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Current Opinion in Immunology

Galectins: emerging regulatory checkpoints linking tumor immunity and angiogenesis

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Immune checkpoints, a plethora of inhibitory pathways aimed at maintaining immune cell homeostasis, may be co-opted by cancer cells to evade immune destruction. Therapies targeting immune checkpoints have reached a momentum yielding significant clinical benefits in patients with various malignancies by unleashing anti-tumor immunity. Galectins, a family of glycan-binding proteins, have emerged as novel regulatory checkpoints that promote immune evasive programs by inducing T-cell exhaustion, limiting T-cell survival, favoring expansion of regulatory T cells, de-activating natural killer cells and polarizing myeloid cells toward an immunosuppressive phenotype. Concomitantly, galectins can trigger vascular signaling programs, serving as bifunctional messengers that couple tumor immunity and angiogenesis. Thus, targeting galectin–glycan interactions may halt tumor progression by simultaneously augmenting antitumor immunity and suppressing aberrant angiogenesis.

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Current Opinion in Immunology 2017, **45**:8–15

This review comes from a themed issue on **Tumour immunology**

Edited by **Dmitry Gabrilovich** and **Robert L Ferris**

http://dx.doi.org/10.1016/j.coi.2016.12.003

0952-7915/Published by Elsevier Ltd.

Galectins as emerging immune checkpoints in cancer

The immune system is equipped with effective weapons to battle against cancer cells, but it is held in check by a number of inhibitory co-receptors including programmed cell death-protein-1 (PD-1) and cytotoxic T lymphocyte–associated antigen-4 (CTLA-4). These regulatory checkpoint pathways which promote immunological homeostasis and safeguard against the detrimental effects of exuberant inflammation can be co-opted by cancer cells to evade immune destruction [1]. Engagement of PD-1 or CTLA-4 by their specific ligands (PD-L1/PD-L2 or CD80/CD86 respectively) results in recruitment of Src homology 2-containing tyrosine phosphatase-2 (SHP2) or protein phosphatase-2A (PP2A), effects that ultimately lead to T-cell exhaustion [1]. Monoclonal antibody (mAb)-based therapies targeting CTLA-4 and/or PD-1/PD-L1 pathways have yielded significant clinical benefits, including durable cancer regression and increased overall survival in patients with various malignancies by unleashing anti-tumor immunity [2]. However, whereas significant clinical responses have been achieved in several patients, others show intrinsic resistance or maintain only short-term benefit as their tumors develop compensatory inhibitory pathways [2]. These include, among others, the lymphocyte activation gene-3 (LAG-3) and the T-cell immunoglobulin and mucin protein-3 (TIM-3) [3]. These co-inhibitory receptors, may act autonomously to control distinct aspects of antitumor immunity, suggesting that combinatorial strategies blocking distinct immune checkpoints may pave the way for successful cancer immunotherapy [2].

Galectins, a family of endogenous glycan-binding proteins, influence a variety of immune cell processes through intracellular or extracellular mechanisms [4]. These lectins can orchestrate immunosuppressive circuits by co-opting selected inhibitory receptors, disrupting co-stimulatory pathways and/or controlling activation, differentiation, and survival of immune cells [4]. According to their structure, galectins are classified into three different families: (a) 'proto-type' galectins (galectin-1, 2, 5, 7, 10, 11, 13, 14 and 15) which display one carbohydrate recognition domain (CRD) that can dimerize; (b) 'tandem-repeat' galectins (galectin-4, 6, 8, 9 and 12) which contain two homologous CRDs in tandem; and (c) the chimera-type galectin-3 which uniquely displays a CRD connected to a non-lectin N-terminal region responsible for oligomerization [5]. In spite of lacking the typical signal sequence required for classical secretion, most galectins are externalized through an unconventional route which precise mechanisms remain uncertain [4].

Galectins were originally discovered by their capacity to bind glyco-conjugates bearing the *N*-acetyl-lactosamine [Galβ(1–4)-GlcNAc; LacNAc] disaccharide; however compelling evidence indicates substantial differences

in glycan-binding specificities of individual members of the galectin family (including preferences for core 2-*O*-glycans, complex branched *N*-glycans or sialylated structures), which might explain differences in their biological activities [4,6,7]. Although these saccharide structures are widely distributed in a range of glycoconjugates, individual galectins may co-opt a particular set of glycosylated receptors, thus emphasizing the importance of protein–protein interactions and glycan density in dictating galectin-receptor preferences [6]. To illustrate this concept, galectin-3 preferentially binds to the T-cell receptor (TCR), CTLA-4, LAG-3, CD71 and CD45 [8,9••,10], whereas galectin-1 interacts with CD45, CD43, CD69 and the pre-B cell receptor (pre-BCR) [10–13] and galectin-9 co-opts TIM-3 and CD44 [14,15••]. Interestingly, some of these receptors including CTLA-4, LAG-3 and TIM-3 have been widely recognized as immune checkpoint molecules capable of de-activating immune cells [2]. Formation of multivalent galectin–glycan complexes contribute to assembly and organization of these receptors, controlling their segregation, internalization and signaling [4,6]. Hence, galectins may twist the fate of immune cells by controlling the stimulatory or inhibitory function of relevant glycosylated receptors.

Interestingly, whereas some galectins are broadly expressed, others are preferentially localized in certain tissues, including galectin-7 in the skin, galectin-12 in adipose tissue and galectin-4 in the gastrointestinal tract [6]. Particularly, galectin-1 is prominent in tolerogenic DCs [16–18] and $CD4^{+}CD25^{+}$ Tregs [19]. Furthermore, several tumors (including melanoma, Hodgkin's lymphoma, lung adenocarcinoma, breast adenocarcinoma, neuroblastoma, glioblastoma, ovary carcinoma, T-cell lymphoma and pancreatic adenocarcinoma) foster immune evasive programs through mechanisms involving galectin-1 [20–23,24•,25–27,28•,29,30••], galectin-3 [31,32••] and galectin-9 [33]. These galectin-driven regulatory circuits may also control formation of aberrant vascular networks [24,34–37], suggesting a galectin-mediated cross-talk between immune and vascular circuits in the tumor microenvironment (TME). In this review we discuss the relevance of galectin–glycan interactions as emerging regulatory checkpoints that control antitumor responses and link immunosuppression to angiogenesis.

Controlling T-cell fate and function

It has become increasingly clear that galectins play key roles in shaping T-cell biology by influencing T-cell activation, signaling and survival [6]. Galectin-1 antagonizes TCR-transmitted signals promoting contraction of the CD8T cell compartment [38]. Moreover, galectin-3 prevents TCR, CD4 and Lck clustering into GM1-enriched membrane microdomains, thereby adjusting T-cell signaling threshold through *N*-glycan-dependent mechanisms [39,40]. In addition, galectin-3 favors T-cell anergy of human tumor-infiltrating lymphocytes (TILs) by distancing the TCR from CD8 molecules [41]. More recently, Petit et al. added mechanistic insights to this picture showing that galectin-3 limits the formation of a functional secretory synapse in CD8 TILs by preventing optimal lymphocyte-function-associated antigen-1 (LFA-1) triggering and reducing adhesion to target cells [32••]. Moreover, galectin-3 destabilizes the immunologycal synapse by promoting TCR downmodulation through intracellular mechanisms involving interactions with Alix, an adaptor endocytic protein [42].

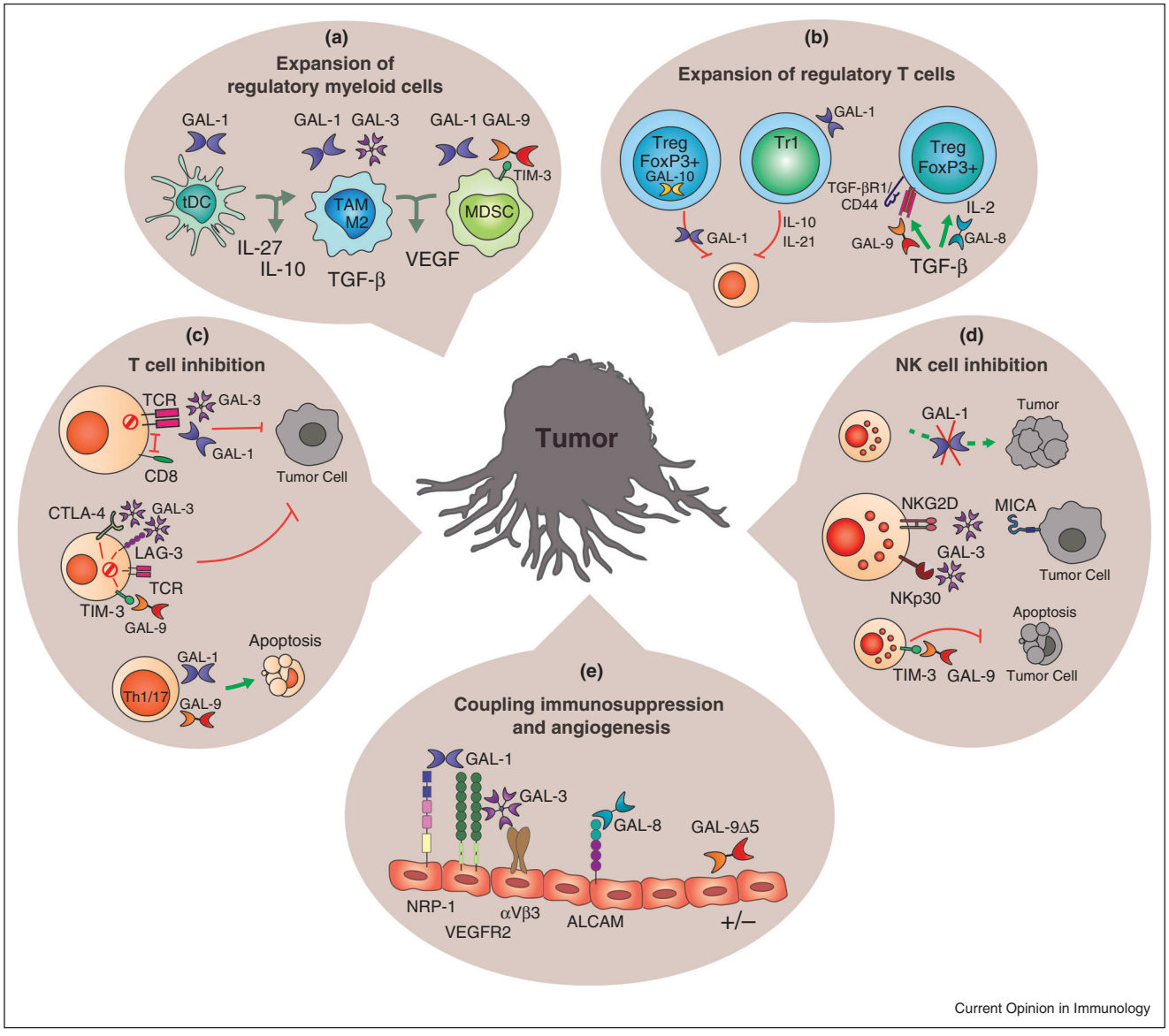
Interestingly, galectins also play direct inhibitory roles by engaging relevant glycosylated receptors, preventing their endocytosis and promoting their retention on the cell surface. In this regard, galectin-3-*N*-glycan complexes trap CTLA-4 on the surface of T cells and prolong inhibitory signals triggered by this immune checkpoint receptor [8]. Moreover, galectin-3 dampens antitumor responses through interactions with LAG-3 on the surface of CD8T cells, whereas galectin-9 binds to TIM-3 leading to T-cell exhaustion [9••,14], an effect that is counteracted by human leukocyte antigen B (HLA-B)-associated transcript 3 (Bat3) [43].

Galectin–glycan interactions may also control T-cell viability. Galectin-1 triggers T-cell apoptosis through binding to *N*-glycans and *O*-glycans on CD45, CD43 and CD7 or by sensitizing resting T cells to Fas-induced death [10,44]. Interestingly, galectin-1 selectively eliminates Th1-differentiated and Th17-differentiated cells as they express the repertoire of glycans required for galectin-1 binding; in contrast Th2 cells are protected from galectin-1-induced death via α2,6 sialylation of surface glycoproteins [45]. Likewise, galectin-9 deletes Th1, Th17 and CD8T cells through glycosylation-dependent binding to TIM-3 [14,46,47], while it augments secretion of T-cell-derived pro-inflammatory cytokines through TIM-3-independent pathways [48]. Interestingly, intracellular galectin-1 can sensitize T cells to apoptosis induced by extracellular galectin-1 [49], suggesting that inhibition of intracellular or extracellular galectin-1 may contribute to augment T-cell responses. In contrast, intracellular galectin-3 protects T cells from extracellular apoptotic stimuli [50], whereas, extracellular galectin-3 directly kills activated T cells [10]. Finally, galectins may also control T-cell differentiation, as galectin-1 and -9 interrupt generation of Th17 effector cells through mechanisms involving CD69 [12] or TIM-3 [46]. Thus, galectins limit effector antitumor T-cell responses through modulation of activation, differentiation, signaling and survival (Figure 1).

Fine-tuning the Treg compartment

Immune checkpoint pathways favor immune evasion programs not only by promoting exhaustion of effector T cells, but also by shaping the Treg compartment [2].

Figure 1



Decisive roles of galectins in tumor immunity and angiogenesis. Galectins (Gals) are key components of the tumor microenvironment (TME) that influence: **(a)** Expansion of regulatory myeloid cells, Gal-1 promotes differentiation of IL-27- and IL-10-producing tolerogenic DCs (tDCs), whereas Gal-1 and Gal-3 contribute to M2 macrophage polarization and recruitment of immunosuppressive tumor-associated macrophages (TAM). Gal-1 also promotes recruitment of myeloid-derived suppressor cells (MDSCs) and Gal-9 enhances their regulatory capacity; **(b)** Expansion of regulatory T cells, Gal-10 and Gal-1 contribute to the immunosuppressive potential of $CD4^+CD25^+$ $Foxp3^+$ Tregs. Further, Gal-1, Gal-8 and Gal-9 promote expansion of these cells. Mechanistically, Gal-8 and Gal-9 amplify TGF-β_1-induced Treg differentiation. Moreover, Gal-1 contributes to Tr1 differentiation by modulating IL-10 and IL-21 expression; **(c)** T-cell inhibition, Gal-1 and Gal-3 can impair TCR-mediated signaling and activation. On the other hand, Gal-3 and Gal-9 can engage immune inhibitory checkpoints including CTLA-4, LAG-3 or TIM-3. Finally, Gal-1 and Gal-9 selectively delete Th1 and Th17 effector cells; **(d)** NK cell inhibition, Gal-1 inhibits NK cell recruitment to TME and suppresses NK cell functionality. Gal-3 inhibits NK cell activity by decreasing affinity of MICA for NKG2D receptor or by inhibiting NKp30 signaling. Gal-9 impairs NK cell cytotoxicity and cytokine production; **(e)** Angiogenesis, Gal-1, Gal-3 and Gal-8 promote angiogenesis through binding to VEGFR2/neuropilin-1 (NRP-1), $\alpha_v\beta_3$ integrin and activated leukocyte cell adhesion molecule (ALCAM) respectively. Gal-9Δ5 isoform exhibits dual pro-angiogenic or anti-angiogenic effects depending on the concentration and environmental context.

$Foxp3^+$ Tregs express galectin-1 and -10 which support Treg suppressive function [19,51]. Moreover, galectin-1 facilitates expansion of both $Foxp3^+$ Tregs and $Foxp3^-$ Tr1 cells in models of infection, breast cancer, autoimmunity and pregnancy [17,25,52,53]. Upon engagement of CD45, galectin-1 instructs a regulatory T-cell signature characterized by high IL-10 and IL-21 expression and modulation of the c-Maf/aryl hydrocarbon

receptor pathway [54]. Moreover, in response to microbiota-induced inflammation, γδ-T cells become regulatory by secreting high amounts of galectin-1, which impairs antitumor immunity and accelerates malignant progression [28•]. On the other hand, galectin-9 acts synergistically with TGF-β_1 to reinforce differentiation of inducible Tregs (iTregs) by forming a tri-molecular complex with CD44 and TGF-βRI and facilitating Smad3 phosphorylation [15••]. Likewise, galectin-8 promotes iTreg differentiation by modulating TGF-β_1 and IL-2 signaling [55]. In contrast, galectin-3 negatively regulates the frequency and function of these cells [56,57]. Thus, galectins can shape the fate of Foxp3$^+$ and Foxp3$^-$ Tregs by tuning their differentiation and immunosuppressive capacity (Figure 1).

Enhancing the regulatory function of myeloid cells

Regulatory myeloid cells including tumor-associated macrophages (TAMs) and neutrophils (TANs), tolerogenic DCs (tDCs) and myeloid-derived suppressor cells (MDSCs) are recruited, activated and expanded in response to tumor-derived factors, negatively regulating antitumor immunity [5]. Galectin-1 promotes differentiation of IL-27-producing tDCs, which in turn induce the expansion of Tr1 cells [16,17,58]. Interestingly, this lectin can also inhibit trans-endothelial migration of inflammatory DCs without altering trafficking of tDCs through mechanisms involving differential *O*-glycosylation of CD43 [59••]. Notably, tumor-driven unremitting expression of special AT-rich sequence-binding protein 1 (Satb1) transcription factor in inflammatory DCs tilts the balance toward an immunosuppressive DC phenotype characterized by high galectin-1 expression [18]. Furthermore, this lectin contributes to polarize macrophages and microglia toward pro-resolving and anti-inflammatory M2 phenotypes [11,60,61] and facilitates MDSC recruitment [62]. On the other hand, galectin-3-*N*-glycan lattices enhance the immunosuppressive activity of macrophages by augmenting surface residency of TGF-βR and prolonging delivery of TGF-β-dependent inhibitory signals [63]. Moreover, this chimera-type lectin suppresses expansion of plasmacytoid DCs [9••]. Finally, galectin-9 favors expansion of CD11b$^+$Ly-6G$^+$ MDSCs and inhibits antitumor responses through TIM-3-dependent mechanisms [33]. Thus, galectin–glycan interactions may control antitumor responses by polarizing tumor-associated myeloid cells toward regulatory profiles (Figure 1).

De-activation of NK cells

NK cell functions are governed by an intricate network of stimulatory and inhibitory receptors and an extensive array of checkpoints. Whereas galectin-1 inhibits recruitment of NK cells in Kaposi's sarcoma, it impairs their antitumor activity in a glioblastoma model through a mechanism involving Gr1$^+$CD11b$^+$ myeloid cells [27,37]. Moreover, galectin-3 antagonizes NK cell-mediated antitumor immunity by diminishing the affinity of MHC I-related chain A (MICA) for the NKG2D receptor [64] or by acting as an inhibitory ligand of the NKp30 receptor [65]. Furthermore, galectin-9 impairs NK cell cytotoxicity and IFN-γ production through TIM-3-independent mechanisms [66]. Thus, galectins may interfere with NK cell-mediated antitumor immunity by modulating their recruitment, lytic activity and cytokine production (Figure 1).

Linking immunosuppression and angiogenesis in TME

As immunosuppression and angiogenesis may occur simultaneously during tumor growth and could be mediated by the same cytokines and cell types, a bidirectional cross-talk has been proposed between immune and vascular programs operating in TME [67]. The most relevant example is VEGF-A, which in addition to its well-known pro-angiogenic activity, induces the accumulation of regulatory myeloid cells. Moreover, hypoxia, the most important stimulus leading to angiogenesis dramatically alters the function of MDSCs, via induction of HIF-1α [68]. Strikingly, these immunoregulatory cells also display pro-angiogenic effects [67]. Galectins are multifaceted players displaying both immunoregulatory and pro-angiogenic function. Notably, exposure of endothelial cells (ECs) to immunosuppressive or hypoxic conditions caused similar changes in the EC glycome, including lower expression of α2,6-linked sialic acid, increased branching of β1,6 N-glycan structures and elongation of poly-LacNAc residues [24•]. This glycan profile, which facilitates galectin binding to vascular cells, recapitulates that found on Th1-differentiated and Th17-differentiated cells [45], mature DCs [16,69] and M1-polarized microglia/macrophages [11] suggesting the occurrence of common glycan profiles governing both immune and vascular programs.

In addition to its broad immunosuppressive activity, galectin-1 binds to VEGFR2 and neuropilin-1 on ECs and promotes angiogenesis [24•,34,37], thus mirroring the effects of VEGF. Disruption of galectin-1 binding to complex *N*-glycans attenuates both cancer-induced immunosuppression and neovascularization [24•,37]. Moreover, targeting this lectin early during tumor progression promotes vessel normalization, leading to amelioration of tumor hypoxia and increased influx of immune cells to TME [24]; thus emphasizing the cross-talk between immune and vascular compartments mediated by galectin–glycan interactions. On the other hand, galectin-3 favors vascularization through binding to integrin $\alpha_v\beta_3$ [70] or by sustaining the pro-angiogenic capacity of TAMs [71]. Moreover galectin-8 promotes angiogenesis via cross-linking of activated leukocyte cell adhesion molecule (ALCAM) [72] and lymphangiogenesis through binding to podoplanin and integrins $\alpha_1\beta_1$ and

$\alpha_5\beta_1$ [73••]. Finally, the galectin-9Δ5 isoform exhibits dual effects, either promoting or suppressing angiogenesis depending on its concentration and environmental context [74]. Thus, galectin–glycan interactions influence tumor vascularization by engaging different receptors and signaling pathways (Figure 1), thus bridging immune and vascular compartments in the TME.

Galectins and resistance to anti-cancer therapies

Galectins have been emerged as critical mediators of resistance to a number of anticancer therapies [24,75••,76–78]. We have identified a glycosylation-based mechanism mediated by galectin-1-*N*-glycan interactions that confers resistance to anti-angiogenic therapies. In tumors resistant to anti-VEGF treatment, galectin-1 binds directly to non-sialylated complex *N*-glycans on VEGFR2 and promotes receptor phosphorylation and signaling. In contrast, in anti-VEGF sensitive tumors, ECs display high amounts of α2,6-linked sialic acid which prevents galectin-1 binding and angiogenesis [24•]. Moreover, galectin-1 confers resistance to anti-CD20 (rituximab) immunotherapy through the control of antibody-mediated tumor phagocytosis [75••] and controls sensitivity of chronic myeloid leukemia cells to imatinib through modulation of multidrug resistance protein-1 (MDR-1) [76]. Furthermore, this endogenous lectin mediates radiotherapy-related systemic lymphopenia via intra-tumoral immunosuppression and enhanced angiogenesis [77]. Likewise, galectin-3 confers resistance to anti-leukemia therapy [78] and reprograms MDR-1 through specific interactions with Na+/K+-ATPase and P-glycoprotein [79]. Thus, galectins emerge as critical determinants of sensitivity to several anticancer modalities, including immunotherapy, anti-angiogenic therapy, radiotherapy and chemotherapy.

Conclusions and future perspectives

As cancer immunotherapies induce durable clinical benefit in only a fraction of patients, most research efforts are being focused on the design of combinatorial approaches that could enhance the efficacy of these treatments [2]. Here we review the emerging role of galectins as regulatory checkpoints that foster immune evasive programs and control the fate and function of antitumor immune cells, linking tumor immunosuppression and angiogenesis. These glycan-binding proteins may serve as soluble ligands of established checkpoint receptors including LAG-3, CTLA-4 and TIM-3 or may hierarchically trigger immune inhibitory signals that control tumor progression. Because of their dual immunosuppressive and pro-angiogenic activities [24•], targeting galectin–glycan interactions has emerged as a promising therapeutic approach, either alone or in combination with other treatment modalities. In this regard, a number of galectin inhibitors with different degrees of selectivity for individual members of the family have been considered, including glycan-based inhibitors (*e.g.* synthetic glycoamines), allosteric antagonists or peptidomimetics such as anginex, 6DBF7 or OTX008, natural or modified polysaccharides such as citrus pectin and anti-galectin neutralizing antibodies [37,80]. Future studies are awaited to further understand the role of galectins as emerging regulatory checkpoints in the TME and to establish their clinical relevance in cancer immunotherapy.

Acknowledgements

Work in G.A.R's lab is supported by grants from the Argentinean Agency for Promotion of Science and Technology (PICT V 2014-3687; PICT 2012-2440), CONICET, University of Buenos Aires, Bunge & Born Foundation and Sales Foundation. A.G.B is a recipient of the Genentech Postdoctoral Cancer Immunotherapy Fellowship from the Society of Immunotherapy of Cancer (SITC, USA).

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