Chimera and Tandem-Repeat Type Galectins

Subjects: Oncology

Contributor: Sheng Yan , Ka Wai Lee , Sze Kwan Lam , Frankie Chi Fat Ko , James Chung Man Ho

In humans, a total of 12 galectins have been identified. These galectins play important roles in controlling immune responses within the tumour microenvironment (TME) and the infiltration of immune cells, including different subsets of T cells, macrophages, and neutrophils, to fight against cancer cells. However, these infiltrating cells also have repair roles and are hijacked by cancer cells for pro-tumorigenic activities. Upon a better understanding of the immunomodulating functions of galectin-3 and -9, their inhibitors, namely, GB1211 and LYT-200, have been selected as candidates for clinical trials. The use of these galectin inhibitors as combined treatments with current immune checkpoint inhibitors (ICIs) is also undergoing clinical trial investigations. Through their network of binding partners, inhibition of galectin have broad downstream effects acting on $CD8^+$ cytotoxic T cells, regulatory T cells (Tregs), Natural Killer (NK) cells, and macrophages as well as playing pro-inflammatory roles, inhibiting T-cell exhaustion to support the fight against cancer cells.

galectins intracellular secretory immune checkpoint inhibitors immuno-oncology therapy

1. Introduction

The function of the immune system in fighting cancer cells has been of long-standing interest in the context of cancer therapies [1], with research dating back to William B. Coley's study in 1819 [2]. Since the first ICI, Cytotoxic-T-lympocyte-antigen-4 (CTLA-4), also known as ipilimumab (Yervoy), was tested and approved for the treatment of metastatic melanoma in 2015 [3][4], the number of checkpoint inhibitors has increased. In particular, when programmed cell death/ligand-1 (PD-1/PD-L1) immune checkpoint proteins are identified, these checkpoint inhibitors are now the standard regimen for immuno-oncology (I-O) therapy when tackling different solid tumours [5]. The most common PD-1 and PD-L1 inhibitors are pembrolizumab (Keytruda), nivolumab (Opdivo), cemiplimab (Libtayo), atezolizumab (Tecentriq), nivolumab (Bavencio), and durvalumab (Imfinzi) [6]. In addition, ipilimumab (Yervoy) is the most common CTLA-4 inhibitor. However, the response rates (RRs) of this approach vary according to types and lines of treatment [7]. It has been found to have a good-to-moderate response, with over a 50% RR against classic Hodgkin's lymphoma, melanoma, and first-line combination-treated non-small cell lung cancer (NSCLC). However, some cancers, such as extensive-stage small cell lung cancer (SCLC), hepatocellular carcinoma (HCC), $PD\text{-}L1^+$ gastric (gastroesophageal junction type), and cervical cancers, have shown less than a 25% RR.

The current challenges to the efficiency of I-O therapies include the exhaustion of cytotoxic T cells [8] and the need to increase the subpopulation of Tregs and other immune cells during immunosuppression [9]. To combat these challenges, other immunomodulators have been identified, such as T-cell immunoreceptor with immunoglobulin

and ITIM domains (TIGIT). TIGIT is found in T cells, NK cells, and tumour cells. The mechanism of immune inactivation may occur through ITIM-dependent negative pathways [10]. Numerous TIGIT antibodies, including BMS-986207, tiragolumab, and vibostolimab, have been developed and tested in clinical trials [11]. The recent results of these trials, such as CITYSCRAPE [12], have generated additional interest in investigating any novel immunosuppressors and their interacting protein mechanisms. The results of the subsequent phase III trials, such as the SKYSCRAPER series (NCT04619707, NCT04513925, NCT046665843, etc.), will need further analysis to enhance this approach to treatment. A list of this series of studies was summarised by Brazel et al. (2023) [13].

Recently, galectins have been identified as immunomodulators [14], joining TIGIT as new potential targets for immunotherapies. The potential candidate reagents and ongoing trial studies are listed in **Table 1**. In particular, newly developed reagents, such as GB1211 [15], a galectin-3 small-molecule inhibitor, and LYT-200, an anti-galectin-9 humanised antibody, are currently on trial (NCT05240131/GALLANT-1 and NCT04666688, respectively). The safety of GB1211 has also been reported, with limited grade 1 and grade 2 adverse effects in healthy participants (NCT03809052) [16]. The efficiency of these candidates in the current trial studies will further support the use of galectins in cancer treatments in combination with PD-1/PD-L1 and TIGIT. Other galectin inhibitors, such as OTX008 and ProLectin-M, have also been investigated in mouse animal model studies [17][18][19][20].

Table 1. Clinical trial studies (extracted from ClinicalTrials.gov, accessed on 11 May 2023).

Target	Drug	Phase	Cancer Type	Intervention
Galectin-1	OTX008	I	Solid tumours	NCT01724320 Status unknown (updated: 2012)
Galectin-3	Belapectin (GR-MD-02)	I	Metastatic melanoma	NCT02117362 Completed (updated: 2019)
		I	Metastatic melanoma, NSCLC, HNSCC	NCT02575404 [21] Active (updated: 2022)
	GB1211	I	Healthy subjects	NCT03809052 [16]

Target	Drug	Phase	Cancer Type	Intervention
				Completed (updated: 2021)
		I/II	NSCLC	NCT05240131 Recruiting (updated: 2023)
	GCS-100	I/II	Relapsed/Refractory diffuse large-B-cell lymphoma	NCT00776802 Withdrawn as funding issue (updated: 2013)
	GM-CT-01	I	Breast, colorectal, head and neck, lung, prostate	NCT00054977 Completed (updated: 2012)
	PectaSol-C, modified citrus pectin (MCP) [22]	N/A	Non-cancer-related: study for high blood pressure control	NCT01960946 Completed (updated: 2021)
	Galactomannan/ ProLectin-M [23]	III	Non-cancer-related: antagonist for COVID-19	NCT05096052 Recruiting (updated: 2022)
Galectin-9	LYT-200 (monoclonal antibody against galectin-9)	I	Acute myeloid leukaemia	NCT05829226 Recruiting

Target	Drug	Phase	Cancer Type	Intervention
				(updated: 2023)
		I/II	Metastatic cancer in head and neck, colorectal, pancreatic, or urothelial origins [24]	NCT04666688 Recruiting (updated: 2023) [25]

ir protein
ytoplasm
ns, which
e (K) and
arginine (I), followed by around 12–16 reticulum (ER). The secretory proteins are then embedded inside lipid bilayer vesicles and transported via budding-off into the Golgi apparatus, after which the vesicles can be further fused with the cell membrane to export the protein outside the cell [26]. However, further analysis of the coding and protein sequence of galectins has resulted in no signal peptide sequence being detected. Their secretory forms are suspected to be produced through the non-canonical secretion pathway [27][28]. Unlike other proteins, their recognition is not based on protein peptides in the form of amino acid chains on the binding partner(s). Galectins contain a carbohydrate-recognition domain (CRD) as a binding motif to recognise the glycosylation sites on other proteins and for binding. The CRD mainly detects glycoproteins and is required for post-translational modification. Their immunological roles have been established and reviewed [29]. Different galectins have been identified that bind with immune cells, such as cytotoxic T cells, dendritic cells, and macrophages, to regulate cancer cell immunosurveillance (also listed in **Table 2**). The roles of these human galectins are discussed in further detail below.

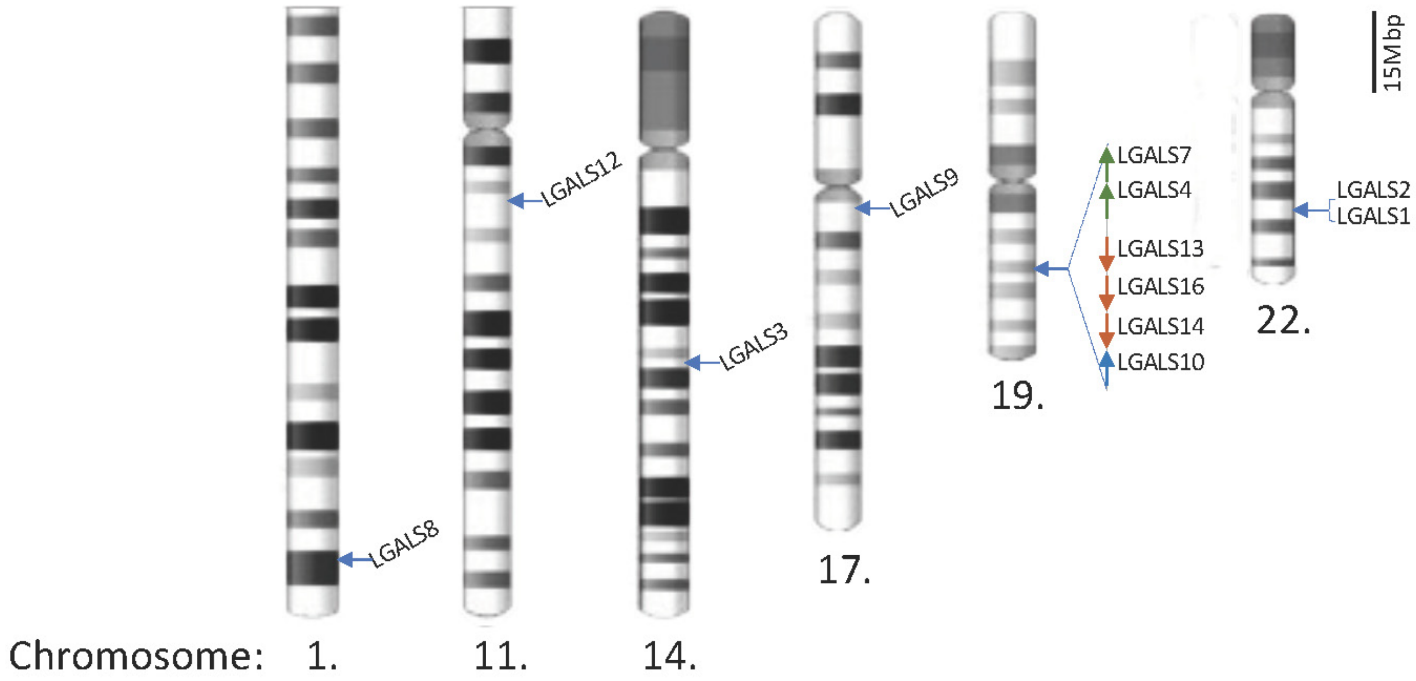

Figure 1. Locations of galectin genes in the human genome. Twenty galectin genes have been identified and are located on chromosomes 1, 11, 14, 17, 19, and 22. The partial q arms of chromosomes 1 and 11 are only shown to

reflect the scale, and the scale bar represents 15 megabase pairs (Mbp) in gene distance.

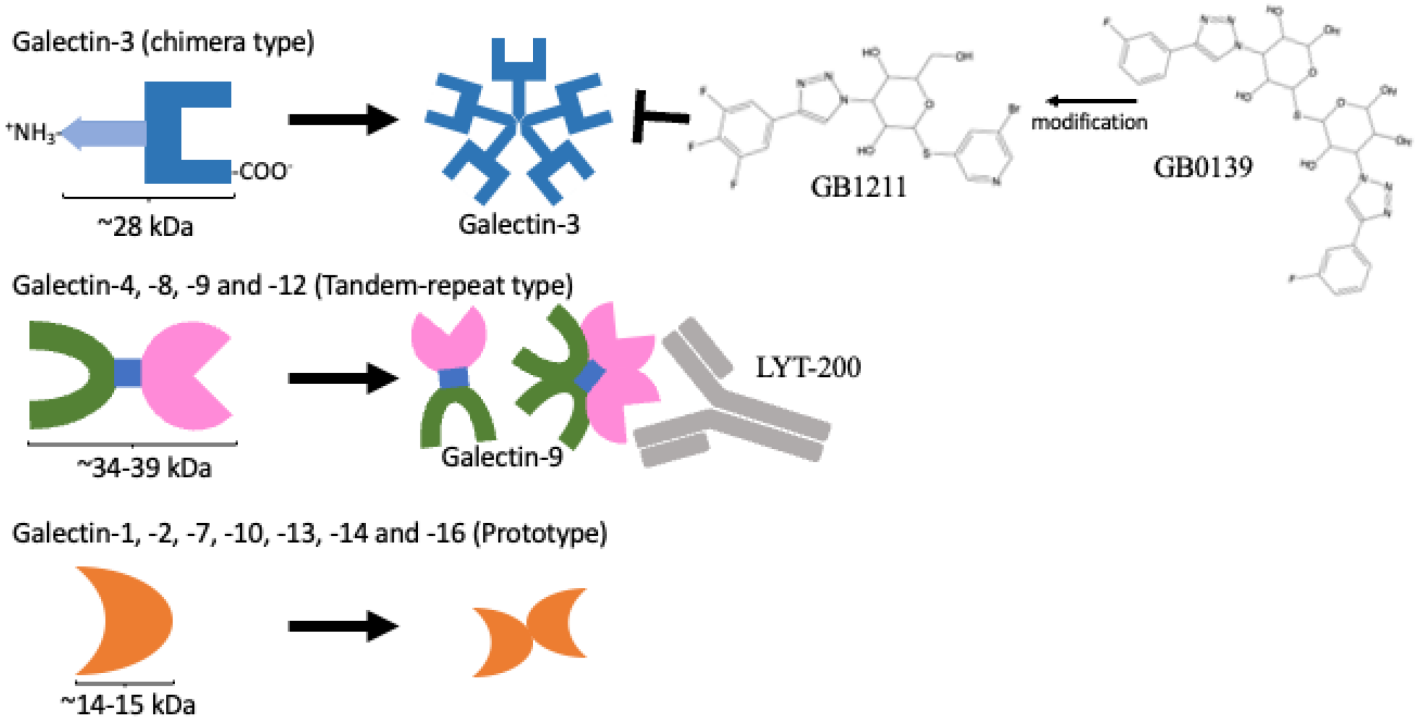

Figure 2. Protein domain structures of galectins and their inhibitors. Only the galectin-3 protein contains CRD domain and an extra amino domain allowing it to form an oligomer (**upper panel**). A protein containing two distinct CRDs, galectin-4, 6, 8, 9, and 12 (**middle panel**). A single CRD protein that can form homo-dimers, galectin-1, 2, 5, 7, 10, 11, 13, 14, and 16 (**lower panel**). GB1211 and LYT-200 represent a newly developed galectin-3-specific inhibitor [15] and a humanised monoclonal antibody against galectin-9, respectively, and are currently under clinical trial.

Table 2. Intracellular and extracellular binding partners of 12 human galectins.

Gene/Protein Name (Chromosome Position [30])	Intracellular (*Cytoplasmic*/*Nucleus*)	Extracellular
LGALS1/Galectin-1 (Chr. 22q13.1)	*Cytoplasmic*: GRP78 [31]	CC and CXC chemokines [35]
	Gemin4 [32]	CD43 [36][37]
	H-Ras [33]	CD45 [36][38]
	PCDH24 [34]	NRP1 [39]
		VEGFR2 [40]

Gene/Protein Name (Chromosome Position [30])	Intracellular (*Cytoplasmic*/*Nucleus*)	Extracellular
LGALS2/Galectin-2 (Chr. 22q13.1)		Binds to surface of $CD14^{(interm.–high)}$ monocyte and promote M1 macrophage differentiation [41]
LGALS3/Galectin-3 (Chr. 14q22.3)	*Cytoplasmic*: Alix (EGFR trafficking) [42][43][44] Gemin4 [32] K-Ras [45][46] PCDH24 [34] *Nucleus*: hnRNPA2B1 [47] Sp1 [48]	CC and CXC chemokines [35] CD29 [49] CD43 [49] CD45 [49] CD71 [49] EGFR [50] Interferon-γ [51] Integrin $\alpha_v\beta_3$ [46] LAG3 [52] MUC1 [53]
LGALS4/Galectin-4 (Chr. 19q13.2)		CD3 [54]
LGALS7/Galectin-7 (Chr. 19q13.2)	*Cytoplasmic*: Bcl-2 [55]	
LGALS8/Galectin-8		αM (CD11b, neutrophils) [56]

Gene/Protein Name (Chromosome Position [30])	Intracellular (*Cytoplasmic*/*Nucleus*)	Extracellular
(Chr. 1q43)		CD166 [57] Podoplanin [58][59]
LGALS9/Galectin-9 (Chr. 17q11.2)	*Cytoplasmic*: Binding to intracellular TIM-3 to modulate mTOR phosphorylation [60] *Cytoplasmic–Lysosomes*: Interact with Lamp2 to regulate lysosomal functions and autophagy [61]	4-1BB [62] CD40 [63] CD44 [64] CD206 [65] Dectin-1 (macrophages) [66] DR3 [67] PD-1 [68] PDI [69][70] TCR [71][72] TIM-3 [68][73][74] VISTA [75]
LGALS10/Galectin-10/Charcot-Leyden crystal protein CLC (Chr. 19q13.2)	*Cytoplasmic–Granules*: Eosinophil-derived neurotoxin EDN (RNS2) and eosinophil cationic protein ECP (RNS3) co-localised with CD63. It is required for the maturation of eosinophil during granulogenesis [76]	
LGALS12/Galectin-12/GRIP1	*Cytoplasmic–Endosome/Lysosomes*:	

Gene/Protein Name (Chromosome Position [30])	Intracellular (*Cytoplasmic*/*Nucleus*)	Extracellular
(Chr. 11q12.3)	VPS13C in lipid droplets and promotes the polarisation to M1 macrophage via TLR4 pathway [77][78]	
LGALS13/Galectin-13/ placental protein 13 (Chr. 19q13.2)	*Nucleus*: HOXA1 [79]	Binds to T lymphocytes and induces apoptosis [80]; Binds to neutrophils and shifts to immunoregulatory phenotype and promotes high PD-L1 expression [81]
LGALS14/Galectin-14 (Chr. 19q13.2)		Binds to T lymphocytes and induces apoptosis [80] c-Rel [82]
LGALS16/Galectin-16 (Chr. 19q13.2)		c-Rel [83]

Remarks: Colour code is based on the galectin's structure: chimera type (galectin-3) is highlighted in light blue; tandem-repeat type is highlighted in light green; prototype has not been highlighted.

3. From Bench to Bedside

3.1. Availability of Galectin-Specific Inhibitors

Luckily, due to the small size of galectin proteins, their protein structures and binding site properties have been revealed by numerous nuclear magnetic resonance (NMR) studies [84]. An understanding of protein structures and functions can expedite the drug development process. Galectin inhibitors can be developed in different forms, including (1) small-molecule carbohydrates, (2) natural polysaccharides and their derivatives, (3) peptides and peptidomimetics, and (4) humanised monoclonal antibodies [85]. Their structures and binding affinities to galectin-1, galectin-3, and galectin-9 were fully reviewed by Mariño et al. (2023) [14]. Interestingly, further NMR spectroscopic

539–

alez, R.; nts with

ancer

ancer: 3, 62,

6. Pandey, P.; Khan, F.; Qari, H.A.; Upadhyay, T.K.; Alkhateeb, A.F.; Oves, M. Revolutionization in Cancer Therapeutics via Targeting Major Immune Checkpoints PD-1, PD-L1 and CTLA-4. Pharmaceuticals 2022, 15, 335.
7. Chang, E.; Pelosof, L.; Lemery, S.; Gong, Y.; Goldberg, K.B.; Farrell, A.T.; Keegan, P.; Veeraraghavan, J.; Wei, G.; Blumenthal, G.M.; et al. Systematic Review of PD-1/PD-L1 Inhibitors in Oncology: From Personalized Medicine to Public Health. Oncologist 2021, 26, e1786–e1799.
8. Chow, A.; Perica, K.; Klebanoff, C.A.; Wolchok, J.D. Clinical implications of T cell exhaustion for cancer immunotherapy. Nat. Rev. Clin. Oncol. 2022, 19, 775–790.
9. Togashi, Y.; Shitara, K.; Nishikawa, H. Regulatory T cells in cancer immunosuppression—implications for anticancer therapy. Nat. Rev. Clin. Oncol. 2019, 16, 356–371.
10. Preillon, J.; Cuende, J.; Rabolli, V.; Garnero, L.; Mercier, M.; Wald, N.; Pappalardo, A.; Denies, S.; Jamart, D.; Michaux, A.C.; et al. Restoration of T-cell Effector Function, Depletion of Tregs, and

analyses and competitive ELISA binding assays for protein structure and a substrate specific using synthetic hydroxypropyl methacrylamide (HPMA) copolymers with multi-Galβ4GlcNAc (LacNAc), which is specific to the CRD of human galectin-1 and galectin-3 with differential affinity at the sub-nanomolar level, have been explored [86]. This kind of assays might allow the identification of potential small-molecular inhibitors for future applications. In addition, peptide inhibitors and monoclonal antibodies are also useful to target secreted galectins and not interfere with the intracellular roles of galectins to minimise the side effects. New development techniques for synthesising glycosyl-side chain molecules and amino acid polypeptides may provide further new targets for galectin-based treatment in future.

3.2. Applications, Safety/Pitfalls/Limitations, and Ongoing Clinical Trials

At present, all ICIs come in the form of antibodies. This creates a limitation for future treatment potential, especially for brain metastasis, where the antibodies are unable to pass through the blood–brain barrier. In addition, the limited availability of immune cells within the central nervous system also imposes a limitation on the use of ICIs in patients with metastases at sites such as the brain and spine. The development of different small-molecule inhibitors for PD-1/PD-L1 is ongoing [87]. Further understanding of the resident and infiltrating immune cells across to the brain/spine via the blood–brain barrier [88] might make the impossible possible.

T-cell exhaustion is a common phenomenon in treatment using ICIs. Galectin inhibitors are a new approach to improve this situation and the durability of the treatment regimen. Combinations of ICIs and other inhibitors in the form of inhibitory antibodies or TKIs have been the subject of ongoing studies [89]. All the components, including cytotoxic T cells, NK cells, and their inhibitory molecules within the TME are required to orchestrate the anti-tumorigenic role of killing tumour cells.

The potential pitfalls of galectin inhibitors in future treatments should be noted in drug development. The first limitation is the unknown factors associated with laboratory settings, such as the differences between mouse models and real humans. The blood components of laboratory mice are different to those of humans. Mice have a high percentage of lymphocytes (around 70% of all leukocytes). In human bodies, the predominant immune cells are neutrophils, which account for over 50% of all leukocytes [90]. This issue is not easily addressed even in humanised mouse models [91]. It is difficult to understand a drug or biological reagent's effect without human trial studies. In addition, as discussed above, most galectins can alter immune responses. For example, galectin-3 has been shown to play important roles against pathogens [92]. For subjects treated with galectin-3 inhibitors, infection-related adverse effects in subsequent phase 2 or 3 trial studies should be noted as a safety parameter. Treatment using galectin-9 inhibitors also raises similar concerns to galectin-3-inhibitor treatment and can affect different types of immune cells. It may also raise additional concerns regarding autoimmune issues.

Although immune-related adverse events (irAEs) are inevitable in ICI treatment, the occurrence of irAEs during treatment reflects better prognosis and overall outcomes [93]. If the irAEs can be better managed and controlled using corticosteroids, this is a good sign of successful treatment. The effective and safe dosages for combined treatment could be evaluated and determined in clinical trial settings and provide guidelines for regimens and any

Direct Killing of Tumor Cells: The Multiple Mechanisms of Action of a TIGIT Antagonist Antibodies. Mol. Cancer Ther. 2021, 20, 121–131.

11. Florou, V.; Garrido-Laguna, I. Clinical Development of Anti-TIGIT Antibodies for Immunotherapy of Cancer. Curr. Oncol. Rep. 2022, 24, 1107–1112.
12. Cho, B.C.; Abreu, D.R.; Hussein, M.; Cobo, M.; Patel, A.J.; Secen, N.; Lee, K.H.; Massuti, B.; Hiret, S.; Yang, J.C.H.; et al. Tiragolumab plus atezolizumab versus placebo plus atezolizumab as a first-line treatment for PD-L1-selected non-small-cell lung cancer (CITYSCAPE): Primary and follow-up analyses of a randomised, double-blind, phase 2 study. Lancet Oncol. 2022, 23, 781–792.
13. Brazel, D.; Ou, S.I.; Nagasaka, M. Tiragolumab (Anti-TIGIT) in SCLC: Skyscraper-02, a Towering Inferno. Lung Cancer Targets Ther. 2023, 14, 1–9.
14. Mariño, K.V.; Cagnoni, A.J.; Croci, D.O.; Rabinovich, G.A. Targeting galectin-driven regulatory circuits in cancer and fibrosis. Nat. Rev. Drug Discov. 2023, 22, 295–316.
15. Zetterberg, F.R.; MacKinnon, A.; Brimert, T.; Gravelle, L.; Johnsson, R.E.; Kahl-Knutson, B.; Leffler, H.; Nilsson, U.J.; Pedersen, A.; Peterson, K.; et al. Discovery and Optimization of the First Highly Effective and Orally Available Galectin-3 Inhibitors for Treatment of Fibrotic Disease. J. Med. Chem. 2022, 65, 12626–12638.
16. Aslanis, V.; Slack, R.J.; MacKinnon, A.C.; McClinton, C.; Tantawi, S.; Gravelle, L.; Nilsson, U.J.; Leffler, H.; Brooks, A.; Khindri, S.K.; et al. Safety and pharmacokinetics of GB1211, an oral galectin-3 inhibitor: A single- and multiple-dose first-in-human study in healthy participants. Cancer Chemother. Pharmacol. 2023, 91, 267–280.
17. Astorgues-Xerri, L.; Riveiro, M.E.; Tijeras-Raballand, A.; Serova, M.; Rabinovich, G.A.; Bieche, I.; Vidaud, M.; de Gramont, A.; Martinet, M.; Cvitkovic, E.; et al. OTX008, a selective small-molecule inhibitor of galectin-1, downregulates cancer cell proliferation, invasion and tumour angiogenesis. Eur. J. Cancer 2014, 50, 2463–2477.
18. Greer, P.F.C.; Rich, A.; Coates, D.E. Effects of galectin-1 inhibitor OTX008 on oral squamous cell carcinoma cells in vitro and the role of AP-1 and the MAPK/ERK pathway. Arch. Oral. Biol. 2022, 134, 105335.
19. Koonce, N.A.; Griffin, R.J.; Dings, R.P.M. Galectin-1 Inhibitor OTX008 Induces Tumor Vessel Normalization and Tumor Growth Inhibition in Human Head and Neck Squamous Cell Carcinoma Models. Int. J. Mol. Sci. 2017, 18, 2671.
20. Leung, Z.; Ko, F.C.F.; Tey, S.K.; Kwong, E.M.L.; Mao, X.; Liu, B.H.M.; Ma, A.P.Y.; Fung, Y.M.E.; Che, C.M.; Wong, D.K.H.; et al. Galectin-1 promotes hepatocellular carcinoma and the combined therapeutic effect of OTX008 galectin-1 inhibitor and sorafenib in tumor cells. J. Exp. Clin. Cancer Res. 2019, 38, 423.

dosage reductions in the case of irAEs. While clinical trials are still ongoing with many unforeseen hurdles to overcome, galectin-3 small-molecule inhibitors, such as belapectin and GB1211, and galectin-9 humanised monoclonal antibodies still provide new opportunities for combination therapies to tackle the current unmet and unresolved issues associated with ICI treatments for many difficult-to-treat cancers.

21. Curti, B.D.; Koguchi, Y.; Leidner, R.S.; Rolig, A.S.; Sturgill, E.R.; Sun, Z.; Wu, Y.; Rajamanickam, V.; Bernard, B.; Hilgart-Martiszus, I.; et al. Enhancing clinical and immunological effects of anti-PD-1 with belapectin, a galectin-3 inhibitor. J. Immunother. Cancer 2021, 9, e002371.
22. Lau, E.S.; Liu, E.; Paniagua, S.M.; Sarma, A.A.; Zampierollo, G.; López, B.; Díez, J.; Wang, T.J.; Ho, J.E. Galectin-3 Inhibition With Modified Citrus Pectin in Hypertension. JACC Basic Transl. Sci. 2021, 6, 12–21.
23. Sigamani, A.; Mayo, K.H.; Miller, M.C.; Chen-Walden, H.; Reddy, S.; Platt, D. An Oral Galectin Inhibitor in COVID-19-A Phase II Randomized Controlled Trial. Vaccines 2023, 11, 731.
24. von Heijne, G. Membrane-protein topology. Nat. Rev. Mol. Cell Biol. 2006, 7, 909–918.
25. Nielsen, H.; Tsirigos, K.D.; Brunak, S.; von Heijne, G. A Brief History of Protein Sorting Prediction. Protein J. 2019, 38, 200–216.
26. Zanetti, G.; Pahuja, K.B.; Studer, S.; Shim, S.; Schekman, R. COPII and the regulation of protein sorting in mammals. Nat. Cell Biol. 2011, 14, 20–28.
27. Popa, S.J.; Stewart, S.E.; Moreau, K. Unconventional secretion of annexins and galectins. Semin. Cell Dev. Biol. 2018, 83, 42–50.
28. Kutzner, T.J.; Higuero, A.M.; Sussmair, M.; Kopitz, J.; Hingar, M.; Diez-Revuelta, N.; Caballero, G.G.; Kaltner, H.; Lindner, I.; Abad-Rodriguez, J.; et al. How presence of a signal peptide affects human galectins-1 and -4: Clues to explain common absence of a leader sequence among adhesion/growth-regulatory galectins. Biochim. Biophys. Acta Gen. Subj. 2020, 1864, 129449.
29. Wan, L.; Hsu, Y.A.; Wei, C.C.; Liu, F.T. Galectins in allergic inflammatory diseases. Mol. Aspects Med. 2021, 79, 100925.
30. Ensembl—Galectins. Available online: https://www.ensembl.org/Homo_sapiens/Search/Results?q=galectins;site=ensembl;facet_species=Human (accessed on 2 April 2023).
31. Zhang, Q.; Ali, M.; Wang, Y.; Sun, Q.N.; Zhu, X.D.; Tang, D.; Wang, W.; Zhang, C.Y.; Zhou, H.H.; Wang, D.R. Galectin-1 binds GRP78 to promote the proliferation and metastasis of gastric cancer. Int. J. Oncol. 2022, 61, 1–18.
32. Park, J.W.; Voss, P.G.; Grabski, S.; Wang, J.L.; Patterson, R.J. Association of galectin-1 and galectin-3 with Gemin4 in complexes containing the SMN protein. Nucleic Acids Res. 2001, 29, 3595–3602.
33. Paz, A.; Haklai, R.; Elad-Sfadia, G.; Ballan, E.; Kloog, Y. Galectin-1 binds oncogenic H-Ras to mediate Ras membrane anchorage and cell transformation. Oncogene 2001, 20, 7486–7493.
34. Ose, R.; Oharaa, O.; Nagase, T. Galectin-1 and Galectin-3 Mediate Protocadherin-24-Dependent Membrane Localization of beta-catenin in Colon Cancer Cell Line HCT116. Curr. Chem. Genom. 2012, 6, 18–26.

35. Eckardt, V.; Miller, M.C.; Blanchet, X.; Duan, R.; Leberzammer, J.; Duchene, J.; Soehnlein, O.; Megens, R.T.; Ludwig, A.K.; Dregni, A.; et al. Chemokines and galectins form heterodimers to modulate inflammation. EMBO Rep. 2020, 21, e47852.

36. Fulcher, J.A.; Chang, M.H.; Wang, S.; Almazan, T.; Hashimi, S.T.; Eriksson, A.U.; Wen, X.; Pang, M.; Baum, L.G.; Singh, R.R.; et al. Galectin-1 co-clusters CD43/CD45 on dendritic cells and induces cell activation and migration through Syk and protein kinase C signaling. J. Biol. Chem. 2009, 284, 26860–26870.

37. Hernandez, J.D.; Nguyen, J.T.; He, J.; Wang, W.; Ardman, B.; Green, J.M.; Fukuda, M.; Baum, L.G. Galectin-1 binds different CD43 glycoforms to cluster CD43 and regulate T cell death. J. Immunol. 2006, 177, 5328–5336.

38. Auvynet, C.; Moreno, S.; Melchy, E.; Coronado-Martinez, I.; Montiel, J.L.; Aguilar-Delfin, I.; Rosenstein, Y. Galectin-1 promotes human neutrophil migration. Glycobiology 2013, 23, 32–42.

39. Hsieh, S.H.; Ying, N.W.; Wu, M.H.; Chiang, W.F.; Hsu, C.L.; Wong, T.Y.; Jin, Y.T.; Hong, T.M.; Chen, Y.L. Galectin-1, a novel ligand of neuropilin-1, activates VEGFR-2 signaling and modulates the migration of vascular endothelial cells. Oncogene 2008, 27, 3746–3753.

40. Croci, D.O.; Cerliani, J.P.; Dalotto-Moreno, T.; Mendez-Huergo, S.P.; Mascanfroni, I.D.; Dergan-Dylon, S.; Toscano, M.A.; Caramelo, J.J.; Garcia-Vallejo, J.J.; Ouyang, J.; et al. Glycosylation-dependent lectin-receptor interactions preserve angiogenesis in anti-VEGF refractory tumors. Cell 2014, 156, 744–758.

41. Yildirim, C.; Vogel, D.Y.; Hollander, M.R.; Baggen, J.M.; Fontijn, R.D.; Nieuwenhuis, S.; Haverkamp, A.; de Vries, M.R.; Quax, P.H.; Garcia-Vallejo, J.J.; et al. Galectin-2 induces a proinflammatory, anti-arteriogenic phenotype in monocytes and macrophages. PLoS ONE 2015, 10, e0124347.

42. Liu, W.; Hsu, D.K.; Chen, H.Y.; Yang, R.Y.; Carraway, K.L., 3rd; Isseroff, R.R.; Liu, F.T. Galectin-3 regulates intracellular trafficking of EGFR through Alix and promotes keratinocyte migration. J. Investig. Dermatol. 2012, 132, 2828–2837.

43. Wang, S.F.; Tsao, C.H.; Lin, Y.T.; Hsu, D.K.; Chiang, M.L.; Lo, C.H.; Chien, F.C.; Chen, P.; Arthur Chen, Y.M.; Chen, H.Y.; et al. Galectin-3 promotes HIV-1 budding via association with Alix and Gag p6. Glycobiology 2014, 24, 1022–1035.

44. Chen, H.Y.; Fermin, A.; Vardhana, S.; Weng, I.C.; Lo, K.F.; Chang, E.Y.; Maverakis, E.; Yang, R.Y.; Hsu, D.K.; Dustin, M.L.; et al. Galectin-3 negatively regulates TCR-mediated CD4+ T-cell activation at the immunological synapse. Proc. Natl. Acad. Sci. USA 2009, 106, 14496–14501.

45. Mysore, V.P.; Zhou, Z.W.; Ambrogio, C.; Li, L.; Kapp, J.N.; Lu, C.; Wang, Q.; Tucker, M.R.; Okoro, J.J.; Nagy-Davidescu, G.; et al. A structural model of a Ras-Raf signalosome. Nat. Struct. Mol. Biol. 2021, 28, 847–857.

46. Seguin, L.; Camargo, M.F.; Wettersten, H.I.; Kato, S.; Desgrosellier, J.S.; von Schalscha, T.; Elliott, K.C.; Cosset, E.; Lesperance, J.; Weis, S.M.; et al. Galectin-3, a Druggable Vulnerability for KRAS-Addicted Cancers. Cancer Discov. 2017, 7, 1464–1479.

47. Fritsch, K.; Mernberger, M.; Nist, A.; Stiewe, T.; Brehm, A.; Jacob, R. Galectin-3 interacts with components of the nuclear ribonucleoprotein complex. BMC Cancer 2016, 16, 502.

48. Jia, W.; Kong, L.; Kidoya, H.; Naito, H.; Muramatsu, F.; Hayashi, Y.; Hsieh, H.Y.; Yamakawa, D.; Hsu, D.K.; Liu, F.T.; et al. Indispensable role of Galectin-3 in promoting quiescence of hematopoietic stem cells. Nat. Commun. 2021, 12, 2118.

49. Stillman, B.N.; Hsu, D.K.; Pang, M.; Brewer, C.F.; Johnson, P.; Liu, F.T.; Baum, L.G. Galectin-3 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death. J. Immunol. 2006, 176, 778–789.

50. Kuo, H.Y.; Hsu, H.T.; Chen, Y.C.; Chang, Y.W.; Liu, F.T.; Wu, C.W. Galectin-3 modulates the EGFR signalling-mediated regulation of Sox2 expression via c-Myc in lung cancer. Glycobiology 2016, 26, 155–165.

51. Gordon-Alonso, M.; Hirsch, T.; Wildmann, C.; van der Bruggen, P. Galectin-3 captures interferon-gamma in the tumor matrix reducing chemokine gradient production and T-cell tumor infiltration. Nat. Commun. 2017, 8, 793.

52. Kouo, T.; Huang, L.; Pucsek, A.B.; Cao, M.; Solt, S.; Armstrong, T.; Jaffee, E. Galectin-3 Shapes Antitumor Immune Responses by Suppressing CD8+ T Cells via LAG-3 and Inhibiting Expansion of Plasmacytoid Dendritic Cells. Cancer Immunol. Res. 2015, 3, 412–423.

53. Piyush, T.; Chacko, A.R.; Sindrewicz, P.; Hilkens, J.; Rhodes, J.M.; Yu, L.G. Interaction of galectin-3 with MUC1 on cell surface promotes EGFR dimerization and activation in human epithelial cancer cells. Cell Death Differ. 2017, 24, 1937–1947.

54. Paclik, D.; Danese, S.; Berndt, U.; Wiedenmann, B.; Dignass, A.; Sturm, A. Galectin-4 controls intestinal inflammation by selective regulation of peripheral and mucosal T cell apoptosis and cell cycle. PLoS ONE 2008, 3, e2629.

55. Villeneuve, C.; Baricault, L.; Canelle, L.; Barboule, N.; Racca, C.; Monsarrat, B.; Magnaldo, T.; Larminat, F. Mitochondrial proteomic approach reveals galectin-7 as a novel BCL-2 binding protein in human cells. Mol. Biol. Cell 2011, 22, 999–1013.

56. Nishi, N.; Shoji, H.; Seki, M.; Itoh, A.; Miyanaka, H.; Yuube, K.; Hirashima, M.; Nakamura, T. Galectin-8 modulates neutrophil function via interaction with integrin alphaM. Glycobiology 2003, 13, 755–763.

57. Renard, H.F.; Tyckaert, F.; Lo Giudice, C.; Hirsch, T.; Valades-Cruz, C.A.; Lemaigre, C.; Shafaq-Zadah, M.; Wunder, C.; Wattiez, R.; Johannes, L.; et al. Endophilin-A3 and Galectin-8 control the clathrin-independent endocytosis of CD166. Nat. Commun. 2020, 11, 1457.

58. Bieniasz-Krzywiec, P.; Martin-Perez, R.; Ehling, M.; Garcia-Caballero, M.; Pinioti, S.; Pretto, S.; Kroes, R.; Aldeni, C.; Di Matteo, M.; Prenen, H.; et al. Podoplanin-Expressing Macrophages Promote Lymphangiogenesis and Lymphoinvasion in Breast Cancer. Cell. Metab. 2019, 30, 917–936.e910.

59. Cueni, L.N.; Detmar, M. Galectin-8 interacts with podoplanin and modulates lymphatic endothelial cell functions. Exp. Cell Res. 2009, 315, 1715–1723.

60. Goncalves Silva, I.; Ruegg, L.; Gibbs, B.F.; Bardelli, M.; Fruehwirth, A.; Varani, L.; Berger, S.M.; Fasler-Kan, E.; Sumbayev, V.V. The immune receptor Tim-3 acts as a trafficker in a Tim-3/galectin-9 autocrine loop in human myeloid leukemia cells. Oncoimmunology 2016, 5, e1195535.

61. Sudhakar, J.N.; Lu, H.H.; Chiang, H.Y.; Suen, C.S.; Hwang, M.J.; Wu, S.Y.; Shen, C.N.; Chang, Y.M.; Li, F.A.; Liu, F.T.; et al. Lumenal Galectin-9-Lamp2 interaction regulates lysosome and autophagy to prevent pathogenesis in the intestine and pancreas. Nat. Commun. 2020, 11, 4286.

62. Madireddi, S.; Eun, S.Y.; Lee, S.W.; Nemcovicova, I.; Mehta, A.K.; Zajonc, D.M.; Nishi, N.; Niki, T.; Hirashima, M.; Croft, M. Galectin-9 controls the therapeutic activity of 4-1BB-targeting antibodies. J. Exp. Med. 2014, 211, 1433–1448.

63. Vaitaitis, G.M.; Wagner, D.H., Jr. Galectin-9 controls CD40 signaling through a Tim-3 independent mechanism and redirects the cytokine profile of pathogenic T cells in autoimmunity. PLoS ONE 2012, 7, e38708.

64. Wu, C.; Thalhamer, T.; Franca, R.F.; Xiao, S.; Wang, C.; Hotta, C.; Zhu, C.; Hirashima, M.; Anderson, A.C.; Kuchroo, V.K. Galectin-9-CD44 interaction enhances stability and function of adaptive regulatory T cells. Immunity 2014, 41, 270–282.

65. Enninga, E.A.L.; Chatzopoulos, K.; Butterfield, J.T.; Sutor, S.L.; Leontovich, A.A.; Nevala, W.K.; Flotte, T.J.; Markovic, S.N. CD206-positive myeloid cells bind galectin-9 and promote a tumor-supportive microenvironment. J. Pathol. 2018, 245, 468–477.

66. Daley, D.; Mani, V.R.; Mohan, N.; Akkad, N.; Ochi, A.; Heindel, D.W.; Lee, K.B.; Zambirinis, C.P.; Pandian, G.S.B.; Savadkar, S.; et al. Dectin 1 activation on macrophages by galectin 9 promotes pancreatic carcinoma and peritumoral immune tolerance. Nat. Med. 2017, 23, 556–567.

67. Madireddi, S.; Eun, S.Y.; Mehta, A.K.; Birta, A.; Zajonc, D.M.; Niki, T.; Hirashima, M.; Podack, E.R.; Schreiber, T.H.; Croft, M. Regulatory T Cell-Mediated Suppression of Inflammation Induced by DR3 Signaling Is Dependent on Galectin-9. J. Immunol. 2017, 199, 2721–2728.

68. Yang, R.; Sun, L.; Li, C.F.; Wang, Y.H.; Yao, J.; Li, H.; Yan, M.; Chang, W.C.; Hsu, J.M.; Cha, J.H.; et al. Galectin-9 interacts with PD-1 and TIM-3 to regulate T cell death and is a target for cancer immunotherapy. Nat. Commun. 2021, 12, 832.

69. Schaefer, K.; Webb, N.E.; Pang, M.; Hernandez-Davies, J.E.; Lee, K.P.; Gonzalez, P.; Douglass, M.V.; Lee, B.; Baum, L.G. Galectin-9 binds to O-glycans on protein disulfide isomerase. Glycobiology 2017, 27, 878–887.

70. Bi, S.; Hong, P.W.; Lee, B.; Baum, L.G. Galectin-9 binding to cell surface protein disulfide isomerase regulates the redox environment to enhance T-cell migration and HIV entry. Proc. Natl. Acad. Sci. USA 2011, 108, 10650–10655.

71. Yang, R.; Sun, L.; Li, C.F.; Wang, Y.H.; Xia, W.; Liu, B.; Chu, Y.Y.; Bover, L.; Vien, L.; Hung, M.C. Development and characterization of anti-galectin-9 antibodies that protect T cells from galectin-9-induced cell death. J. Biol. Chem. 2022, 298, 101821.

72. Colomb, F.; Giron, L.B.; Premeaux, T.A.; Mitchell, B.I.; Niki, T.; Papasavvas, E.; Montaner, L.J.; Ndhlovu, L.C.; Abdel-Mohsen, M. Galectin-9 Mediates HIV Transcription by Inducing TCR-Dependent ERK Signaling. Front. Immunol. 2019, 10, 267.

73. Pang, N.; Alimu, X.; Chen, R.; Muhashi, M.; Ma, J.; Chen, G.; Zhao, F.; Wang, L.; Qu, J.; Ding, J. Activated Galectin-9/Tim3 promotes Treg and suppresses Th1 effector function in chronic lymphocytic leukemia. FASEB J. 2021, 35, e21556.

74. Zhu, C.; Anderson, A.C.; Schubart, A.; Xiong, H.; Imitola, J.; Khoury, S.J.; Zheng, X.X.; Strom, T.B.; Kuchroo, V.K. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat. Immunol. 2005, 6, 1245–1252.

75. Yasinska, I.M.; Meyer, N.H.; Schlichtner, S.; Hussain, R.; Siligardi, G.; Casely-Hayford, M.; Fiedler, W.; Wellbrock, J.; Desmet, C.; Calzolai, L.; et al. Ligand-Receptor Interactions of Galectin-9 and VISTA Suppress Human T Lymphocyte Cytotoxic Activity. Front. Immunol. 2020, 11, 580557.

76. Grozdanovic, M.M.; Doyle, C.B.; Liu, L.; Maybruck, B.T.; Kwatia, M.A.; Thiyagarajan, N.; Acharya, K.R.; Ackerman, S.J. Charcot-Leyden crystal protein/galectin-10 interacts with cationic ribonucleases and is required for eosinophil granulogenesis. J. Allergy Clin. Immunol. 2020, 146, 377–389.e10.

77. Wan, L.; Lin, H.J.; Huang, C.C.; Chen, Y.C.; Hsu, Y.A.; Lin, C.H.; Lin, H.C.; Chang, C.Y.; Huang, S.H.; Lin, J.M.; et al. Galectin-12 enhances inflammation by promoting M1 polarization of macrophages and reduces insulin sensitivity in adipocytes. Glycobiology 2016, 26, 732–744.

78. Hancock-Cerutti, W.; Wu, Z.; Xu, P.; Yadavalli, N.; Leonzino, M.; Tharkeshwar, A.K.; Ferguson, S.M.; Shadel, G.S.; De Camilli, P. ER-lysosome lipid transfer protein VPS13C/PARK23 prevents aberrant mtDNA-dependent STING signaling. J. Cell Biol. 2022, 221, e202106046.

79. Yang, T.; Yao, Y.; Wang, X.; Li, Y.; Si, Y.; Li, X.; Ayala, G.J.; Wang, Y.; Mayo, K.H.; Tai, G.; et al. Galectin-13/placental protein 13: Redox-active disulfides as switches for regulating structure, function and cellular distribution. Glycobiology 2020, 30, 120–129.

80. Balogh, A.; Toth, E.; Romero, R.; Parej, K.; Csala, D.; Szenasi, N.L.; Hajdu, I.; Juhasz, K.; Kovacs, A.F.; Meiri, H.; et al. Placental Galectins Are Key Players in Regulating the Maternal Adaptive Immune Response. Front. Immunol. 2019, 10, 1240.

81. Vokalova, L.; Balogh, A.; Toth, E.; Van Breda, S.V.; Schafer, G.; Hoesli, I.; Lapaire, O.; Hahn, S.; Than, N.G.; Rossi, S.W. Placental Protein 13 (Galectin-13) Polarizes Neutrophils Toward an Immune Regulatory Phenotype. Front. Immunol. 2020, 11, 145.

82. Si, Y.; Li, Y.; Yang, T.; Li, X.; Ayala, G.J.; Mayo, K.H.; Tai, G.; Su, J.; Zhou, Y. Structure-function studies of galectin-14, an important effector molecule in embryology. FEBS J. 2021, 288, 1041–1055.

83. Si, Y.; Yao, Y.; Jaramillo Ayala, G.; Li, X.; Han, Q.; Zhang, W.; Xu, X.; Tai, G.; Mayo, K.H.; Zhou, Y.; et al. Human galectin-16 has a pseudo ligand binding site and plays a role in regulating c-Rel-mediated lymphocyte activity. Biochim. Biophys. Acta Gen. Subj. 2021, 1865, 129755.

84. Miller, M.C.; Nesmelova, I.V.; Daragan, V.A.; Ippel, H.; Michalak, M.; Dregni, A.; Kaltner, H.; Kopitz, J.; Gabius, H.J.; Mayo, K.H. Pro4 prolyl peptide bond isomerization in human galectin-7 modulates the monomer-dimer equilibrum to affect function. Biochem. J. 2020, 477, 3147–3165.

85. Laderach, D.J.; Compagno, D. Inhibition of galectins in cancer: Biological challenges for their clinical application. Front. Immunol. 2022, 13, 1104625.

86. Bertuzzi, S.; Gimeno, A.; Martinez-Castillo, A.; Lete, M.G.; Delgado, S.; Airoldi, C.; Rodrigues Tavares, M.; Blahova, M.; Chytil, P.; Kren, V.; et al. Cross-Linking Effects Dictate the Preference of Galectins to Bind LacNAc-Decorated HPMA Copolymers. Int. J. Mol. Sci. 2021, 22, 6000.

87. Wang, T.; Cai, S.; Cheng, Y.; Zhang, W.; Wang, M.; Sun, H.; Guo, B.; Li, Z.; Xiao, Y.; Jiang, S. Discovery of Small-Molecule Inhibitors of the PD-1/PD-L1 Axis That Promote PD-L1 Internalization and Degradation. J. Med. Chem. 2022, 65, 3879–3893.

88. Croese, T.; Castellani, G.; Schwartz, M. Immune cell compartmentalization for brain surveillance and protection. Nat. Immunol. 2021, 22, 1083–1092.

89. Vafaei, S.; Zekiy, A.O.; Khanamir, R.A.; Zaman, B.A.; Ghayourvahdat, A.; Azimizonuzi, H.; Zamani, M. Combination therapy with immune checkpoint inhibitors (ICIs); a new frontier. Cancer Cell Int. 2022, 22, 2.

90. Eruslanov, E.B.; Singhal, S.; Albelda, S.M. Mouse versus Human Neutrophils in Cancer: A Major Knowledge Gap. Trends Cancer 2017, 3, 149–160.

91. Douam, F.; Ploss, A. A humanized “new-trophil” mouse to study early inflammatory processes. Proc. Natl. Acad. Sci. USA 2022, 119, e2216699119.

92. Diaz-Alvarez, L.; Ortega, E. The Many Roles of Galectin-3, a Multifaceted Molecule, in Innate Immune Responses against Pathogens. Mediators Inflamm. 2017, 2017, 9247574.

93. Socinski, M.A.; Jotte, R.M.; Cappuzzo, F.; Nishio, M.; Mok, T.S.K.; Reck, M.; Finley, G.G.; Kaul, M.D.; Yu, W.; Paranthaman, N.; et al. Association of Immune-Related Adverse Events With Efficacy of Atezolizumab in Patients With Non-Small Cell Lung Cancer: Pooled Analyses of the Phase 3 IMpower130, IMpower132, and IMpower150 Randomized Clinical Trials. JAMA Oncol. 2023, 9, 527–535.