Drugs in Human Semen

• **Goal**: To address recent MHRA and FDA requests for data demonstrating safety of pharmaceuticals regarding male-mediated conceptus exposure, and arrive at an appropriate and data-based use of contraception in clinical trials.

• **Steering Team**

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  - Kimberly Benson, U.S. FDA
  - Bruce Beyer, sanofi-aventis
  - Bill Breslin, Lilly
  - Gary Chellman, Charles River Labs
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  - Jim Kim, HESI
  - Larry Leshin, U.S. FDA
  - Jimmy McBlane, MHRA
  - Graeme Moffat, Amgen
  - Christine Nguyen, U.S. FDA
  - Anthony Scialli, Tetra Tech Sciences
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  - Ulla Wandel-Liminga, Sweden Medical Products Agency
Drugs in Human Semen

- Regional health authority concern for male-mediated embryo/fetal harm following indirect exposure to drugs has lead to requirement for use of barrier protection for men enrolled in clinical trials with pregnant partners.
- Most companies do not routinely assess for semen concentrations of drug.
Three Potential Mechanisms of Embryo/Fetal Exposure Considered

- Vaginal absorption of drug into maternal circulation and subsequent distribution to conceptus – likely most relevant

When requested by regional healthy authorities, using “worst case” assumptions, potential embryo/fetal risk based on known male systemic exposures is modeled. In this approach, only extremely potent teratogens could be a concern.
Diffusion of drug from semen and across cervical barrier

Cervical canal – mucus impervious to sperm/microorganisms during pregnancy

Ejaculate

Drug
Model of counter-current transfer of heat or a substance between vaginal vein blood and uterine arterial blood

Einer-Jensen N, Hunter R 2005;129:9-18
Drugs in Semen

Experimental Approaches:

1) Vaginal dosing of pregnant cynomolgus monkeys with assessment of drug in maternal and fetal plasma.

2) Thalidomide experiments in pregnant rabbits comparing intra-vaginal and oral routes.

3) Use of vital dyes (e.g. trypan blue) placed into vagina of pregnant rats followed by time course evaluation of dye migration in dams.

4) Imaging studies in pregnant mice
Cynomolgus Monkey Study

- Bristol-Myers Squibb
- Amgen
- Charles River Labs
- Objectives:
  - Determine if traditional assumptions and calculations presently used are sufficient to predict potential male-mediated embryo/fetal harm
Cynomolgus Monkey Study

• Study Design
  – 3 pregnant cynomolgus monkeys (GD 60/70)
  – Given 1 mL of 0.75% metronidazole gel vaginally and monitored for leakage (minimal to none)
  – Timed cesarean-section 7 hours after dose for collection of maternal and fetal blood samples and amniotic fluid
  – Plasma and amniotic fluid samples analyzed for parent and metabolite (MS/MS)
Cynomolgus Monkey Study: Metronidazole Concentrations

<table>
<thead>
<tr>
<th>Dam ID</th>
<th>Gestation Day</th>
<th>Maternal Plasma (ng/mL)</th>
<th>Fetal Plasma (ng/mL)</th>
<th>Amniotic Fluid (ng/mL)</th>
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<tbody>
<tr>
<td>1501</td>
<td>GD 70</td>
<td>94.4</td>
<td>105</td>
<td>145</td>
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<td>Maternal/fetal ratio</td>
<td>0.9x</td>
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<tr>
<td>1502</td>
<td>GD 60</td>
<td>756</td>
<td>735</td>
<td>649</td>
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<td></td>
<td>Maternal/fetal ratio</td>
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<tr>
<td>1503</td>
<td>GD 70</td>
<td>494</td>
<td>539</td>
<td>817</td>
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<tr>
<td></td>
<td>Maternal/fetal ratio</td>
<td>0.9x</td>
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</table>
Cynomolgus Monkey Study: [Hydroxymetronidazole]

<table>
<thead>
<tr>
<th>Dam ID</th>
<th>Gestation Day</th>
<th>Maternal Plasma (ng/mL)</th>
<th>Fetal Plasma (ng/mL)</th>
<th>Amniotic Fluid (ng/mL)</th>
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</thead>
<tbody>
<tr>
<td>1501</td>
<td>GD 70</td>
<td>3.66</td>
<td>4.17</td>
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<td>Parent/metabolite ratio</td>
<td>26x</td>
<td>25x</td>
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<td>26.5</td>
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<td>GD 70</td>
<td>19.6</td>
<td>21.3</td>
<td>13.4</td>
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<tr>
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<td>Parent/metabolite ratio</td>
<td>25x</td>
<td>26x</td>
<td>61x</td>
</tr>
</tbody>
</table>
Cynomolgus Monkey Study: Conclusions

- Maternal systemic exposure to metronidazole is variable following vaginal dosing.
- Fetal exposure was equivalent to maternal systemic exposure for parent and metabolite on GD 60 and 70.
- Supports that traditional modeling of fetal exposure to drug in semen would be sufficiently conservative to predict potential fetal risk.
PK Pregnant Rabbit Study
Thalidomide

• Celgene Corp.

• Objective –
  – To compare embryonic concentrations of thalidomide following maternal oral or vaginal administration in rabbits
PK Pregnant Rabbit Study

Study Design

• Oral dosages:
  – 20 mg/kg/day (NOAEL in previous rabbit EFD study)
  – 180 mg/kg/day (malformations seen in previous study)

• Intravaginal (IVg) dosages:
  – 2, 20, and 180 mg/kg/day

• 8 mated rabbits/group (N=2/time point) dosed from GD 7-11

• Sample collection at 1, 3, 6, and 24 hours postdose on GD11 for assay of thalidomide concentration in:
  – Maternal plasma samples
  – Yolk sac cavity (YSC) fluid from each implant (analyzed individually)
  – Embryos (pooled by litter prior to analysis)
PK Pregnant Rabbit Study - Preliminary Outcomes

- Exposures were more variable following intravaginal dosing as compared to oral dosing
- Maternal plasma, YSC fluid, and embryonic exposures (AUC) were lower following IVg administration as compared to oral administration
- No meaningful difference in YSC fluid/maternal plasma AUC ratio between these routes

- Conclusion: There was no difference in uptake of thalidomide into the intrauterine compartment following oral and intravaginal routes of administration.
Pregnant Rat Study using Vital Dyes

- Johnson & Johnson
- Objective: Visually evaluate dye distribution when administered intravaginally to pregnant rats
- Study Design
  - Dye to be administered on GD12
    - Use dyes with various sizes and polarity
  - Animals to be sacrifice at 0.25, 0.5, 1, 2, and 4 h
- Study in progress
Transgenic Pregnant Mouse Imaging Study

- sanofi aventis
- Objective:
  - Visualize potential embryo/fetus exposure to compounds via intravaginal route using optical imaging model
Transgenic Pregnant Mouse Imaging Study

• Study Design
  – Exposure at different stages of estrus cycle and during pregnancy
  – Fluorescence imaging and ex vivo imaging
  – 13 β-actin-luciferase female transgenic mice
  – D-luciferin substrate dosed intravaginally (20 ul of 0.5 mg/kg)
  – Imaged with IVIS-100 between 1 to 10 min
## Imaging Study Results

**Mouse 10, 20 imaged at different estrus stage**

<table>
<thead>
<tr>
<th>Time</th>
<th>Mouse 10</th>
<th>Mouse 20</th>
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</thead>
<tbody>
<tr>
<td>2/18 13:09PM</td>
<td>![Image 1]</td>
<td>![Image 2]</td>
</tr>
<tr>
<td>2/22 9:59AM</td>
<td>![Image 3]</td>
<td>![Image 4]</td>
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<table>
<thead>
<tr>
<th>Cycle</th>
<th>Mouse 10</th>
<th>Mouse 20</th>
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</thead>
<tbody>
<tr>
<td>diestrus</td>
<td>![Image 5]</td>
<td>![Image 6]</td>
</tr>
<tr>
<td>diestrus/metastrus</td>
<td>![Image 7]</td>
<td>![Image 8]</td>
</tr>
</tbody>
</table>

ILSI Health and Environmental Sciences Institute
Imaging Study
Results

Pregnant mice: GT8

Mouse

11
4
17
19
36

Pregnant

No-pregnant
Transgenic Pregnant Mouse Imaging Study - Topline Results

1. Mice in diestrus and proestrus showed imaging signal
2. Mice in estrus/metestrus did not show any imaging signal
3. Pregnant mice indeed showed the imaging signal
Drugs in Semen WG: Next Steps

• Complete vital dye study
• Define next steps for imaging study
• Symposium at Teratology Society June 29, 2011
• Publish Results
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  - Deanna Newcomb

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  - George Scott
  - J. Zalikowski

- **sanofi adventis**
  - Tony DeLise

- **Johnson & Johnson**
  - Graham Bailey