Fasiglifam: Preclinical and Clinical Safety Considerations

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Fasiglifam (TAK-875) is a GPR40 agonist

- Activation of the G-protein coupled receptor 40 (GPR 40) or free fatty acid receptor 1 (FFAR 1) in the β cells of the pancreas by fatty acids or synthetic ligands has been shown to enhance insulin secretion. (Diabetes 2009;58:1067)

- Fasiglifam (TAK-875), an oral, highly potent and selective GPR 40 agonist was developed as a first in class agent. (Diabetes 2010; 59:A165). Fasiglifam was effective in lowering blood sugar in a 12 week phase 2 randomized, double-blind, placebo control and active comparator (glimepiride) trial. (Lancet 2012;379:1403)

- Fasiglifam clinical development program was terminated in phase 3 due to liver safety signals
Faiglifam (TAK-875): Mechanism of Action

TAK-875: agonist of G-protein-coupled receptor 40 (GPR40), also known as free fatty acid receptor (FFAR1)
GLP Toxicology

• Rat 26 Week Study
  – No histological signs of liver toxicity in chronic 26 week study (some transaminase elevations at high multiples of human exposure (72X))
    • Centrilobular hypertrophy consistent with enzyme induction
    • Submandibular gland: Eosinophilic bodies in the granular ducts – only toxicologically significant change
    • NOAEL 60 mg/kg in both sexes
      – Ratio of animal:human exposure 7.9X M and 18.1X F
        » Exposure multiples based on data obtained in subjects with T2DM who were dosed with 50 mg fasiglifam once daily for 14 consecutive days
GLP Toxicology

- Dogs 39 week plus 13 week recovery study
  - Liver: hyperplasia of bile duct, inflammatory cell infiltration in Glisson’s sheath, necrosis of bile duct epithelia, foreign body granuloma, foreign material in bile duct, perivascular cell infiltration in central vein- considered species specific
  - Kidney: dilatation of tubules, basophilia of tubules, foreign body granuloma, granular cast
  - Pancreas: decreased zymogen granules
  - Thymus: atrophy
  - NOAEL 40 mg/kg (M) and 80 mg/kg (F)
    - Ratio of animal:human exposure 7.4X M and 10.2 F
TWO DOGS WITH LIVER CLIN PATH SIGNALS BY DAY 14  
(600MG/KG; 50X HUMAN CSS)

ALT

AST

GGT

ALP

Time

Time
TAK-875 Treatment (Dog)
13 Week Study 600 mg/kg (52X)
Crystal Formation within areas of Granulomatous Inflammation in Dog Liver
Investigative Studies

Crystalline material isolated from dog liver

- Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI ToF MS) analysis indicated that the crystalline material in the liver sections of affected dogs contained fasiglifam and fasiglifam glucuronide (fasiglifam-G).

- There were no dog-specific metabolites in the plasma, liver, or bile; and no evidence of covalent binding of fasiglifam-G (an acyl glucuronide) to plasma or liver proteins in dogs.

- Liver toxicity observed in fasiglifam-treated dogs was due to high concentrations of fasiglifam and fasiglifam-G in bile that exceeded the solubility limit of these compounds. As a result, crystal formation in the biliary tree occurred and led to the histological finding of granulomatous inflammation in the liver.
## Minimal Mitochondrial Toxicity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Condition</th>
<th>Cell Viability EC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>Ratio Glu/Gal</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpromazine</td>
<td>Glucose</td>
<td>14.69</td>
<td>1.2</td>
<td>Non-mitotoxicant control</td>
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<tr>
<td></td>
<td>Galactose</td>
<td>12.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papaverine</td>
<td>Glucose</td>
<td>27.99</td>
<td>12.6</td>
<td>mitotoxicant control</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>2.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAK-875</td>
<td>Glucose</td>
<td>117.00</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>106.50</td>
<td></td>
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<tr>
<td>TAK-875-GLU</td>
<td>Glucose</td>
<td>195.50</td>
<td>1.5</td>
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<td></td>
<td>Galactose</td>
<td>134.80</td>
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![Graphs showing TAK-875 and TAK-875-GLU effects](image)
Hepatocellular Bile Salt Transport Mechanisms

BS = bile salt
BSEP = bile salt export pump
MRP = multidrug resistance associate protein

Blood or Sinusoidal Space

Canaliculus

Blood acid synthesis from cholesterol
Phase II conjugation of bile acids

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TAK875 and 875G are Potent Inhibitors of Key Liver Transporters

<table>
<thead>
<tr>
<th>Compound</th>
<th>hMRP2</th>
<th>hMRP3</th>
<th>hMRP4</th>
<th>hBSEP</th>
<th>dBsep</th>
<th>rBsep</th>
<th>hNTCP</th>
<th>OATP1B1</th>
<th>OATP1B3</th>
<th>OAT1</th>
<th>OAT2</th>
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<tbody>
<tr>
<td>TAK-875</td>
<td>-</td>
<td>14.7</td>
<td>11.7</td>
<td>14.3</td>
<td>16.1</td>
<td>20.6</td>
<td>2</td>
<td>2.43</td>
<td>11.6</td>
<td>12</td>
<td>4.44</td>
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<tr>
<td>TAK-875-Glu</td>
<td>9.0</td>
<td>3.36</td>
<td>6.65</td>
<td>41.6</td>
<td>18.2</td>
<td>82.5</td>
<td>2.4</td>
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</tbody>
</table>

TAK875 Css ~9.4 μM at 50 mg/Day;
TAK-875 is in high risk zone for BSEP Inhibition

Comparison of the Css/BSEP IC₅₀ Ratio with the BSEP IC₅₀ Value Alone for 109 Marketed or Withdrawn Drugs

TAK875 in high risk zone


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Hepatocellular Bile Salt Transport Mechanisms - TAK-875

BS = bile salt
BSEP = bile salt export pump
MRP = multidrug resistance associate protein

P - parent
G - glucuronide
Potential Mechanism
BSEP and MRP Inhibition

• Bile acids can cause toxicity due to detergent properties

• MRP can compensate for BSEP inhibition
  – inhibiting both MRP and BSEP prevents compensation

• Considered a susceptibility factor for DILI

• Recent literature on BSEP/MRP inhibition links Css/IC50 ratio to liver toxicity
  – Morgan et al. 2013
  – Köck et al 2014

• Alternatively, bile acid alterations might lead to change in TAK-875 bile solubility
Decrease in Dog Bile Acid Concentrations

TAK-875 solubility may be affected by the decrease in bile acids in bile!
TAK-875 Cardiovascular Outcomes Trial

Overview

Objective: To demonstrate that treatment with TAK-875 compared with placebo when given in combination with Standard of Care in subjects with T2DM and cardiovascular disease is not associated with an excess risk of CV events

Study Design Schematic:

Exclusion criteria included: ALT or AST > 3X ULN at screening, Total bilirubin >ULN (exception Gilbert’s Syndrome), active HBV or HCV infection requiring anti-viral treatment
# TAK-875 Cardiovascular Outcomes Trial

<table>
<thead>
<tr>
<th>Follow Up</th>
<th>Placebo (n=1603)</th>
<th>Fasiglifam (n=1604)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT or AST &gt; 3 x ULN (n/N %)</td>
<td>0.5</td>
<td>2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT or AST &gt; 5 x ULN (n/N %)</td>
<td>0.1</td>
<td>1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT or AST &gt; 10 x ULN (n/N %)</td>
<td>0.1</td>
<td>0.3</td>
<td>0.125</td>
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<tr>
<td>ALT or AST &gt; 3x ULN and Total Bilirubin &gt; 2 x ULN (n/N %)</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>Maximal Bilirubin &gt; 2 x ULN (n/N %)</td>
<td>0</td>
<td>0.1</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Cardiovascular Outcomes Trial study case:

Lab Values (x ULN)

- ALT (SGPT)
- AST (SGOT)
- Alkaline Phosphatase
- Bilirubin Total

Subject with ALT > 3xULN or AST > 3xULN or TBILI > 2xULN

Subject ID = TAK-875_306-3109032

- TAK-875 50 mg
- non-serious AE - transaminases increased reported on day 29

Alternative etiology: none (viral hepatitis serology [A,B,C,E, CMV,EBV] and autoimmune hepatitis serology negative); abdominal ultrasound- liver steatosis
LSEC adjudication category: Possible
Japan Phase 3 Study Objective and Design

Objective: To evaluate the efficacy and safety of once daily TAK-875 25 mg and 50 mg orally administered for 24 weeks in type 2 diabetic patients with inadequate glycemic control despite diet therapy and/or exercise therapy.

Design:

<table>
<thead>
<tr>
<th>(4 weeks) Screening</th>
<th>(24 weeks) Double-Blind Treatment Period</th>
<th>(1 week) Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAK-875 25 mg</td>
<td>TAK-875 50 mg</td>
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<tr>
<td>TAK-875 25 mg</td>
<td>TAK-875 50 mg</td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
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</tbody>
</table>

Randomization

End of treatment

Exclusion criteria included ALT or AST ≥ 2.5 x ULN, or total bilirubin ≥ 1.5 mg/dL during the screening period.
## Japan Phase 3 Study for 24 Weeks: Subjects with Markedly Abnormal Liver Tests

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>TAK-875</th>
<th>TAK-875</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>25 mg</td>
<td>50 mg</td>
</tr>
<tr>
<td>Any Elevation After Treatment (%)</td>
<td>1 (1.5)</td>
<td>3 (4.8)</td>
<td>4 (6.5)</td>
</tr>
<tr>
<td>ALT &gt; 3 x ULN</td>
<td>1 (1.5)</td>
<td>3 (4.8)</td>
<td>4 (6.5)</td>
</tr>
<tr>
<td>ALT &gt; 5 x ULN</td>
<td>1 (1.5)</td>
<td>1 (1.6)</td>
<td>0</td>
</tr>
<tr>
<td>ALT &gt; 3 x ULN with Total bilirubin &gt; 2 x ULN</td>
<td>0</td>
<td>1 (1.6)</td>
<td>0</td>
</tr>
<tr>
<td>Total bilirubin &gt; 2 x ULN</td>
<td>0</td>
<td>1 (1.6)</td>
<td>0</td>
</tr>
</tbody>
</table>
Japan Phase 3 study case: ALT >3X ULN and T Bili > 2X ULN

Withdrawal case-

1004-001: TCI2012A00049 (Serious)  
AE: Hepatic function abnormal  
(Meeting the criteria of biochemical Hy’s law)  
25 mg

Alternative etiology: gall stone disease  
LSEC adjudication category: Unlikely

![Graph showing liver enzyme levels over time]

- TAK-875
- ALT
- AST
- ALP
- T-bil
- an OTC stomach medicine
- amlodipine, rosuvastatin, eicosapentaenoic acid

Study Day

ALT 10xULN

ALT 3xULN

ALT 1xULN
Key Messages

• Liver identified as a target organ in nonclinical GLP studies
  – TAK-875 crystal formation thought to cause liver toxicity in dogs at high doses.
  – Safety margin projected for human efficacious dose
  – No similar crystallization observed in rat

• Post Phase 3 termination, additional investigative studies were performed

• Inhibition of hepatobiliary transporters are a known susceptibility factor for DILI

• TAK-875 (or its glucuronide) inhibits hepatobiliary transporters in vitro at clinically relevant concentrations
  – BSEP, MRP2, MRP3, MRP4, NTCP, OATP1B1, OATP1B3, OAT1 and OAT2

• TAK-875 alters bile acid homeostasis in vivo (surrogate for hepatobiliary transporter inhibition) and causes an increase in serum bile acid in mice, rats and dogs at exposures exceeding clinical steady state concentrations

• In in vivo dog studies, serum bile acid elevations preceded overt liver injury suggesting a temporal and mechanistic relationship

• Bile acid quantification of dog bile demonstrated a decrease in TAK-875 treated animals, suggesting that solubility of TAK-875 could be altered in TAK-875 treated animals

• TAK-875 development was terminated based on an a liver safety signal.