“NUCLEAR RECEPTORS MODULATORS AND TREATMENT OF CHOLANGIOPATIES”

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DISCLOSURES

I serve as member of the Scientific Advisor Board of Intercept Pharma Inc, New York, NY, USA

My presentation includes mention of off-label or investigational use drugs: INT-747 (FXR) and INT-777 (TGR5)
Patterns of Hepatic Fibrosis Development

A. Biliary Type

B. Post-necrotic

C. Vascular Type

D. ASH/NASH

Pericellular “Chicken Wire”

Pinzani M. and Rombouts K., Dig Liv Dis 2004; 36:231-242
PRIMARY BILIARY CIRRHOSIS (PBC)

Tipically progressive portal-to-portal fibrosis (portal-to-central only in late stage)

Onion-like peribiliary fibrosis with subsequent involvement of portal tracts

PRIMARY SCLEROSING CHOLANGITIS (PSC)
**Prevalent Mechanisms of Fibrogenesis in Different CLDs**

- Chronic direct/indirect damage (Viral, Autoimmune) → Chronic activation of the wound healing process
- Chronic Toxic Damage and/or metabolic overload (ETOH Abuse, metals, steatohepatitis) → Oxidative stress
- Ductular reaction / chronic cholestasis → Oxidative Stress, “Pro-inflammatory” Cholangiocytes, Disturbance of the normal Epithelial-Mesenchymal Equilibrium (???)
Bile duct damage and proliferation

Cholangiocytes Promote Fibrogenesis by Secreting Pro-Inflammatory and Pro-Fibrogenic Factors

ROS                          Bile Acids
Activation, Proliferation, Chemotaxis, Contraction, Secretion of Chemokines

“Pro-Inflammatory” Cholangiocytes

PDGF-BB
VEGF
MCP-1
ET-1
CTGF
TGF-β
IL-6
TNFα
Origin of Hepatic MFs Following Liver Injury

Roles of MFs in human liver diseases (established or suggested)

- **Pro- Fibrogenic**
  - proliferation (all MFs)
  - migration (all MFs)
  - ECM synthesis (all MFs)
  - contractility (HSC/MFs)
  - proinflammatory (HSC/MFs)

- **Pro-angiogenic**
  - documented for HSC/MFs and IF/MFs

- **Immunomodulatory**
  - documented for HSC/MFs

- **In liver regeneration**
  - proposed for HSC/MFs

- **As Stem Cells**
  - suggested for HSC/MFs

Parola M and Pinzani M. Fibrogenesis & Tissue Repair 2009
Bile Acids Induce Hepatic Stellate Cell Proliferation via Activation of the Epidermal Growth Factor Receptor

Svegliati-Baroni G et al., Gastroenterology 2005; 128:1042

Bile Acid-induced Epidermal Growth Factor Receptor Activation in Quiescent Rat Hepatic Stellate Cells Can Trigger Both Proliferation and Apoptosis

Sommerfeld A et al., J Biol Chem 2009; 24: 22173
**Endocrine Hormone Receptors**
1. Estrogen Receptor-β (ER-β)
2. Estrogen Receptor-α (ER-α)
3. Glucocorticoid Receptor (GR)
4. Mineralcorticoid Receptor (MR)
5. Androgen Receptor (AR)
6. Progesterone Receptor (PR)
7. Retinoic Acid Receptor (RAR)
8. Tyroid Hormone Receptor (TR)
9. Vitamin-D Receptor (VDR)

**Orphan Nuclear Receptors**
1. Chicken Ovalbumin Upsteram (COUP)
2. Dosage-sensitive Sex Reversal (DAX)
3. Germ Cell Nuclear Factor (GCNF)
4. Liver Related Homologue-1 (LRH-1)
5. NGF-induced clone B (NGFI-B)
6. Photoreceptor Nuclear Receptor (PNR)
7. Reverse ErbA (RevErbA)
8. Small Heterodimer Partner
9. Steroidogenic Factor-1 (SF-1)

**Adopted Orphan Receptors**
1. Androstan Receptor (CAR)
2. Estrogen Related Receptor-α (ERR)
3. Farnesoid X Receptor (FXR)
4. Hepatocyte Nuclear Factor-4 (HNF-4)
5. Liver X Receptor (LXR)
6. Peroxisome Proliferator-Act Rec (PPAR)
7. Pregnane X Receptor (PXR)
8. Retinoid X Receptor (RXR)

**HUMAN NUCLEAR RECEPTOR SUPERFAMILY**

**METABOLIC NUCLEAR RECEPTORS**
Control of bile acid biosynthesis, disposal and transport

From Zollner and Trauner, Br J Pharmacol 2009
Targets for Nuclear Receptor Ligands in Cholestasis

- Decrease FIBROSIS
- Decrease BA-UPTAKE
- Decrease BA-SYNTHESIS
- Increase DETOXIFICATION
- Increase BASOLATERAL EXCRETION
- Increase RENAL ELIMINATION

From Zollner and Trauner, Br J Pharmacol 2009
Targets for Nuclear Receptor Ligands in Cholestasis

From Zollner and Trauner, Br J Pharmacol 2009
Nuclear Receptor Ligands Potentially Employable for the Treatment of Cholestatic Diseases

- **FXR**
  - CDCA, DCA, CA, synthetic: GW4064, 6-ECDCA
- **PXR**
  - Rifampicin in humans, phenobarbital, dexamethasone, statins, St. John’s wort, clotrimazole pregnenolone-16\(\alpha\)-carbonitride (PCN) in rodents
- **VDR**
  - 1\(\alpha\),25-dihydroxy-vitamin, D\(\alpha\), LCA
- **PPAR-\(\alpha\)**
  - Fatty acids, fibrates, statins, eicosanoids, leukotriens, NSAIDs, WY-14643

### Table: Nuclear Receptor Ligands for Cholestatic Disorders

<table>
<thead>
<tr>
<th>Drug</th>
<th>Nuclear receptor target</th>
<th>Cholestatic disorder</th>
<th>ClinicalTrials.gov weblink</th>
</tr>
</thead>
<tbody>
<tr>
<td>INT-747 (6-ECDCA)</td>
<td>FXR</td>
<td>PBC/monotherapy</td>
<td><a href="HTTP://CLINICALTRIALS.GOV/CT2/SHOW/NCT00570765">HTTP://CLINICALTRIALS.GOV/CT2/SHOW/NCT00570765</a></td>
</tr>
<tr>
<td>INT-747 (6-ECDCA)</td>
<td>FXR</td>
<td>PBC/combination therapy with UDCA</td>
<td><a href="HTTP://CLINICALTRIALS.GOV/CT2/SHOW/NCT00550862">HTTP://CLINICALTRIALS.GOV/CT2/SHOW/NCT00550862</a></td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>PPAR(\alpha)</td>
<td>PBC</td>
<td><a href="HTTP://CLINICALTRIALS.GOV/CT2/SHOW/NCT00575042">HTTP://CLINICALTRIALS.GOV/CT2/SHOW/NCT00575042</a></td>
</tr>
</tbody>
</table>
FXR
(Farnesoid-X-Receptor)
THE MULTIFUNCTIONAL ROLE OF FXR SIGNALING

**Bile Acids Metabolism**
- CYP7A1*
- CYP8B1*
- SHP
- BSEP
- IBABP
- NTCP*

**Lipid Metabolism**
- APO-A1* (?)
- PLTP
- LPL
- CEPT
- SREBP-1*
- VLDL rec.

**Hepatic Stellate Phenotype**
- SHP
- AP-1*
- α-SMA*
- Colla-1*
- TIMP-1*

**Carbohydrate Metabolism**
- PEPCK*
- TRB3
- FBP1*

* SHP-dependent Modulation
FXR IS EXPRESSED IN RAT HSC AT EARLY STAGES OF CULTURE AND IS UPREGULATED UPON ACTIVATION

**Expression of Ntcp, FXR, Shp and Collagen1α1 mRNA in Quiescent and Activated Mouse HSC Compared to Whole Mouse Liver Tissue**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mouse HSC</th>
<th>5d culture</th>
<th>10 d culture</th>
<th>P1 SFIF</th>
<th>10%FBS</th>
<th>LT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Col 1α2</strong></td>
<td>0.22</td>
<td>0.23</td>
<td>7.80</td>
<td>11.26</td>
<td>17.09</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>FXR</strong></td>
<td>0.01</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>11.90</td>
</tr>
<tr>
<td><strong>SHP</strong></td>
<td>0.005</td>
<td>0.026</td>
<td>0.019</td>
<td>n.d.</td>
<td>n.d.</td>
<td>5.906</td>
</tr>
<tr>
<td><strong>VDR</strong></td>
<td>n.d.</td>
<td>n.d.</td>
<td>11.07</td>
<td>17.54</td>
<td>30.65</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>Ntcp</strong></td>
<td>0.01</td>
<td>n.d.</td>
<td>0.001</td>
<td>n.d.</td>
<td>0.002</td>
<td>53.30</td>
</tr>
</tbody>
</table>

Data are presented as ratio to 28S rRNA expression
n.d. : not detectable

Expression of Bile Acid Transport and Detox Systems and their Regulatory NR in Activated Human HSC Compared to Primary Human Hepatocytes (PHH) and Whole Human Liver Tissue (LT)

<table>
<thead>
<tr>
<th>Gene</th>
<th>hHSCs (n=6)</th>
<th>PHH (n=3)</th>
<th>LT (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTCP</td>
<td>n.d.</td>
<td>5.6±3.1</td>
<td>12.6±8.0</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>n.d.</td>
<td>2.5±1.7*</td>
<td>10.5±4.2</td>
</tr>
<tr>
<td>BSEP</td>
<td>n.d.</td>
<td>1.1±0.5#</td>
<td>4.2±0.3</td>
</tr>
<tr>
<td>MRP2</td>
<td>0.05±0.06**</td>
<td>3.5±3.4</td>
<td>6.5±3.0</td>
</tr>
<tr>
<td>MRP3</td>
<td>0.4±0.3#</td>
<td>1.3±0.9#</td>
<td>3.0±0.8</td>
</tr>
<tr>
<td>MRP4</td>
<td>1.0±1.0</td>
<td>0.04±0.02*</td>
<td>0.07±0.06</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>n.d.</td>
<td>0.7±0.7</td>
<td>0.7±0.2</td>
</tr>
<tr>
<td>UGT2B4</td>
<td>n.d.</td>
<td>1.4±0.7#</td>
<td>4.6±0.1</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>0.02±0.04**</td>
<td>1.0±0.2#</td>
<td>7.3±2.0</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>0.3±0.4**</td>
<td>5.8±6.6</td>
<td>374±208</td>
</tr>
<tr>
<td>FXR</td>
<td>0.06±0.01**</td>
<td>0.8±0.3</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>SHP</td>
<td>0.03±0.01**</td>
<td>1.5±1.0</td>
<td>3.9±0.3</td>
</tr>
<tr>
<td>VDR</td>
<td>16.1±8.3**</td>
<td>0.7±0.6</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>CAR</td>
<td>n.d.</td>
<td>1.1±0.6</td>
<td>10.7±1.6</td>
</tr>
<tr>
<td>PXR</td>
<td>0.5±0.4**</td>
<td>8.4±3.8</td>
<td>30.0±11.1</td>
</tr>
<tr>
<td>LXRα</td>
<td>0.3±0.07**</td>
<td>0.8±0.3#</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>LXRβ</td>
<td>4.8±1.9#</td>
<td>1.4±1.0</td>
<td>1.0±1.0</td>
</tr>
</tbody>
</table>

Data are presented as ratio to 28S rRNA expression
n.d. : not detectable

*p<0.05: HSCs vs. PHH
#p<0.05: HSCs or PHH vs. LT

Fickert P., et al., Am J Pathol 2009; 175: 2392-2405
DEVELOPMENT OF SPONTANEOUS LIVER FIBROSIS IN FXR -/- MICE COMPARED TO WILD TYPE FXR+/+

Fickert P., et al., Am J Pathol 2009; 175: 2392-2405
XENOBiotic-INDUCED CHOLANGiopathy WITH PARTIAL BILIARY OBSTRUCTION AND CHOLESTASIS – DUCTULAR REACTION AND ONION LIKE FIBROSIS

INCREASED BILIARY PRESSURE, LOW INFLAMMATION, DUCTULAR REACTION AND BILIARY-TYPE FIBROSIS

DDC: 3,5-DIETHOXYCARBONYL-1,4-DIHYDROCOLLIDINE
FXR -/-

INCREASED BILIARY PRESSURE
CHOLESTASIS
CELL DAMAGE

DECREASED
FIBROSIS BILIARY TYPE
FIBROSIS ONION-LIKE

DECREASED
DUCTULAR REACTION

DECREASED
COLANGITIS
BILE DUCT OBSTRUCTION
CHOLESTASIS
CELL DAMAGE

INCREASED

Pinzani M – AISF MONO 2010

Fickert P., et al., Am J Pathol 2009; 175: 2392-2405
FARNESOID-X-RECEPTOR CRITICALLY DETERMINES THE FIBROTIC RESPONSE IN MICE

1. - THE DEVELOPMENT OF FIBROSIS LARGELY DEPENDS ON THE EXTENT OF DUCTULAR REACTION

2. – IN THE FXR -/- MICE THE REDUCTION OF DUCTULAR REACTION IS LIKELY DUE TO DECREASED BILIARY PRESSURE DUE TO DECREASED BILIARY SECRETION

3. - THE DEVELOPMENT OF FIBROSIS DOES NOT REFLECT THE DEGREE OF CHOLESTASIS AND CELL DAMAGE AND MAY NOT DEPEND ON BILE SALT TOXICITY

4. – IN THE PRESENCE OF A NORMAL FXR PHENOTYPE, STIMULATION WITH FXR AGONISTS MAY HAVE BENEFICIAL EFFECTS THAT ARE INDEPENDENT OF THE EFFECT ON FIBROSIS (HSC: NO SUFFICIENT FXR EXPRESSION, NON EFFECT OF FXR -/- IN NON CHOLESTATIC MODELS)

Is Hepatic Hemodynamics Still Metabolically Regulated in Cirrhotic Liver?

**Bile Acids Metabolism**
- CYP7A1*
- CYP8B1*
- SHP
- BSEP
- IBABP
- NTCP*

**Lipid Metabolism**
- APO-A1* (?)
- PLTP
- LPL
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**Hepatic Stellate Phenotype**
- SHP
- AP-1*
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- Colla-1*
- TIMP-1*

**Carbohydrate Metabolism**
- PEPCK*
- TRB3
- FBP1*

**FXR Agonist, i.e. INT 747**

- Significant reduction of HVPG in cirrhotic pts
  - Jalan R. et al. AASLD 2009, EASL 2010

**FXR Signaling**

- Prevention of HSC activation
- Influence on glucose homeostasis through the induction of PPARα

- Downregulation of hepatic fatty acid biosynthesis and VLDL formation
Bile Acids as Metabolic Hormones: the Discovery of TGR5

Cell Metabolism 10, 167–177, September 2, 2009

Genes involved in Energy Balance
TGR5 Expression in Normal and BDL Rat Liver

Keitel V., et al. BBRC 2008;372: 78-84
The Membrane-bound Bile Acid Receptor TGR5 is Localized in the Primary Cilium of Cholangiocytes

Liver Sinusoidal Cell
- eNOS
  - Increased NO production

Monocytes/Macrophages Kupffer Cells
- Decreased synthesis:
  - IL-1 alpha
  - IL-1 beta
  - IL-6
  - IL-8
  - TNF-alpha

Intestine (??)

Brain (??)

Liver Sinusoidal Cell

Target Genes
- cAMP

PKA

CREB-P

TGR5

Pinzani M – AISF MONO 2010
1. Complete resequencing of TGR5 performed in 267 PSC patients and 274 healthy controls.

2. Six nonsynonymous mutations were identified in addition to 16 other novel single-nucleotide polymorphisms.

3. All these mutations are associated with a reduced or abolished TGR5 function.
NUCLEAR AND BILE ACID RECEPTORS

Bile acids are key metabolic regulators and represent the ideal link between the digestive tract and metabolic homeostasis.

The current and future acquisitions in the interaction between bile acid and bile acid receptors, either nuclear or membrane, will likely lead to a new dimension in Hepato/Gastroenterology.