Evaluating the MMHP as a Safety Assessment Tool for DILI

HESI Genomics MMHP Workshop
Washington, DC

Nov. 28, 2012
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DILI = Drug-Induced Liver Injury

• Predominantly characterized in humans by hepatocellular injury observed as an increase in serum aminotransferase enzymes (ALT and AST), but severe DILI can occur that is fatal or requires liver transplantation
  – Typically DILI is reversible with cessation of dosing

• During clinical drug development it is rare to see severe hepatotoxicity; usually manifests as mild elevations in ALT and AST
  – Often not predicted by preclinical studies
  – Depending on the therapeutic indication and patient population, this can halt further development

• Severe liver injury often manifests post-marketing, and is termed idiosyncratic DILI based on the low incidence (< 1 in 10,000)
  – Dependent on individual susceptibility
  – Hy’s Law used as an indicator of a drug’s potential to cause severe liver injury
DILI is Expensive!


Better preclinical tools are needed to predict DILI

DILI in clinical development can cost >$500M!

*N. Terblanche Journal of Commercial Biotechnology 2008 14(3): 201-12*
Can Preclinical Genetic Diversity Help?

Current mouse strains used for risk assessment:

- **CD-1**: Pharmaceutical Industry, Outbred line
- **B6C3F1**: National Toxicology Program, F1 hybrid

But.....there are hundreds of inbred strains of mice

**Mouse Diversity Panel (MDP)**

Increasing genetic diversity in preclinical species may translate to improved predictivity of human risk

Mouse photo: http://jaxmice.jax.org/findmice/why.html
Mouse Model of the Human Population

- The Mouse Model of the Human Population (MMHP) consists of a panel of ~35 inbred mouse strains encompassing a genetic diversity equal to or greater than that found in the human population.

- The Hamner Institutes successfully used this model to map polymorphisms that infer susceptibility to acetaminophen-induced liver injury.
  - Demonstrated that genetic variation in the CD44 gene was associated with susceptibility in humans.

(Harrill et al, Genome Research, 2009)
Initial Pfizer/Hamner Collaboration

Rationale:
The project seeks to address the ongoing issue of attrition due to hepatotoxicity, especially for those compounds where hepatotoxicity is only identified upon entry into clinical trials.

Project Plan Outline:
• A two-phase collaboration was initiated to evaluate DILI in the MMHP
  – Phase 1 - Assess variability of liver injury for 3 DILI compounds (troglitazone, isoniazid, and flucloxicillin) in dose range-finding studies in five inbred strains
  – Phase 2 - If liver injury is successfully demonstrated in Phase 1, select 1 compound for full screen assessment in all 35 inbred strains
    • Variable strain-specific phenotype data generated from the full screen would power a whole genome association and eQTL mapping studies to identify genes that underlie predisposition to DILI
  – Collaboration opened to HESI in 2010 to educate on this approach and broaden the scope of endpoints evaluated in the full screen assessment
Project Expectations & Results

Expectations:
- Mouse strains would demonstrate strain-dependent differences in liver toxicity when treated with a hepatotoxicant
  - Differences could be used to understand mechanism of toxicity and identify potential biomarkers for the toxicity through GWAS and transcriptomic analyses

Results:
- Clear strain-dependent differences were observed microscopically in the liver following treatment of INH
- Results suggested the MMHP could be a useful tool for understanding DILI risk

Graph courtesy of Alison Harrill
Extended Pfizer/Hamner Collaboration

**Objective:**
- Test 4 additional DILI-associated compounds in the MMHP in order to further evaluate this model

**Expectations:**
- Testing multiple compounds in this model would identify mouse strains that demonstrate increased susceptibility to liver injury
  - These strains could be used to screen compounds in early preclinical development …
- GWAS analysis would identify genetic biomarkers associated with susceptibility to liver injury
  - These biomarkers could be tested for clinical relevance
- The MMHP would be a useful tool for addressing compounds that fail for DILI during clinical development or for idiosyncratic DILI following market approval
Extended Pfizer/Hamner Collaboration

- Two compounds that have completed dosing and preliminary analysis…

  - **Thelin (sitaxetan)** – Pfizer drug for pulmonary arterial hypertension withdrawn from the market in 2010 for poor hepatotoxicity risk/benefit profile

  - **PF-04287881** – Pfizer drug candidate demonstrating mild increases in serum transaminase levels in early clinical development

<table>
<thead>
<tr>
<th>Inbred Mouse Strains</th>
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<tbody>
<tr>
<td>129S1/SvImJ</td>
<td>LP/J</td>
</tr>
<tr>
<td>A/J</td>
<td>MA/MyJ</td>
</tr>
<tr>
<td>AKR/J</td>
<td>MRL/MpJ</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>NOD/LtJ</td>
</tr>
<tr>
<td>BTBR T+ tf/J</td>
<td>NON/LtJ</td>
</tr>
<tr>
<td>BUB/BnJ</td>
<td>NOR/LtJ</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>NZB/B1NJ</td>
</tr>
<tr>
<td>C57Bl/6J</td>
<td>NZW/LacJ</td>
</tr>
<tr>
<td>C57BLKS/J</td>
<td>P/J</td>
</tr>
<tr>
<td>C57BR/cdJ</td>
<td>PL/J</td>
</tr>
<tr>
<td>C58/J</td>
<td>PWK/PhJ</td>
</tr>
<tr>
<td>CBA/J</td>
<td>RIIIS/J</td>
</tr>
<tr>
<td>CE/J</td>
<td>SEA/GnJ</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>SJL/J</td>
</tr>
<tr>
<td>FVB/NJ</td>
<td>SM/J</td>
</tr>
<tr>
<td>I/LnJ</td>
<td>SWR/J</td>
</tr>
<tr>
<td>KK/HiJ</td>
<td>WSB/EiJ</td>
</tr>
<tr>
<td>LG/J</td>
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Thelin

- Thelin is an endothelin receptor antagonist, and there was a known risk of liver injury with this class of drugs when Thelin was brought to market (i.e., bosentan).
- During a Phase 3 compassionate use study with Thelin, one subject died of hepatic failure, however, the risk benefit ratio for Thelin looked better than other marketed drugs in this class.
- Preclinical studies were conducted in mice, rats, and dogs, including a repeat-dose toxicity study in C57BLK/6 mice where Thelin was administered at dosages up to 500 mg/kg.
  - *In mice, mortality was observed at dosages >400 mg/kg, but 200 mg/kg was tolerated; slight hepatocellular hypertrophy was observed in the liver at all dosages tested.*
  - *Minimal/slight hepatocellular hypertrophy was also observed in rats and dogs at the highest dosages tested.*
- Thelin is a potent BSEP inhibitor.
Dosage selected for MMHP study was 300 mg/kg

Study Design:

- Compound administered daily by oral gavage
- Blood collected at 2 hr post-dose on Day 7 for determination of plasma drug concentration (dried blood spot analysis)
- Serum collected at necropsy for clinical chemistry analysis; parameters analyzed included ALT, AST, total bilirubin, cholesterol, and albumin
- Liver tissue collected for histopathological evaluation at necropsy
Dehydration and lethargy seen in some strains on days 5-7; increase in liver to body weight ratio in all strains (ranging from 129% in the KK/HIJ strain to 254% in the P/J strain)
Thelin Changes in Serum ALT

Increased serum ALT changes were only observed with a few strains, and were of minimal magnitude.

*represents p<0.05 for two-tailed t-test within each strain

Graph courtesy of Hong Wu
Serum cholesterol changes (< 50% increase or decrease) observed in some strains

*represents p<0.05 for two-tailed t-test within each strain

Graph courtesy of Hong Wu
Thelin concentration at 2 hr post-dose on day 7 is **NOT** correlated with changes in serum cholesterol.
Thelin Microscopic Findings

- Microscopic findings in the liver were **hepatocellular hypertrophy**, **increased mitosis in hepatocytes**, **subcapsular necrosis**, and **periportal vacuolation**
  - Hepatocellular hypertrophy was diffuse and observed across all strains; the severity was strain dependent with the highest severity observed in the NZW/LacJ mice.
  - Subcapsular necrosis was a secondary finding resulting from increased parenchymal pressure and was generally associated with higher grades of hypertrophy.
  - The increased mitosis and presence of periportal vacuolation was mild in severity and variable between strains.

![BALB/cJ Vehicle](Image1) ![Thelin](Image2)

![400X](Image3)

*Pictures courtesy of Lisa Kurtz, Alison Harrill*
Top genes identified in GWAS are associated with cholesterol regulation (Ingenuity Pathway Analysis).

Genomic loci associated with variable cholesterol levels using genome wide association (GWA) analysis (EMMA).

Taken From Poster Presented at 2012 SOT Meeting, San Francisico, CA
Outcome of GWAS Analysis With Cholesterol Phenotype

- Using less stringent criteria (non-bonferroni corrected) for GWAS, 171 genes with associated SNPs were identified
  - Metacore pathway analysis of the 171 genes identified key pathways that included: *regulation of fatty acid metabolic process, positive regulation of lipid metabolic process, cellular response to xenobiotic stimulus, negative regulation of cholesterol storage, regulation of lipid transport*

  One gene was common in all of significant pathways (*p*< 10e-07)

  alpha 2-macroglobulin receptor (also known as low density lipoprotein receptor-related protein and CD91)

<table>
<thead>
<tr>
<th>Genes</th>
<th>Included genomic regions</th>
<th>Genotype</th>
<th>Cholesterol (p value; two way ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2MR</td>
<td>rs50266218 (coding non-syn)</td>
<td>G</td>
<td>p=0.0319</td>
</tr>
<tr>
<td></td>
<td>rs50266218 (coding non-syn)</td>
<td>C</td>
<td>p=0.0319</td>
</tr>
</tbody>
</table>

Data courtesy of Hong Wu
Thelin Summary

• Study findings support a general liver adaptive response to Thelin, but NOT an overtly toxic phenotype in any of the strains tested (enzyme induction?)
  ➢ GWAS and pathway analysis results supported this

• A consistent hepatocellular hypertrophy phenotype was observed in all strains demonstrating lack of phenotypic diversity between mouse strains

• MMHP diversity model non-informative about Thelin-induced liver injury risk
  • Is a phenotype more reflective of the clinical phenotype needed for model success?
  • BSEP inhibitors may have a different phenotype in mice compared to humans based on physiological differences between mice and humans (eg – bile acid composition, flow rate)

• GWAS and pathway analysis did identify potential genomic biomarkers related to the phenotype observed
PF-04287881

• Preclinical IND-enabling studies used rat as the rodent species; compound never administered to mice
  • *Minimal, non-adverse increases in ALT and AST (<2X control mean) at highest dosage tested (300 mg/kg) in a rat 4-week toxicity study; non-adverse microscopic findings of kupffer cell vacuolation at 300 mg/kg, and multinucleate hepatocytes at >100 mg/kg*

• Dose range-finding study conducted in CD-1 mice to identify an appropriate dosage for MMHP study

Dosages tested: 150, 300, and 600 mg/kg
  – compound administered daily by oral gavage
PF-04287881 Dose Range-Finding Study in CD-1 Mice

- Clear liver injury phenotype observed including a dose-dependent increase in serum ALT

- Mice at 300 mg/kg and 600 mg/kg had mild to moderate centrilobular hepatocellular hypertrophy, and at 600 mg/kg had minimal centrilobular hepatocellular vacuolation around the central vein

Graph courtesy of Lisa Kurtz
• 600 mg/kg dosage selected for MMHP study

Study Design:

- Compound administered daily by oral gavage
- Blood collected at 2 and 24 hr post-dose on Day 7 for determination of plasma drug concentration (dried blood spot analysis)
- Serum collected at necropsy for clinical chemistry analysis; parameters analyzed included ALT, AST, alkaline phosphatase, total bilirubin, cholesterol, and albumin
- Liver tissue collected for histopathological evaluation at necropsy
** The C58/J and WSB/EiJ strains were not tested for ALP due to insufficient sample volume

Graph courtesy of Merrie Mosedale
Serum ALT Levels Do Not Correlate with AUC

The average total plasma exposure of PF-04287881 during the 2 h to 24 h period post final dose on Day 7 is represented as plasma AUC

Graph courtesy of Merrie Mosedale
Microscopic Findings Correlate With Serum ALT Increases

PF-04287881-related changes in the liver corresponded to one or more of the following findings singly or in combination:

- Hepatocellular hypertrophy
- Kupffer cell vacuolation
- Hepatocyte necrosis/apoptosis (single cell)

Two strains did not have any microscopic findings

Graphs courtesy of Merrie Mosedale
GWAS Results With Serum ALT and AST

Phenotype: ALT

JAX SNP ID: JAX00124192 (intergenic)

5% FDR Threshold (Step Up), $-\log_{10}(p) = 5.00728922800102$

Phenotype: AST

5% FDR Threshold (Step Up), $-\log_{10}(p) = 6.7148591832764$

The same SNP was highly associated with both phenotypes!

Merrie Mosedale
PF-04287881 Summary

- Strain-dependent liver injury phenotype observed with PF-04287881 that better represents human phenotype
- Additional candidate gene analysis underway
- Follow-up study conducted using 4 strains with a range of relevant phenotypes

**Goals:**
- Better characterize vacuolation
  - Phospholipidosis?
- Confirm source of serum ALT

<table>
<thead>
<tr>
<th></th>
<th>ALT Fold Change ('881 treated/vehicle)</th>
<th>Kupffer cell vacuolation</th>
<th>Single cell Necrosis</th>
<th>Hepatocellular hypertrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA/MyJ</td>
<td>1.5-fold</td>
<td>positive</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>WSB/EiJ</td>
<td>3-fold</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>NZW/LacJ</td>
<td>14-fold</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>SM/J</td>
<td>3-fold</td>
<td>negative</td>
<td>negative</td>
<td>positive</td>
</tr>
</tbody>
</table>

**Additional endpoints:**
- EM on liver and additional target organs (lung and spleen; based on studies in rat)
- miR-122, GLDH, albumin
- Transcript profiling
Pfizer In-House MMHP Evaluation

• A Pfizer Phase 2 clinical study for PF-04191834 was terminated in Dec 2010 due to liver enzyme elevation detected in 6 subjects (ALT or AST >3x ULN, or recurrent increases >1.5x ULN).

• PF-04191834 was very non-toxic in preclinical studies
  • NOAEL in rat 2 and 6-week repeat-dose toxicity studies was 2000 mg/kg (no adverse findings reported)
  • NOAEL in dog 2 and 6-week repeat-dose toxicity studies was 1000 mg/kg (no adverse findings reported)

- A decision was made to conduct a dose range-finding study using 4 DILI-sensitive mouse strains identified from acetaminophen- and isoniazid-induced liver injury studies in MMHP
  - A full MMHP study with all mouse strains would be conducted if results of this study identified a liver injury phenotype
Design for Dose Range-Finding Study

<table>
<thead>
<tr>
<th>Strain</th>
<th>INH (n=34)</th>
<th>APAP (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZW/LacJ</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>FVB/NJ</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ indicates strain ranked in the top 6 of all strains based on pathological severity
- indicates strain ranked in the bottom half of all strains based on pathological severity

- PF-04191834 administered daily by oral gavage for 14 consecutive days
  ➢ Dosages: 500, 1000, 2000 mg/kg/day
  ➢ 5 female mice per group (female rats had higher exposure in repeat-dose toxicity studies)
  ➢ Standard clinical path at necropsy; liver collected for histopathologic evaluation
  ➢ TK samples collected at 5 time points post-dose on Day 14
PF-04191834 Study Results

- Slight increase in alkaline phosphatase in NZW/LacJ strain only
- No microscopic findings in the liver in any strain

Dosage (mg/kg):   0         500      1000      2000

* *p<0.05 vs. control mice
* Student t-test
* Data shown as mean ± SD

Graph courtesy of Hong Wu
PF-04191834 Summary

• PF-04191834 administration resulted in an ~29 % increase in ALP levels at 1000 and 2000 mg/kg in NZW/LacJ mice
  ➢ No increase in exposure between 1000 and 2000 mg/kg
  ➢ FVB/NJ mice had significantly higher exposure than NZW/LacJ mice at 500 and 2000 mg/kg groups (p<0.05), without toxicity findings

• PF-04191834 did not cause liver injury in mouse strains showing increased sensitivity to liver injury in MMHP studies with APAP and INH

• Based on findings in these 4 strains, a full MMHP study was not considered likely to provide understanding PF-04191834-induced liver injury observed in the clinic
MMHP - Current Conclusions

- MMHP can have impact/application to the Pfizer portfolio
  - Tool for looking at mechanisms of toxicity and identifying genetic biomarkers associated with specific endpoints
  - Strains with increased susceptibility to liver injury are being identified and can be used to assess compounds in early preclinical development (better assessment of safety margin)
  - Low risk tolerance for liver injury in target population
  - Slight changes in transaminases observed in toxicity studies that raise questions, but are not considered adverse

<table>
<thead>
<tr>
<th>Top 5 MMHP Strains Susceptible to Liver Injury Based on Microscopic Findings in the Liver</th>
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</thead>
<tbody>
<tr>
<td>Isoniazid</td>
</tr>
<tr>
<td>LG/J</td>
</tr>
<tr>
<td>Sm/J</td>
</tr>
<tr>
<td>NZW/LacJ</td>
</tr>
<tr>
<td>DBA/2J</td>
</tr>
<tr>
<td>BUB/BnJ</td>
</tr>
</tbody>
</table>
MMHP - Current Conclusions

• A clinically-relevant phenotype is needed in the mouse for MMHP success
• MMHP is likely a less useful tool for idiosyncratic DILI or DILI observed in early phase clinical development than expected

Remaining Question:
  • Is this model more applicable to other toxicities?
Acknowledgements

Hamner Institutes for Health Sciences
Alison Harrill
Lisa Kurtz
Merrie Mosedale
Paul Watkins

Pfizer
Hong Wu
Karamjeet Pandher
Peter Schmidt
Leslie Obert
Rich Giovanelli
Brian Rago
Jason Barricklow
Mathew Pletcher
Michael Lawton
Jon Cook
Drug Safety Technology Committee