Agenda

• Galectins – a structural perspective
• Galcetin-3 – molecular targets for therapeutic intervention
• Galectin-3 – Function in disease
• Pharmacological Targeting of Galectin-3
  • Preclinical Results
  • Clinical Results in NASH-cirrhosis
• Summary
What are Galectins?

- Family of highly conserved, molecular effectors that mediate various biological processes
  - Currently 15 mammalian galectins have been reported
  - Many more are found in different organisms
- Function by interacting with cell surface glycoconjugates
  - Usually target β-galactoside epitopes
- Involved in various biological functions and pathologies
  - Galectins are a focus for therapeutic discovery
  - Clinical interventions against cancer, fibrosis and other pathological disorders
Galectins - Structural

• Three groupings
  • Prototype – single core domain referred to as CRD
    • Gal-1, -2, -5, -7, -10, -11, -13, -14
  • Chimera – C-terminal CRD linked to a lengthy, collagen-like, dynamic and structurally aperiodic N-terminal tail
    • Gal-3
  • Tandem Repeats – two homologous, distinct, CRDs that are connected by linker polypeptide chains
    • Gal-4, -6, -8, -9, -12

• General Features
  • Well-defined CRD with highly conserved amino acid sequence
  • $\beta$ – sandwich structure
Galectin Oligomer States

- β-sandwich structure consists of 11 strands
  - 6 of strands define the sugar binding face (S-face) of the CRD
  - 5 of remaining strands define the opposing F-Face
- High resolution structures of the CRDs of human galectins have been reported
- CRD structures are highly conserved
- Lactose is the minimal carbohydrate ligand necessary for binding to galectins
Galectin-3 Carbohydrate Binding

- Lactose binding affinity
  - Gal-3: $26 \times 10^{-6}$ to $0.6 \times 10^{-3}$ M
- Gal-3
  - $\alpha$-galactomannan binds to the F-face of the CRD in both Gal-3 and Gal-1 (e.g., GM-CT-01)
  - Rhamnogalacturonan polysaccharide binds relatively strongly to the N-terminal sequence of Gal-3 NT (e.g., GR-MD-02)
  - Oligosaccharide branching can enhance binding affinity for Gal-1, Gal-3, and Gal-9

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- Pharmacological Targeting
  - Preclinical
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Galectin Functions in Cancer

- Galectin expression varies from cell type
- All cells express at least one galectin
- Galectins are synthesized on cytosolic ribosomes
- Galectins control cell-cell and cell-matrix interactions, adhesion, proliferation, apoptosis, pre-mRNA splicing, immunity and inflammation

Tumorigenicity
- Pro: ↑
- Anti: ↓
Galectin Function in Fibrosis

- Fibrosis represents the end result of chronic inflammatory reactions induced by a variety of stimuli
- Mechanisms driving fibrogenesis are distinct from those regulating acute inflammatory reactions
- Studies indicate that Gal-3 is increased in fibrosis affecting many different tissues
  - Liver, Kidney, Lung, Heart and Nervous System
- Galectin-3 sits at the nexus of a variety of downstream processes involved in fibrosis in numerous tissues
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Gal-3 Plays a Critical Role in Fibrosis

- Galectin-3 expression is up-regulated in human liver fibrosis.
  (a) Galectin-3 expression in normal human liver.
  (b) Galectin-3 in cirrhosis secondary to hepatitis C infection.
- Galectin-3 expression is temporally and spatially related to fibrosis in a reversible rat model of liver fibrosis.
  i. (C-Upper) Collagen stained with PSR.
  ii. (C-Lower) Galectin-3 immunohistochemistry.
  iii. Control (olive oil vehicle only).
  iv. (C-Middle) Peak fibrosis in rat liver after 12 weeks of twice weekly i.p. CCL4.
  v. (C-Right) Resolution, 24 weeks after cessation of CCL4-induced liver injury.

Henderson, et al 2006
Galectin-3 Critical Role in Fibrosis

Galectin-3 mediated activation of HSCs *in vivo* is a central mechanism underlying hepatic fibrosis.

- Hepatic stellate cell (HSC) is the key fibrogenic cell liver
- HSCs activation to proliferative, fibrogenic, and contractile myofibroblasts with increased expression of -smooth muscle actin (-SMA),
- After CCL4 administration, SMA expression was markedly increased in WT compared with *Galectin-3 null* mice
- The transcripts for -SMA mRNA were significantly increased in WT animals after CCL4 treatment
- Decrease in mRNA expression was paralleled by a decrease in hepatic -SMA protein expression assessed by Western blot

Henderson et al, 2006
Galectin-3 has important effects on two key cell types in liver fibrosis - macrophages and stellate cells.
What is the Role of TGF-β?

- TGF-β is a major profibrogenic cytokine and is thought to be a key mediator of fibrosis in many different organs; but, is it?
- TGF-β mRNA expression was markedly elevated after hepatic injury.
- However, expression of TGF-β was similar in whole liver homogenates from fibrotic liver in WT and Galectin-3 null mice and secretion of TGF-β was the same in WT and Galectin-null macrophages and HSCs.
- Smad-2 and Smad-3 signaling in HSCs was similar between the two genotypes when stimulated with TGF-β.
- However, despite similar levels of TGF-β and intact TGF-β signaling pathways, the absence of Galectin-3 markedly inhibited the fibrotic phenotype in vitro and in vivo in animal models.
- TGF-β failed to transactivate galectin-3-null hepatic stellate cells, in contrast with wildtype hepatic stellate cells.
- Furthermore, galectin-3 siRNA treatment in vitro and in vivo inhibited hepatic stellate cell activation.

**These data demonstrate that TGF-β stimulated HSC activation and procollagen production requires the presence of Galectin-3.**

Henderson et al, 2006; 2009
Mechanisms of Galectin-3-mediated regulation of alternative macrophage (M2) activation.

A Galectin-3 feedback loop drives alternative macrophage activation.

Henderson et al, 2009
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• Galectin-3 Function in disease

**Pharmacological Targeting**
  • Preclinical
  • Clinical Results in NASH-cirrhosis
Approaches to targeting Galectin-3 with pharmacological agents

• Disaccharides such as lactose and n-acetyl lactosamine bind to the canonical carbohydrate recognition domain
• Derivative disaccharides are in phase 1 development for lung fibrosis (inhaled formulation) (TD-139; Galecto Biotech, licensed by BMS)
• Monosaccharides bind much less avidly to the CRD and neither mono or disaccharides are orally bioavailable
• Anti-galectin antibodies have reportedly been explored, but there are no reported successes
• Small organic molecules have not been identified that interact with CRD, but allosteric approaches may be tractable (internal data; Galectin Sciences, LLC)
• Galectin Therapeutics approached inhibition using naturally occurring plant polysaccharides as starting materials to produce specific Galectin-3 inhibitors
• Broad composition of matter and methods of use patents
The theoretical basis for complex carbohydrate drugs is that terminal galactose residues bind to galectin proteins in the context of a macromolecular structure, similar to the situation with glycoproteins.

**Characteristics of binding**
- Binds to canonical CRD but also to F-face of CRD
- Each molecule of GR-MD-02 binds ~5 galectin-3 molecules
- 50% Gal-3 is bound ~2.9 μM
- Gal-3 > Gal-1 >> Gal-7

**Critical chemical properties**
- Average molecular weight
- % total Gal + Arab
- Gal/Ara ratio
- % GalA methoxylated
The GR-MD-02 is a **non-biological complex drug substance** which is a polymer comprised of a repeating unit, which is represented by the structure diagram below:

The active moiety is defined analytically by the combination of the following key characteristics of the compound, which are controlled by established specifications:

- **Identity (2D NMR)**
- **Purity** (content based on Size Exclusion Chromatography with RI detector)
- **Carbohydrate composition**, including Galactose and Arabinose (based on Ion Chromatography with PAD detector)
- **Degree of Esterification** (based on analyses by 2D NMR of the degree of methyl galacturonate vs. glucorinate)
- **Molecular Weight** (based on Size Exclusion Chromatography with MALS detector)
Interaction of GR-MD-02 with Galectin-3

NMR-HSQC (nuclear magnetic resonance-heteronuclear single quantum coherence).

- Many resonances within the CRD (carbohydrate recognition domain) are shifted, at both the canonical carbohydrate binding site on the β-sandwich S-face and the opposing F-face.
- Binding stoichiometry was approximately 5 molecules of Gal-3 per one molecule of GR-MD-02.
- The concentration of GR-MD-02 at which 50% Gal-3 is bound is about 2.9 µM.
- Binding to Gal-3 is somewhat more avid than binding to Gal-1 and much greater than binding to Gal-7.
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GR-MD-02 Has Therapeutic Effect On NASH With Fibrosis In Mouse Model*

*Traber PG and Zomer E. Therapy of Experimental NASH and Fibrosis with Galectin Inhibitors. PLOS ONE 2013;8:e83481
GR-MD-02 Markedly Improved NASH in the Mouse Model, with similar improvements to those seen in Galectin-3 null mice

Journal of Hepatology 2011 vol. 54 | 975–983
GR-MD-02 Prevented and Completely Reversed Fibrosis in NASH Mouse Model and Efficacy Not Dependent on When Treatment was Started

Given IV twice weekly. These results have been confirmed in repeat studies with different dosing regimens.
Galectin-3 Expression Is Increased in a Subset of Macrophages in NASH Mice

No major change in total number of macrophages, as shown with macrophage staining, but increase in gal-3 expression.
Galectin-3 is Expressed In Liver Macrophages And Is Markedly Increased In Human NASH

Kupffer cells = liver resident macrophages  Portal macs = macrophages located in portal regions
Treatment with GR-MD-02 Markedly Reduces Gal-3 Expressing Macrophages in NASH Mice
Activated stellate cells have a myofibroblast phenotype and are primary cellular source of fibrotic tissue.
Dose response of once weekly doses of GR-MD-02 in mouse NASH shows efficacy in reducing fibrosis down to 10 mg/kg per week.

The difference in the results of Sirius red staining and hydroxyproline content in the 10 mg/kg group may be related to differences in the sensitivity and reproducibility of the two the methods, with the biochemical method generally felt to be more reliable than the histological method.
Dose Response Effect On Liver Hydroxyproline and iNOS Expression Defines Therapeutic Window
Blood and Tissue Levels Of Galectin-3 Are Not Correlated In Mouse NASH Model Nor Human NASH

Marked changes in expression of galectin-3 in liver macrophages are not reflected in changes in blood galectin-3

Liver Gal-3 Staining

Liver Tissue Level

Blood Level

- No evidence for correlation between blood galectin-3 levels and disease activity or fibrosis stage in patients with NASH\(^1\)
- Blood Galectin-3 levels in humans are correlated with obesity\(^1,2\) and diabetes\(^2\)

\(^1\) Yilmaz, et al. Clinical Biochemistry 2011
GR-MD-02 Reversed Cirrhosis And Improved Portal Hypertension In Rat Model*

GR-MD-02 reversed cirrhosis and reduced portal inflammation and hepatocyte ballooning

Liver histology from each animal was evaluated in a blinded fashion by an experienced pathologist. Statistical analysis was done using Mann-Whitney test for non-parametric measurements and graph shows median with interquartile range.

Preclinical Data Shows That GR-MD-02 Can Reverse NASH, Fibrosis, and Cirrhosis

<table>
<thead>
<tr>
<th>Effect</th>
<th>NASH mouse(^1)</th>
<th>Cirrhotic rat(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduces inflammation</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Reduces fat</td>
<td>X</td>
<td>N/A</td>
</tr>
<tr>
<td>Reduces cell death</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Prevents fibrosis</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Reverses fibrosis</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Reduces portal pressure</td>
<td>N/A</td>
<td>X</td>
</tr>
<tr>
<td>Targets macrophages in liver</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Reduces galectin-3 in liver</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

\(^1\)Traber PG and Zomer E. Therapy of Experimental NASH and Fibrosis with Galectin Inhibitors. PLOS ONE 2013;8:e83481


N/A = not applicable
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NASH-CX Phase 2b Clinical Trial

• Enrolled 162 Patients
  • NASH cirrhosis with portal hypertension
  • Well compensated disease with no complications of cirrhosis

• Primary Endpoint
  • Portal pressure (HVPG—hepatic venous pressure gradient)
  • Change in baseline adjusted HVPG from beginning to end of study
  • FDA views this endpoint as a potentially acceptable surrogate for outcomes for registration trials in this patient population.

• Secondary Endpoints
  • Liver biopsy for staging of fibrosis
  • FibroScan® for measuring liver stiffness which is related to fibrosis
  • Methacetin breath test which measures liver function
  • Patient outcomes

• Top line data reported in December 2017 [see our website for additional information]
43% of patients without varices in the GR-MD-02 2mg/kg group showed a ≥2 mmHg and ≥20% decrease from baseline compared to 13% in the placebo group.

**Percentage of Patients Who Had a Clinically Relevant Reduction in HVPG With:**

- ≥ 2 mmHg Decrease From Baseline AND
- ≥ 20% Decrease From Baseline

<table>
<thead>
<tr>
<th>Group</th>
<th>0%</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>4/31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GR-MD-02 2 mg/kg</td>
<td>10/23</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Highlights**

- Chi Square

High bar to demonstrating efficacy that is contingent on clinically important reduction in HVPG from baseline.
Patients in the 2 mg/kg treatment group showed statistically significant improvement of liver cell death on liver biopsy\(^1\)

In the total population there was improvement in cell death, a critical feature of NASH

\(^1\) ITT population

Ordinal Logistic Regression Analysis

Highlights

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SEM</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td>GR-MD-02 2 mg/kg</td>
<td>0.03</td>
<td>53</td>
</tr>
<tr>
<td>GR-MD-02 8 mg/kg</td>
<td>0.09</td>
<td>54</td>
</tr>
</tbody>
</table>
Highlights of Results NASH-CX – reported Dec. 2017

- **NASH-CX is the first clinical trial to show positive results in compensated NASH cirrhosis without esophageal varices**
  - Clinically meaningful effect in reducing portal pressure in a subgroup of patients
  - Improvement in liver cell death, a key component of NASH in the total population
  - Reduction in the development of new esophageal varices
  - Drug was safe and well-tolerated
  - Following meeting with FDA in May 2018, determined to be Phase 3-ready
  - The presence of varices is part of STD care for patients and can easily be done with endoscopy
  - 50% of NASH cirrhosis patients do not have varices when diagnosed
  - Further awareness of NASH will lead to early diagnosis which will increase the number of patients without varices

- **These results will propel development program to the next stage**
  - Proceeding with plans for a phase 3 clinical trial program

- **End of Phase 2 meeting held with FDA gave guidance for Phase 3.**
  - Development of varices or change in HVPG could be meaningful surrogate endpoints.
  - Prevention of formation of large varices, since they require intervention, may be acceptable outcome measure

- See further detailed information on clinical trial results and FDA discussions on our website
NASH Cirrhosis Therapy Program with GR-MD-02: Summary

- Unmet medical need with a very large potential market
- Competitively well positioned in NASH cirrhosis
- Reversal of fibrosis/cirrhosis in preclinical models
- Strong patent portfolio supporting composition of matter, production, and use of GR-MD-02
- Extensive pre-clinical and early clinical data demonstrates strong safety profile and tolerability
- Phase 2b clinical trial readout December 2017
- Phase 3 trial being planned based on FDA guidance and KOL inputs
Galectin-3 is at the apex of multiple pathophysiological processes in NASH and fibrogenesis

<table>
<thead>
<tr>
<th>Cause of Liver Injury</th>
<th>Mediators</th>
<th>Inflammation</th>
<th>Fibrosis</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metabolic Syndrome</td>
<td>Glucose Intolerance</td>
<td>Fat Accumulation</td>
<td>Impaired Lipid Metabolism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyperglycemia</td>
<td>Adipo-Cytokines</td>
<td>Free Fatty Acids</td>
</tr>
</tbody>
</table>

Potential sites of anti-galectin activity

- **Macrophage effects:**
  - ↓ pro-inflammatory cell type
  - ↓ recruitment of monocytes
  - ↓ inflammatory cytokines

- **Hepatocyte/Macrophage effects:**
  - ↓ scavenger receptor activity
  - ↓ ALE and AGE uptake
  - ↓ cellular toxicity

- **Stellate Cell effects:**
  - ↓ activation
  - ↓ TGF-β1 receptor activity
  - ↓ collagen production

- **Extracellular matrix/collagen dissolution:**
  - ↑ restorative macrophage activity
  - ↑ matrix metalloproteases

Blue is hypothesis
Summary

• Galectin-3 is critical to the fibrotic process
  • Galectin-3 also plays an important role in various cancers
• Preclinical models demonstrate that a galectin antagonist (GR-MD-02) can reverse fibrosis in various animal models
  • Liver, Lung, Kidney and Heart
• Mechanistic models were presented to demonstrate that Galectin-3 is at the nexus of organ fibrosis
• Clinical results in patients with NASH cirrhosis demonstrate efficacy of GR-MD-02 in reducing portal pressure in well compensated cirrhotic patients without varices at baseline and reducing hepatocyte ballooning
Critical Role of Galectin-3

Hepatocyte damage
Inflammation

Oxidative stress
and cofactors

Hepatocyte growth
arrest/lipoapoptosis

Rise of fibrogenic
cholangiocytes
ductular reaction

Tweak
(inflammatory cells)

EMT-like
changes

Galectin 3

PDGF-BB, ET-1
TGFβ1, TGFβ2
CTGF, MCP-1

HGF, bFGF,
IL-6, Shh

Galectin 3

Activated
cholangiocytes

De novo expression of
integrin αβ6

Activated
myofibroblasts

αβ6 inhibitors

Cirrhosis/HCC

Modified from Shuppan et al 2017
Thanks for your attention

For further information see www.galectintherapeutics.com