaded with the anticancer drug bufalin (BUF) and tested as a drug delivery system for castration-resistant prostate cancer (CRPC). Biodegradable BUF-G3-C12 nanoparticles exhibited controlled drug release and enhanced tumour reduction in mice bearing DU145 prostate cancer cells[263].

Biological agents

To avoid 'off-target' effects and achieve higher selectivity, efforts are underway to develop biological agents that target individual members of the galectin family, including galectin-specific neutralizing mAbs, decoys, aptamers and RNA silencing strategies using siRNA[9,98,100,264–267]. Both RNA interference (RNAi) and CRISPR technologies have been used to inhibit the expression and function of specific galectins in several experimental models[268]. GAL1 silencing with RNA technologies led to dramatic antitumour effects in several cancer models[9,98,114,125,126,128,256] by unleashing antitumour immunity and suppressing aberrant vascularization[9,98,266]. More recently, administration of a GAL1-targeted DNA aptamer resulted in antitumour effects via its capacity to restore immune function in vivo[267]. Despite promising results in preclinical models, more investigations into the mechanisms that underlie nucleic acid-based targeting strategies will be required to translate these findings into clinically important therapies.

Over the past two decades, mAb-based therapies that target immune checkpoint or pro-angiogenic pathways have generated unprecedented success in treating a broad range of malignancies[269]. mAb-based therapeutics directed against galectins could be an important approach, as these agents are target specific and well tolerated in humans[270]. To date, several GAL1-neutralizing mAbs have been developed, characterized and functionally evaluated in in vitro and in vivo models[9,98,100,271,272]. For example, anti-GAL1 mAbs inhibit GAL1-mediated apoptosis of Epstein–Barr virus (EBV)-specific $CD8^+$ T cells, suggesting that they could prevent EBV-induced immune evasion in post-transplant lymphoproliferative disorders (PTLDs)[271]. Moreover, these mAbs restrained tumour growth in models of Kaposi's sarcoma, lung adenocarcinoma, melanoma and T cell lymphoma[9,98]. Because GAL1 is known to activate VEGFR2 signalling[9], the ability of anti-GAL1 mAb to prevent resistance to VEGF blockade was assessed in various models. Blockade of GAL1–*N*-glycan interactions using a functional blocking mAb not only circumvented resistance to anti-VEGF but also normalized aberrant tumour vasculature and promoted an increased influx of immune cells to the tumour bed[9]. Given these promising findings, additional mAbs specific for GAL1 have been designed and found to have therapeutic potential[100,272]. Interestingly, mAb-mediated GAL1 blockade enhanced the immunostimulatory effect of anti-PD1 therapy in HNSCC by preventing T cell exclusion from tumour sites[92]. Moreover, a recombinant vaccine protein consisting of bacterial sequences fused to GAL1 was recently described to generate high anti-GAL1 antibody levels in experimental models of melanoma, associated with increased cytotoxic T cell infiltration and decreased tumour burden[273].

Based on the oncogenic roles of GAL3 triggered upon interaction with *N*-glycans on MUC16 (ref. [274]), Stasenko et al.[275] evaluated the therapeutic potential of anti-GAL3 mAbs in high-grade serous ovarian cancer and other MUC16/CA-125-expressing malignancies. The GAL3-blocking mAb 14D11 recognizes both human and mouse GAL3, but not other galectin family members. Administration of this agent extended overall survival in tumour-bearing mice and delayed the occurrence of lung metastasis in breast cancer models[275]. Recently, anti-GAL3 mAbs developed by the company TrueBinding were patented for the treatment of liver fibrosis, pulmonary fibrosis, NASH and various neoplastic conditions[276].

Furthermore, given the tolerogenic activities of GAL9 in Dectin-1-expressing macrophages[124], a fully human IgG4 mAb to GAL9 known as LYT200 was developed by PureTech Health, and was the first anti-galectin mAb to reach clinical evaluation. This agent is in phase I/II clinical trials either alone or in combination with chemotherapy (gemcitabine/nab-paclitaxel) or anti-PD1 mAbs as a potential treatment for metastatic solid tumours (NCT04666688, Table 1). In 2021, the FDA designated LYT200 an orphan drug for the treatment of pancreatic cancer. Additionally, anti-GAL9-based therapies (including LYT200) exhibit cytotoxic effects in models of human T cell acute lymphoblastic leukaemia (T-ALL)[277]. Thus, GAL1-, GAL3- or GAL9-blocking mAbs, either alone or in combination, represent an array of multifunctional and selective strategies that can reprogramme the tumour, immune and vascular landscapes in a broad range of malignancies.

Another strategy for GAL3 blockade was the development of an N-terminally truncated form of this lectin (GAL3C); the N-terminal fragment is not involved in carbohydrate recognition but is essential for cooperative binding and cross-linking. As a consequence, GAL3C functions as a competitive inhibitor of GAL3 by preventing homophilic cross-linking following its interactions with glycan structures. Recombinant GAL3C has advantages such as a relatively simple and controlled production process and high selectivity compared with other synthetic inhibitors and natural polysaccharides[278]. GAL3C exhibited antitumour and antimetastatic activities in models of breast[279] and ovarian[278] cancer via mechanisms involving CD44 inactivation and inhibition of the effects of MUC1, including abrogation of AKT and nuclear factor-κB (NF-κB) downstream signalling pathways. Furthermore, intravenous administration of GAL3C in a multiple myeloma model resulted in

diminished tumour growth and increased the therapeutic impact of the proteasome inhibitor bortezomib[280]. However, a major limitation of GAL3C is its rapid clearance; hence, efforts are underway to explore the effects of GAL3C conjugation with other molecules to increase its molecular weight and stability. In this regard, the fusion of GAL1 with the Fc region of human IgG1 (GAL1hFc) resulted in enhanced stability and lifespan of this lectin accompanied by more pronounced immunoregulatory activity[134]. Thus, structural modifications might improve galectin-mediated therapeutic efficacy, mitigate 'off-target' effects and amplify clinically relevant antagonistic or agonistic activities.

Future potential of galectin inhibitors

Several challenges need to be addressed to accelerate the progress of galectin inhibitors into clinical settings. Some challenges are specific to particular therapeutic modalities; for example, compared with peptides, pectins or mAbs, small-molecule inhibitors such as galactosides or lactosides are more challenging to synthesize, and the development of new synthetic methods for these types of compound is currently an active area of research[281]. Other challenges are common to some or all of the modalities, including thorough understanding of their selectivity, biodistribution and safety profiles, and dissection of specific mechanisms of action. Below, we compare the advantages and limitations of various galectin-targeted therapeutic modalities and consider their future clinical potential.

Understanding selectivity

A key challenge in the design of galectin inhibitors is to avoid 'off-target' effects by preventing binding to other cellular proteins, including other family members. Although GAL1, GAL3 and GAL9 seem to share a common pro-fibrotic and pro-tumoural function, they might have contrasting roles in other pathological conditions. Moreover, given their structural similarities and wide tissue distribution, selective targeting of specific galectins could also become relevant in fibrotic or neoplastic diseases. To achieve selective targeting, it might be necessary to exploit the differences in galectin expression pattern and biological activity as well as differences in their ligand-binding specificity, capacity to cross-link individual glycosylated receptors and extracellular or intracellular distribution. Moreover, biochemical factors (stability, redox status, pH and multimerization) that govern their glycan-dependent or independent interactions should also be considered[282–284].

The extensive investigations into higher affinity and more selective small-molecule galectin inhibitors has led to an improved understanding of the crucial interactions that underlie their inhibitory activity and enhanced selectivity[179]. The use of GB0139 (**11**) for IPF and GB1211 for NASH laid the foundations for translational studies focused on small-molecule galectin inhibitors. GB0139 was first described as a potent inhibitor of GAL3 but also exhibited affinity for GAL1 (ref. [144]). This finding highlighted the potential use of these compounds for therapeutic targeting of multiple galectins, including those with pro-fibrotic activity.

Regarding peptidomimetics, most of the beneficial effects of Anginex have been associated with GAL1 inhibition, although affinity for GAL2, GAL7, GAL8N and GAL9N has also been demonstrated[285], suggesting that Anginex and related compounds have limited selectivity. Additional work is needed to explore the selectivity of peptide-based inhibitors towards different galectins and potential 'off-target' effects.

Modified natural complex polysaccharides such as MCPs or galactomannans are well tolerated in humans with few adverse effects. However, they vary substantially in molecular weight and degree of esterification, and their non-synthetic nature limits the thorough structural characterization required to understand their selectivity. Of particular note, the affinity of galectins for pectins and galactomannans has been questioned[218]. GCS-100 has been proposed as a GAL3 inhibitor, although its selectivity with respect to other galectins remains unknown. Further biochemical characterization of galectin–polysaccharide interactions is required, as well as in vivo studies on the biological activities of these compounds in galectin-null mice, as the possibility that polysaccharides could interact with other key mediators of disease pathogenesis has not been ruled out[218].

Despite its enhanced selectivity when compared with other inhibitors, truncated GAL3C has not yet reached clinical trials. The specificity of anti-GAL1 and anti-GAL3 mAbs has been demonstrated in in vitro assays[9,35,272,275], although further studies performed in vivo are needed to examine potential 'off-target' effects.

For these highly specific biological agents, it is crucial to recognize that neoplastic diseases present a case-by-case challenge; in some cases, selectivity for a specific galectin might not be crucial because two or more galectins exert similar biological activities (for example, immunosuppression driven by GAL1, GAL3 and/or GAL9). In these cases, overall galectin activities could be blocked using a pan-galectin inhibitor. The varied expression levels, diverse cell sources and possible opposing functions of galectins based on extracellular or intracellular localization should be examined in individual cancers. Recent advances in human organoids and humanized animal models, together with the development of single-cell technologies[286–288] and machine learning algorithms, will be invaluable to discern the source and cell-specific function of these lectins in the tumour microenvironment. Furthermore, the exquisite regulation by glycan epitope density on specific receptors as well as the multivalent nature of these interactions should also be considered[58]. In this respect, multivalent glycodendrimers, sugar calixarenes and nanotechnology-based developments could be useful to understand the natural clustering and presentation of ligands on the target cell surface, providing crucial mechanistic insights into their multivalency. A low-affinity inhibitor might be presented in clusters, which could increase its potency. As an example, lactose calixarenes were described as selective GAL4 inhibitors in vitro, with 300-fold improvement in binding[289]. However, these large, polar and labile molecules could present a difficult challenge owing to their ADME(T) properties.

Overall, a deeper understanding of the multifunctionality and disease-specific expression profiles of the various galectins, improved characterization of the cellular glycome and further identification of galectin binding partners and receptors will be essential for the design of novel galectin inhibitors with various degrees of selectivity.

Identifying mechanisms of action

The mechanisms of action of currently available galectin inhibitors differ considerably from one another, and, in some cases, remain a matter of ongoing debate. Although plant-derived polysaccharides such as MCPs or galactomannans have reached clinical trials, their specific targets and/or mechanisms of action remain unclear[218,290–292]. The apparent contradiction between the limited interactions with galectins and the health-promoting effects of these polysaccharides was evaluated by Miller et al.[231,293,294], who suggested that the biological effects do not involve interactions with canonical galectin CRDs. Indeed, Stegmayr et al.[218] have proposed that Davanat inactivates GAL1 by an oxidative mechanism. Likewise, the negative charge of pectins suggests that their

Glossary

ADME(T)
Acronym for the study of absorption, distribution, metabolism, excretion and (toxicity), key processes in drug pharmacokinetics.

Antennae
Branches of the core *N*-glycan structure (Manα1–3(Manα1–6) Manβ1–4GlcNAcβ1–4GlcNAcβ1–Asn-X-Ser/Thr) initiated by the action of *N*-acetylglucosaminyltransferases, resulting in mono-, di-, tri- and tetra-antennary complex *N*-glycans.

Cluster glycoside effect
Increased binding activity observed in multivalent versus monovalent interactions on a per mole or valence-corrected basis, previously known as 'avidity'. Multivalent interactions are typically stronger than the sum of individual monovalent interactions.

Child–Pugh
Classification that assesses liver function and prognosis in patients with cirrhosis. Based on a score that includes serum albumin and serum bilirubin levels, ascites and encephalopathy, it includes three classes (A, B, C). Classes B and C are the most severe forms of hepatic dysfunction, with life expectancy of 4–14 years (B) and 1–3 years (C).

Cyclodextrins
Cyclic oligosaccharides composed of α(1–4)-linked D-glucopyranose units that bear a hydrophilic outer surface and a hydrophobic central cavity.

Doxorubicin
Anthracycline antibiotic with broad use as a chemotherapeutic agent.

F-face of the GAL3 β-sandwich
All members of the galectin family, including GAL3, present a conserved carbohydrate recognition domain with an 11-stranded, β-sandwich fold that presents two faces: the six β-stranded S-face (key for interaction with β/α-galactosides), and an opposing F-face (five β-strands) where some larger polysaccharides can interact.

Glycoconjugates
Macromolecules that contain carbohydrates in their structure. Carbohydrates can be covalently linked to proteins or lipids.

Glycodendrimers
Radially symmetrical, carbohydrate-based repetitively branched molecules with well-defined, homogeneous and monodisperse structure.

Idiopathic pulmonary fibrosis
(IPF). A chronic, progressive inflammatory lung disease that causes fibrosis.

Mucin 1
Glycoconjugate that belongs to the family of large, highly glycosylated proteins that contain tandem repeats of amino acids rich in serine and threonine (mucins). These Ser/Thr residues can be O-glycosylated with the monosaccharide *N*-acetylgalactosamine (GalNAc) and further elongated (O-GalNAc glycans). This modification is characteristic of mucins and is therefore termed mucin-type O-glycosylation. Mucins can be secreted or be transmembrane proteins.

N-acetyllactosamine
(LacNAc). A Galβ(1–3)GlcNAc (type 1) or Galβ(1–4)GlcNAc (type 2) disaccharide.

Nonalcoholic steatohepatitis
(NASH). An inflammatory condition in which patients build up an excess of fat in the liver, leading to damage of this organ. This pathology is not related to alcohol consumption and results in progressive fibrosis that can lead to cirrhosis and hepatocellular carcinoma.

Neoglycoproteins
Synthetic or modified glycoproteins. This term typically refers to natural proteins that have been modified by the addition of structurally well-defined glycans.

Small interfering RNA
(siRNA). A class of double-stranded RNA molecules, usually about 20–25 nucleotides in length, designed to target a specific mRNA for degradation. Thus, siRNA prevents the production of specific proteins based on the nucleotide sequences of their corresponding mRNA. The process is called RNA interference (RNAi).

Sorafenib
An anticancer agent that inhibits multiple kinases and affects the vascular endothelial growth factor (VEGF), Raf–Ras and FMS-like tyrosine kinase 3 (FLT3) pathways.

Thomsen–Friedenreich antigen
(Tf antigen). The core 1 O-GalNAc glycan Galβ(1–3)GalNAc, also known as TF(a), T antigen, Thomsen–Friedenreich disaccharide and/or CD176. In some publications the definition of Tf antigen includes the covalently bound Ser/Thr (α-linkage).

Topomimetic
Agent with stereostructural elements that imitate the topographical features of proteins or peptides.

Tumour-associated carbohydrate antigens
(TACAs). Glycan structures that are frequently found in tumour cells as a consequence of malignancy. They include under- or overexpression of naturally occurring glycans, but also neo-expression of specific carbohydrate structures.

Valency
Number of individual structural units connected to a core structure in a multivalent ligand.

therapeutic effects could be dependent on charge-based interactions instead of specific intermolecular affinities. Other studies of pectin-derived polysaccharides focused on their direct cytotoxic effects involving apoptosis and autophagy[213,295]. Moreover, it is not clear whether these high molecular weight polysaccharides target the intracellular functions of galectins via internalization (as observed in the case of dextrans), followed by selective distribution to various subcellular compartments[296]. Additionally, these macromolecules might function by sequestering galectins in the extracellular space, thereby modifying their distribution between the intracellular and extracellular compartments. Although this is an attractive hypothesis, intracellular levels of GAL3 did not change upon the addition of MCPs[218]. Additional studies focused on the mechanisms that underlie the uptake, function and target engagement of plant-derived polysaccharides will be necessary to improve our understanding of their therapeutic potential.

Interestingly, regarding peptidomimetics, the DBF-based analogues 6DBF7 and DB21 inhibited tumour growth in mice to an even greater extent than Anginex, possibly owing to their increased bioavailability

and/or potential differences in mechanisms of action[242,247]. Whereas Anginex paradoxically enhances GAL1 binding to several oligosaccharides and glycoproteins[285], the peptidomimetics 6DBF7 and DB21 behave as non-competitive allosteric inhibitors of GAL1 that reduce its affinity for lactose[247]. Topomimetics OTX008 and PTX013 also function as allosteric inhibitors of GAL1, albeit via an interaction site that differs from the one used by Anginex derivatives (Fig. 5). Like 6DBF7 and DB21, and in contrast to parental Anginex, these compounds decrease the affinity of the GAL1 CRD for lactose[254]. Collectively, these results suggest that structural modification of these peptides results in diversification of their mechanisms of action. Interestingly, using a recombinant adeno-associated virus to deliver Anginex revealed that this compound inhibited VEGF expression and the AKT, JNK and NF-κB downstream signalling pathways[245]. These GAL1-independent effects, together with the ability of Anginex to disrupt EC membranes via electrostatic interactions[297], point to alternative modes of action. Additional in vivo studies in GAL1-deficient mice might help to unravel these effects.

Clinical outlook

The development of small-molecule galectin inhibitors has progressed towards high-affinity agents, surmounting limitations such as low stability and lifespan and leading to two pioneering compounds currently under clinical evaluation: the TDG derivative GB0139 for IPF, and the α-thiogalactoside GB1211 for NASH, liver fibrosis and NSCLC. Given the results obtained in clinical trials, their therapeutic potential is promising in both fibrosis and cancer. For example, a phase II trial to evaluate the safety and efficacy of GB1211 in combination with pembrolizumab in HNSCC and melanoma is expected to begin in 2023.

Regarding polysaccharides, clinical evaluation of GCS-100 and galactomannan Davanat has not been resumed, whereas PectaSol-C is commercially available as a dietary supplement for health and oncology support. Future studies will be crucial to unveil the full potential of the galactoarabino-rhamnogalacturonate belapectin, which is currently in phase IIb/III clinical trials for the prevention of eosophageal varices in NASH, and in the early stages of evaluation for melanoma, NSCLC and HNSCC in combination with pembrolizumab.

Among peptide and peptidomimetics, only OTX008 has been clinically evaluated, and this was more than a decade ago. Although the results of this phase I study documented its safety and low toxicity, no further advances have been described. Despite considerable efforts, our understanding of the mechanistic basis of the antitumour effects of peptide and peptidomimetic drugs beyond their specific interactions with galectins remains incomplete[252,253].

Anti-galectin mAbs have been introduced more recently to reinforce antitumour immunity, reprogramme pathological angiogenesis and restrain tumour metastasis[9,98,124,272]. This concept is based on findings that suggest that anti-galectin mAbs have multifunctional roles as cancer therapeutics and that their use helps to avoid the accumulated toxicity associated with administration of multiple mAbs. The anti-GAL9 mAb LYT200 is under clinical assessment and is an example of the emerging strategy of targeting glycosylation-related immune checkpoints in cancer[14,298]. The use of intrabodies designed to target the intracellular functions of galectins, or nanobodies[299] capable of reaching areas within tumours and fibrotic foci, might be an important breakthrough in the coming years. Moreover, functionalized nanoparticles containing galectin-specific siRNA[265], aptamers[267] and scFv fragments[300] might be alternative agents to target galectin–glycan interactions.

Finally, results obtained in clinical trials for NASH and IPF should facilitate the clinical translation of galectin blockers designed to treat other fibrotic conditions. For example, GAL3 has a crucial role in cardiac fibrosis leading to heart failure[301], and clinical trials designed to evaluate PectaSol-C in patients with high blood pressure have been completed (NCT01960946)[302]. Moreover, inhibition of GAL3 with GB0139 (**11**) resulted in diminished VEGF-driven angiogenesis in a mouse model of corneal fibrosis[303]. In this regard, GAL1 has been proposed as a relevant target in renal fibrosis[304], atherosclerosis and abdominal aortic aneurysms[34], highlighting novel therapeutic opportunities for its inhibitors. Additionally, recognition of the central role of galectins in the pathogenesis of autoimmune inflammation[16] and neurodegeneration[30,31] has led to the design of galectin-based agonists and antagonists to treat these chronic inflammatory conditions. In this regard, TB006, an anti-GAL3 mAb developed by TrueBinding, is in a phase II clinical trial for treatment of acute ischaemic stroke (NCT05156827) and a phase I/II trial for Alzheimer disease (NCT05074498).

In conclusion, the rapid pace of advances in glycobiology, the promising performance of some galectin inhibitors in preclinical studies and clinical trials, and the urgent need for alternative therapies for cancer and fibrotic diseases should help to accelerate the translation of anti-galectin therapeutics into clinical practice.

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