What Fetal Exposure/Outcome Could Occur from Drugs in Semen? Studies with Metronidazole and Thalidomide

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Background

- HESI DART Committee initiative
- Concern about potential embryo-fetal harm following seminal exposure to drugs with teratogenic potential
- Amount of drug in semen is generally very low (based on ejaculate volume)
- Distribution mechanisms that could theoretically result in higher than expected concentrations in the intrauterine compartment
- Survey of literature showed variable semen:plasma ratios – up to 10-fold (Klemmt and Scialli, BDR-B, 2005)
Metronidazole
Cynomolgus Monkey Study

• Objectives:
  – Determine if a systemic exposure-based model is sufficient to predict potential male-mediated embryo/fetal effects by performing a PK study in pregnant cynomolgus monkeys with intravaginal administration of metronidazole

• Work performed by:
  – Bristol-Myers Squibb
  – Amgen
  – Charles River Labs
Metronidazole
Cynomolgus Monkey Study Design

• 3 naïve pregnant cynomolgus monkeys (GD 60/70/71)
• Given 1 mL of 0.75% metronidazole gel vaginally and monitored for leakage (minimal to none)
  – Clinical formulation for vaginal use
  – Metronidazole kinetics following vaginal administration in humans published – well absorbed and distributed
• 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)
No effect of fetal blood collection site on concentration values:

<table>
<thead>
<tr>
<th>Site of collection for fetus from dam 1503</th>
<th>Metronidazole (ng/mL)</th>
<th>Metabolite (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilicus</td>
<td>552</td>
<td>21.6</td>
</tr>
<tr>
<td>Umbilical cord</td>
<td>542</td>
<td>20.4</td>
</tr>
<tr>
<td>Decapitation</td>
<td>523</td>
<td>21.3</td>
</tr>
</tbody>
</table>

**No fetal blood could be obtained via cardiac puncture or the vena cava.**
## 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>
</tr>
</thead>
<tbody>
<tr>
<td>1501</td>
<td>GD 70</td>
<td>94.4</td>
<td>105</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>Maternal/fetal ratio</td>
<td>0.9x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1502</td>
<td>GD 60</td>
<td>756</td>
<td>735</td>
<td>649</td>
</tr>
<tr>
<td></td>
<td>Maternal/fetal ratio</td>
<td>1.0x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1503</td>
<td>GD 71</td>
<td>494</td>
<td>539</td>
<td>817</td>
</tr>
<tr>
<td></td>
<td>Maternal/fetal ratio</td>
<td>0.9x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Maternal and fetal exposures to metronidazole are variable following vaginal dosing
- Maternal to fetal exposure ratios were equivalent between animals
### 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>
</tr>
</thead>
<tbody>
<tr>
<td>1501</td>
<td>GD 70</td>
<td>3.66</td>
<td>4.17</td>
<td>2.01</td>
</tr>
<tr>
<td></td>
<td>Parent/metabolite ratio</td>
<td>26x</td>
<td>25x</td>
<td>72x</td>
</tr>
<tr>
<td>1502</td>
<td>GD 60</td>
<td>26.5</td>
<td>21.1</td>
<td>8.69</td>
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<tr>
<td></td>
<td>Parent/metabolite ratio</td>
<td>29x</td>
<td>31x</td>
<td>75x</td>
</tr>
<tr>
<td>1503</td>
<td>GD 71</td>
<td>19.6</td>
<td>21.3</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>Parent/metabolite ratio</td>
<td>25x</td>
<td>26x</td>
<td>61x</td>
</tr>
</tbody>
</table>

- Fetal and maternal exposures of metabolite were also similar on GD 60-71.
- Therefore, parent to metabolite ratios were similar in maternal and fetal plasma samples.
Metronidazole
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 60-71
• Supports the use of a systemic exposure-based model for prediction of fetal exposure to drug in semen and potential fetal risk
Thalidomide Rabbit Studies
Thalidomide in Semen

Background

• Rabbit seminal transfer study of thalidomide\textsuperscript{1}
  – Radioactivity detected in rabbit seminal fluid
  – Semen to plasma concentration ratio was 1.3-1.4

• Human seminal transfer study of thalidomide\textsuperscript{2}
  – 100 mg dose resulted in:
    • Plasma concentrations of 10-350 ng/mL
    • Semen levels of 10-250 ng/g (max. 1.0 \(\mu\)g/4 g of ejaculate)
  – 400 mg dose can result in approximately 4.0 \(\mu\)g thalidomide/ejaculate

\textsuperscript{1}Lutwak-Mann C et al. Thalidomide in rabbit semen. Nature 1967;214:1018-1020
\textsuperscript{2}Teo SK et al. Thalidomide is distributed into human semen after oral dosing. Drug Metabolism and Disposition 2001;29:1355-1357
Thalidomide Rabbit Studies

• Rabbit used as a model in this investigation:
  – Sensitive species to thalidomide-induced embryopathy
  – Seminal transfer data in this species
  – Intravaginal dosing is feasible

• Rabbit toxicokinetic (TK) study:
  – Measured and compared drug levels in maternal and embryonic compartments following oral and intravaginal routes of exposure

• Rabbit embryofetal development (EFD) study:
  – Compared embryo-fetal development outcome following oral and intravaginal administration
TK Study Design

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

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

• Eight 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)
Absorption via IVg route was more variable, less complete, and slower than via oral route, resulting in lower, more variable exposures, and relatively flat conc vs time curves. 24-hour samples from 2 mg/kg IVg group were BQL.
# Plasma PK Data Following Oral or Intravaginal Administration

<table>
<thead>
<tr>
<th>Dose</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>$T_{\text{max}}$ (hr)</th>
<th>AUC (ng*hr/mL)</th>
<th>Dose adjusted AUC&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg/kg PO</td>
<td>3880</td>
<td>3</td>
<td>37800</td>
<td>1890</td>
</tr>
<tr>
<td>180 mg/kg PO</td>
<td>19300</td>
<td>3</td>
<td>287000</td>
<td>1590</td>
</tr>
<tr>
<td>2 mg/kg IVg</td>
<td>250</td>
<td>1</td>
<td>1070</td>
<td>535</td>
</tr>
<tr>
<td>20 mg/kg IVg</td>
<td>534</td>
<td>1</td>
<td>10600</td>
<td>530</td>
</tr>
<tr>
<td>180 mg/kg IVg</td>
<td>6470</td>
<td>6</td>
<td>87300</td>
<td>485</td>
</tr>
</tbody>
</table>

<sup>a</sup> Dose adjusted AUC = AUC / dose

- Plasma exposure data (AUC) show good dose proportionality for both oral and IVg dosing.
- Plasma exposure (AUC) via IVg route was approximately 30% relative to the oral route.
- $C_{\text{max}}$ via IVg route was 3-fold to 7-fold lower than oral route.
- The 180 mg/kg IVg dose resulted in plasma concentrations in the range of those observed following the 20 and 180 mg/kg oral doses, suggesting these groups may be most useful for making comparisons between routes of administration.
Plasma, Embryo and YSC Fluid Concentrations Following Oral or Intravaginal Dosing

- Solid lines are plasma concentrations, dashed lines are YSC fluid concentrations and dotted lines are embryo concentrations;
- Embryo conc data are in ng/mg of weighed sample, so direct comparisons to plasma and embryonic fluid concentrations should not be made. However, the graph shows that the relative concentrations of thalidomide in plasma, YSC fluid and embryo at these doses were similar between oral and IVg routes of administration.
Technical challenges Collecting Embryo Concentration Data

- Collection of whole embryos was difficult at GD11. Large variability in weights of collected embryos indicates that some embryos were only partially collected.
- Embryos were pooled by litter prior to assay
- Assay requires rinsing of embryos in Sorensen’s buffer
- Embryos on GD11 contain high fluid contents
- Lack of intact embryo samples, and variable fluid and water contents in the collected samples resulted in variable/unreliable embryo concentration data
Significance of Yolk Sac Cavity Fluid

• Early Rabbit Embryo Development:
  (Carney et al. BDR Part C 72:345-360, 2004)
  – Prior to GD13, the rabbit embryo and visceral yolk sac are bathed in the same fluid compartment (yolk sac cavity)
  – The yolk sac cavity fluid compartment in the rabbit is large (~1 mL by GD10)

• Drug exposure of the embryo should be related to the drug concentration in the YSC fluid, which can be measured more directly and accurately

• Ratio of drug concentrations in YSC fluid to plasma would be a good measurement of any differences in uptake into the intrauterine compartment between the two routes of administration
YSC Fluid and Embryo Exposure following Oral or Intravaginal Administration

<table>
<thead>
<tr>
<th></th>
<th>20 mg/kg PO</th>
<th>180 mg/kg PO</th>
<th>2 mg/kg IVg</th>
<th>20 mg/kg IVg</th>
<th>180 mg/kg IVg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Fluid</td>
<td>Embryo</td>
<td>Plasma</td>
<td>Fluid</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>3880</td>
<td>2230</td>
<td>3630</td>
<td>19300</td>
<td>10000</td>
</tr>
<tr>
<td></td>
<td>18400</td>
<td>250</td>
<td>107</td>
<td>1060</td>
<td>534</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
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<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>AUC0-t (ng*hr/mL)</td>
<td>37800</td>
<td>25100</td>
<td>39900</td>
<td>287000</td>
<td>160000</td>
</tr>
<tr>
<td></td>
<td>256000</td>
<td>1070</td>
<td>524</td>
<td>2260</td>
<td>10600</td>
</tr>
<tr>
<td></td>
<td>5720</td>
<td>25800</td>
<td>87300</td>
<td>53500</td>
<td>112000</td>
</tr>
<tr>
<td>Fluid to plasma AUC ratio</td>
<td>NA 0.66</td>
<td>NC</td>
<td>NA 0.56</td>
<td>NC</td>
<td>NA 0.49</td>
</tr>
<tr>
<td>Ratio of IVg AUC to Oral AUC</td>
<td>NA NA NA</td>
<td>NA NA NA</td>
<td>NA NA 0.28</td>
<td>0.23 0.65</td>
<td>0.30 0.33</td>
</tr>
</tbody>
</table>

a. Embryo concentration data are ng/mg of dry tissue, so direct comparisons to plasma concentrations could not be made
b. 24 hour samples were BQL, AUC values are for 0-6 hrs; NA: not applicable; NC: not calculated

- YSC Fluid and embryo T\text{max} values were similar to maternal plasma T\text{max} values
- YSC fluid to plasma AUC ratios were similar across doses and routes of administration (0.49-0.66)
TK Study Summary

• Plasma, YSC fluid and embryo exposures were lower following IVg administration as compared to oral administration
• Exposures were more variable following intravaginal dosing as compared to oral dosing
• YSC fluid is the appropriate compartment for measurement of embryo exposure at this developmental stage
• No meaningful difference in YSC fluid/plasma AUC ratio between oral and IVg routes (range of 0.49 to 0.66)

• Conclusion: There was no difference in uptake of thalidomide into the intrauterine compartment following oral and intravaginal routes of administration.
EFD Study Design

• Intravaginal (IVg) dose:
  – 2 mg/kg/day thalidomide (>10,000-fold greater than dose calculated based on thalidomide concentration in human ejaculate)

• Oral dose:
  – 2 mg/kg/day thalidomide
  – 180 mg/kg/day thalidomide (positive control group)

• Number of animals:
  – EFD evaluation – 20/group
  – TK evaluation – 4/group

• Dosed from GD 7-19

• Evaluation:
  – Maternal effects
  – Embryo-fetal development
  – TK
EFD Study – In-life Results

- No treatment-related effects in the 2 mg/kg/day oral or intravaginal groups
- Treatment-related findings in the positive control group only:
  - ↓ weight gain, in part due to lower gravid uterine weights (↑ postimplantation loss and ↓ fetal weights)
  - Slightly ↓ food consumption
EFD Study – Uterine Findings

<table>
<thead>
<tr>
<th></th>
<th>0 mg/kg/day Intravaginal</th>
<th>2 mg/kg/day Oral</th>
<th>2 mg/kg/day Intravaginal</th>
<th>180 mg/kg/day Oral</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Females on Study</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>No. Not Pregnant</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>No. Pregnant</td>
<td>20</td>
<td>18</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Pregnancy Index (%)</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>90</td>
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<tr>
<td>No Early Deliveries</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. Females with All Resorptions</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>No. Females Pregnant by Stain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No. Females with Viable Fetuses</td>
<td>19</td>
<td>18</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>No. Corpora Lutea Per Animal</td>
<td>9.7</td>
<td>9.0</td>
<td>9.3</td>
<td>9.8</td>
</tr>
<tr>
<td>No. Implantation Sites Per Animal</td>
<td>8.7</td>
<td>8.1</td>
<td>7.9</td>
<td>8.7</td>
</tr>
<tr>
<td>Preimplantation Loss (%/Animal)</td>
<td>10.23</td>
<td>9.39</td>
<td>9.65</td>
<td>6.10</td>
</tr>
<tr>
<td>No. Viable Fetuses Per Animal</td>
<td>8.4</td>
<td>7.7</td>
<td>7.1</td>
<td>5.7</td>
</tr>
<tr>
<td>Fetal Sex Ratio (% Males/Animal)</td>
<td>47.4</td>
<td>48.9</td>
<td>54.6</td>
<td>44.5</td>
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<tr>
<td>Postimplantation Loss (%/Animal)</td>
<td>3.33</td>
<td>4.49</td>
<td>14.64</td>
<td>38.94</td>
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<td>No. Nonviable Fetuses Per Animal</td>
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<tr>
<td>Litter Size (No./Animal)</td>
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<td>7.7</td>
<td>7.1</td>
<td>5.7</td>
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<td>No. Early Resorptions Per Animal</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
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<tr>
<td>No. Late Resorptions Per Animal</td>
<td>0.2</td>
<td>0.1</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Total No. Resorptions Per Animal</td>
<td>0.3</td>
<td>0.4</td>
<td>0.8</td>
<td>3.1</td>
</tr>
</tbody>
</table>

- No treatment-related effects in the 2 mg/kg/day oral or intravaginal groups
- Positive control group
  - ↑ postimplantation loss (early resorptions) and ↓ viable fetuses
EFD Study – Developmental Findings

- No treatment-related effects in the 2 mg/kg/day oral or intravaginal groups
- Positive control group: Classic thalidomide-induced dysmorphology
  - External malformations: Fore- and hind-limb abnormalities, dome shaped head, short tail, umbilical omphalocele, cranial abnormalities
  - Visceral malformations: Colon, trachea, heart and kidney abnormalities, absent ureters, hydrocephaly, microphthalmia, absent lungs, diaphragmatic hernia
  - Skeletal malformations: Fused, absent, bent or misshapen bones of limbs, pectoral and/or pelvic girdle, skull, tail and sternebrae

<table>
<thead>
<tr>
<th>Treatment Level</th>
<th>No. Litter Evaluated</th>
<th>No. Fetuses Evaluated</th>
<th>Total External Malformations</th>
<th>Total External Variations</th>
<th>Total Visceral Malformations</th>
<th>Total Visceral Variations</th>
<th>Total Skeletal Malformations</th>
<th>Total Skeletal Variations</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Litter Evaluated</td>
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<td>160</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>No. Fetuses Evaluated</td>
<td>18</td>
<td>138</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
<td>4 (2.5)</td>
<td>3 (2.2)</td>
<td>10 (6.3)</td>
<td>82 (59.4)</td>
</tr>
<tr>
<td>Total External Malformations</td>
<td></td>
<td></td>
<td></td>
<td>86 (84.3)</td>
<td></td>
<td>70 (68.6)</td>
<td></td>
<td>98 (96.1)</td>
</tr>
<tr>
<td>Total External Variations</td>
<td></td>
<td></td>
<td></td>
<td>0 (0.0)</td>
<td></td>
<td>0 (0.0)</td>
<td></td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total Visceral Malformations</td>
<td></td>
<td></td>
<td>2 (1.8)</td>
<td>3 (2.2)</td>
<td>9 (5.6)</td>
<td>14 (10.1)</td>
<td>12 (8.7)</td>
<td>82 (59.4)</td>
</tr>
<tr>
<td>Total Visceral Variations</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Skeletal Malformations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Skeletal Variations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No. Litter Evaluated:
- 0 mg/kg/day Intravaginal: 19
- 2 mg/kg/day Oral: 18
- 2 mg/kg/day Intravaginal: 14
- 180 mg/kg/day Oral: 17

No. Fetuses Evaluated:
- 0 mg/kg/day Intravaginal: 160
- 2 mg/kg/day Oral: 138
- 2 mg/kg/day Intravaginal: 114
- 180 mg/kg/day Oral: 102

Total External Malformations:
- 0 mg/kg/day Intravaginal: 0 (0.0)
- 2 mg/kg/day Oral: 1 (0.7)
- 2 mg/kg/day Intravaginal: 0 (0.0)
- 180 mg/kg/day Oral: 86 (84.3)

Total External Variations:
- 0 mg/kg/day Intravaginal: 0 (0.0)
- 2 mg/kg/day Oral: 0 (0.0)
- 2 mg/kg/day Intravaginal: 0 (0.0)
- 180 mg/kg/day Oral: 0 (0.0)

Total Visceral Malformations:
- 0 mg/kg/day Intravaginal: 4 (2.5)
- 2 mg/kg/day Oral: 3 (2.2)
- 2 mg/kg/day Intravaginal: 2 (1.8)
- 180 mg/kg/day Oral: 70 (68.6)

Total Visceral Variations:
- 0 mg/kg/day Intravaginal: 9 (5.6)
- 2 mg/kg/day Oral: 14 (10.1)
- 2 mg/kg/day Intravaginal: 6 (5.3)
- 180 mg/kg/day Oral: 61 (59.8)

Total Skeletal Malformations:
- 0 mg/kg/day Intravaginal: 10 (6.3)
- 2 mg/kg/day Oral: 12 (8.7)
- 2 mg/kg/day Intravaginal: 4 (3.5)
- 180 mg/kg/day Oral: 91 (89.2)

Total Skeletal Variations:
- 0 mg/kg/day Intravaginal: 81 (50.6)
- 2 mg/kg/day Oral: 82 (59.4)
- 2 mg/kg/day Intravaginal: 65 (57.0)
- 180 mg/kg/day Oral: 98 (96.1)
EFD Study – TK Results

<table>
<thead>
<tr>
<th></th>
<th>Oral 2 mg/kg/day</th>
<th>Intravenous 2 mg/kg/day</th>
<th>Oral 180 mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestation Day 7</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>197 ± 50.9</td>
<td>119 ± 65.4</td>
<td>14900 ± 1590</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt;</td>
<td>1060 ± 376</td>
<td>875 ± 313</td>
<td>228000 ± 25000</td>
</tr>
<tr>
<td><strong>Gestation Day 18</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>318 ± 91.7</td>
<td>175 ± 159</td>
<td>16600 ± 2750</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt;</td>
<td>1590 ± 816</td>
<td>1650 ± 8.66</td>
<td>230000 ± 116000</td>
</tr>
<tr>
<td><strong>Gestation Day 19 (3 hr post-dose)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dam (ng/mL)</td>
<td>225 ± 111</td>
<td>157 ± 111</td>
<td>13800 ± 3360</td>
</tr>
<tr>
<td>Fetus (ng/mL)</td>
<td>126 ± 62.6</td>
<td>86.6 ± 64.1</td>
<td>9690 ± 2410</td>
</tr>
<tr>
<td>Fetal: Maternal Plasma Ratio</td>
<td>56.32 ± 5.07</td>
<td>57.59 ± 19.33</td>
<td>70.48 ± 4.67</td>
</tr>
</tbody>
</table>

- **Comparison of oral and intravaginal administration at 2 mg/kg/day**
  - Similar AUC values
  - C<sub>max</sub> values: Intravaginal was 55-60% of oral
  - Fetal to maternal plasma concentration ratios were similar (0.56 and 0.58)

- **C<sub>max</sub> and AUC values were greater than dose proportional for oral doses of 2 and 180 mg/kg/day**
Human Exposure Prediction

• Thalidomide human semen study
  – 100 mg dose resulted in:
    • Plasma concentrations of 10-350 ng/mL
    • Semen levels of 10-250 ng/g (max. 1.0 μg/4 g of ejaculate)
  – 400 mg dose can result in approximately 4.0 μg thalidomide/ejaculate

• PK modeling of 4.0 μg intravaginal dose
  – Assumptions: CL/f – 10.9 L/hr; V/F = 16.0 L; 100% bioavailability (highest possible exposure)
  – Predicted AUC is 0.37 ng*hr/mL and C_{max} is <0.2 ng/mL
Application of Data

• NOAEL (oral) for developmental effects:
  – Rabbit: 20 mg/kg/day ($C_{\text{max}} = 824$ ng/mL; AUC = 4180 ng•hr/mL on GD 19 in the previous rabbit EFD study)

• Exposure margin in rabbit at NOAEL:
  – Highest predicted human AUC following semen exposure is 0.37 ng•hr/mL, with a $C_{\text{max}}$ of <0.2 ng/mL, resulting in estimated exposure margins of >4000 for $C_{\text{max}}$ and >10,000 for AUC
Overall Conclusion

• Compared to the oral route, intravaginal administration of thalidomide resulted in:
  – lower systemic exposures
  – similar uptake from systemic circulation into intrauterine compartment
  – no difference in embryofetal developmental toxicity
• PK modeling using human semen data and assuming 100% bioavailability shows very large exposure margins (>4000X) at the rabbit NOAEL for teratogenicity
• No developmental effects observed at a dose >10,000-fold higher than the expected amount of thalidomide in human semen
• These large exposure multiples diminish the concern for drug exposure via semen even if there is any currently unknown vaginal transport mechanism
• Approach taken in this investigation can be of general application to other drugs in development with similar safety concerns
Acknowledgements
(Thalidomide Rabbit Studies)

• Celgene Corporation
  – Gondi Kumar
  – Matthew Hoffmann
  – Rodrigo Laureano
  – Yongkai Sun

• MPI Research
  – Formulation development
  – Study conduct