Preclinical evaluation of the role of galectin-1 in tumor immune evasion associated mechanisms using a highly selective galectin-1 small molecule inhibitor

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Introduction

Galectin-1 (Gal-1) is a β-galactoside-binding lectin that is highly expressed in the tumour microenvironment of some aggressive cancers, and whose high expression correlates strongly with poor survival. Following a data mining exercise, we found strong evidence of the potential role of Gal-1 in the establishment of a fibrotic tumour stroma and mechanisms associated with tumour invasion, metastasis and angiogenesis (right). However, there was limited data regarding the role of Gal-1 in immune escape by tumours to make any strong conclusions around this specific tumour microenvironment mechanism of action. To further strengthen the potential role of Gal-1 in tumour immune evasion, we completed a series of preclinical *in vivo* and *in vitro* activities, using a highly selective Gal-1 small molecule inhibitor developed at Galecto Biotech, GB1908.

Methods

To examine Gal-1 inhibition *in vivo*, we investigated the effects of the highly selective Galectin-1 inhibitor, GB1908, in a syngeneic mouse model of lung adenocarcinoma. Tumour volume was measured throughout the study, and RNA profiling of tumours was implemented upon completion of the in-life phase. Plasma samples were taken on the first and sixth (data not shown) day of dosing, at 4 hours post oral administration, to determine the plasma concentration of GB1908.

To evaluate Gal-1 inhibition on the T cell compartment, relevant to the clinical tumour microenvironment, we used an *in vitro* human cell system including H1299 lung cancer *cells,* human peripheral blood mononuclear cells (PBMCs, containing mitogen stimulated T cells) and either primary human fibroblasts (stroNSCLC model) or endothelial cells (VascNSCLC model). After treatment with or without GB1908, a selection of biomarkers were profiled to indicate the potential benefit of Gal-1 inhibition within the tumour microenvironment.

Results

Oral administration of GB1908 reduced mouse lung adenocarcinoma growth and showed substantial changes to gene expression profiles strongly associated with NK cell activity . No additional cytotoxic T cell related gene changes were uncovered in the GB1908 treated tumours. This result was expected due to the T-cell independent nature of the model and the lack of previous efficacy observed with checkpoint inhibitors as single agents

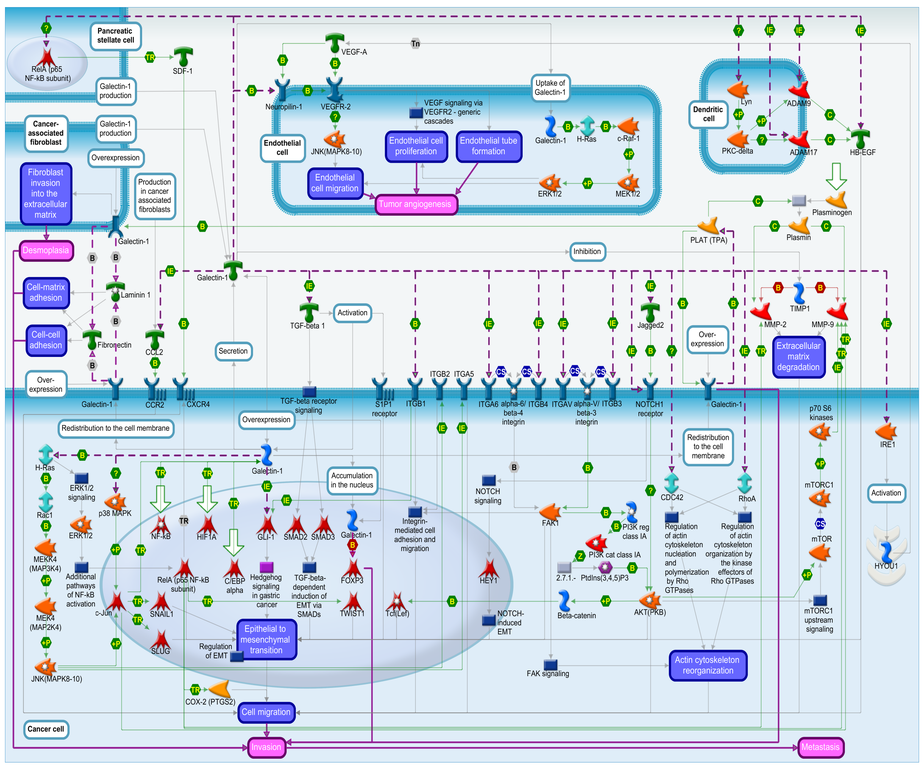
In two separate *in vitro IO* model systems we found a variety of immune suppressive proteins to be inhibited following 48 hour incubations with GB1908, including IL17, IL10 and IL6.

Conclusions

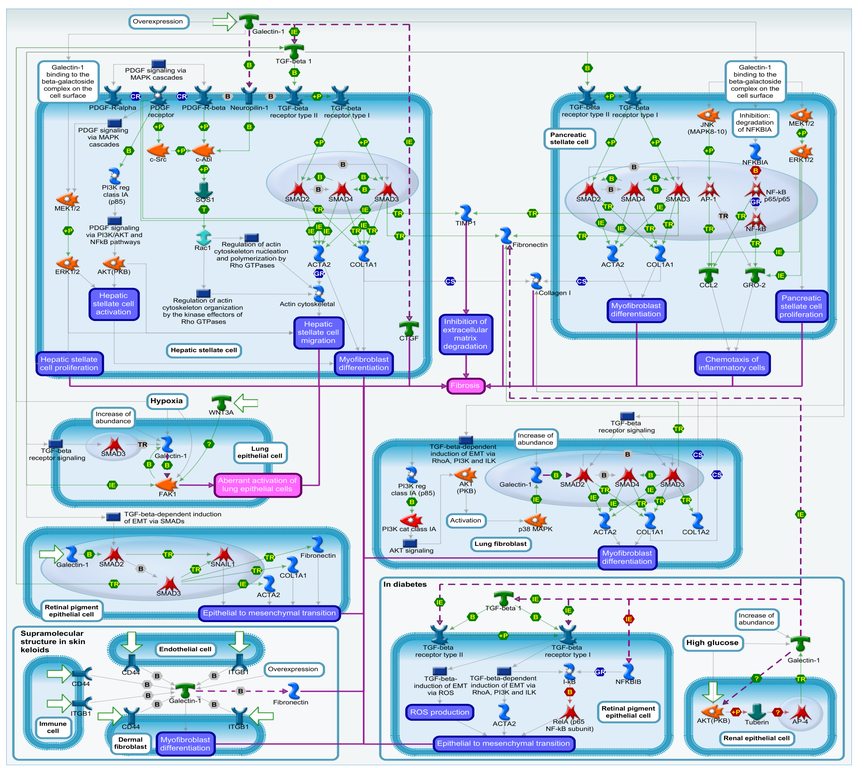
In summary, the data presented suggests highly selective Gal-1 inhibition could provide an effective monotherapy (or in combination with immune checkpoint inhibitors) to boost immune infiltration and/or activation in the tumour microenvironment of lung adenocarcinoma, and potentially other aggressive cancers.

Roles for Gal-1 in fibrosis and tumor invasion, metastasis and angiogenesis.

Role of Gal-1 in tumour invasion, metastasis, and angiogenesis

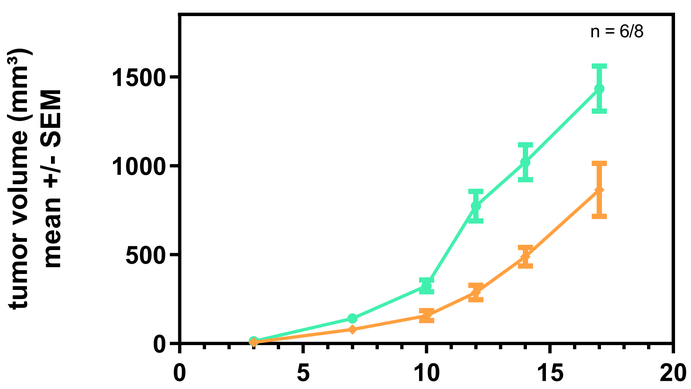


Role of Gal-1 in fibrosis



Data mining of published and clinical biomarker datasets were used to generate disease-specific pathway map reconstructions for Gal-1.

Gal-1 inhibition reduces tumor growth *in vivo* in a mouse model of lung adenocarcinoma



Lewis Lung Carcinoma mouse model

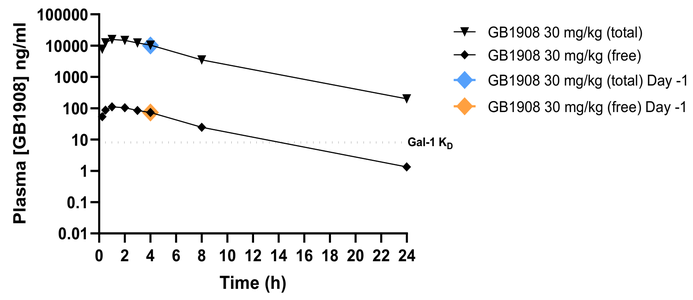
- Vehicle dosed orally, BID, from Day -1 to Day 17 (n=8)
- GB1908 (30mg/kg) dosed orally, BID, from Day -1 to Day 17 (n=6)
- Lewis Lung Carcinoma (LL/2) cells inoculated on Day 0 (all animals)

Treatment with GB1908 at 30 mg/kg (bid) significantly reduced tumor volume by 40% at day 17, in comparison to vehicle treatment.

Gal-1 inhibition alters some immune cell gene profiles within the tumor

Gene/s of interest (example)	Expression changes observed
TNF family member genes (TNF)	Yes
Cytotoxic genes (Granzyme B)	Yes
Additional Cytotoxic genes (Perforin)	No
T cell activation genes (LAG3)	No
NK specific genes (NKG7, NCR1)	Yes

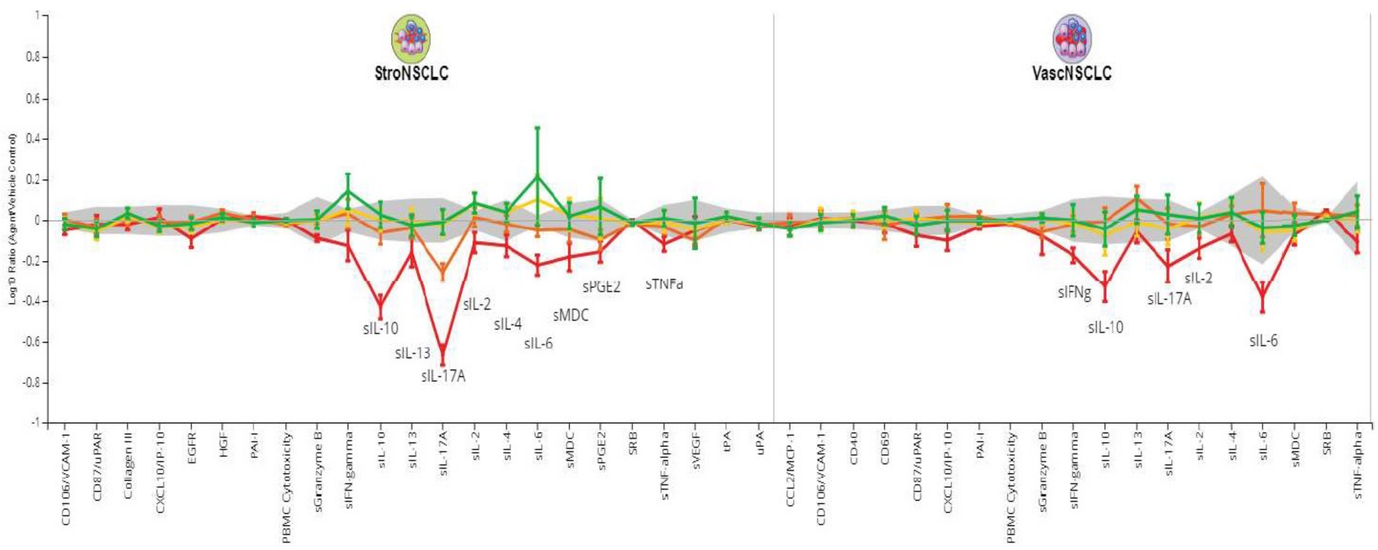
RNAseq analysis of tumors showed substantial changes in NK cell and TNF family gene profiles, when comparing vehicle to GB1908-treated tumors. (Tumors removed 4 hours post final dose).



Black lines: concentration profiles of total and free GB1908 measured in mouse plasma over 24 hours following a 30 mg/kg oral dose in a separate PK investigation.

Blue diamond = Total and Orange diamond = Free GB1908 concentrations at 4 hours after a single dose of GB1908 (30 mg/kg) on Day -1 (similar profile observed on day 5)

Gal-1 inhibition blocks immunosuppressive cytokine release in *in vitro* immuno-oncology cell models



Treatment of the “stroNSCLC” and “VascNSCLC” in vitro model systems with 10µM GB1908 (left, red line) inhibited the expression of numerous immune-suppressive proteins, notably IL17, IL10 and IL6.

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