Case Study:
Developmental Effects of an Immunomodulatory Biologic
HESI ITC Developmental Immunotoxicity Testing of Pharmaceuticals Workshop

May 3-4, 2010
Washington, DC

Suzanne E. Parker
Biogen Idec
- Species Selection
- Technical Issues with Fetal Assessments
- Disparate Species Effects in EFD Studies
- Potential Adverse Effects on Development of the Fetal Immune System
  - Implications for Immunotox Testing in Developmental Studies
Drug

- Ig-Receptor Fusion Protein
  - CH2 & CH3 regions of human IgG1, receptor antagonist

- Indication: Autoimmune disease

- Inhibits 2 pathways involved in:
  - Establishment and regulation of lymphoid organ microenvironments required for efficient operation of immune system
  - Embryo/Neonate: essential for development of lymph nodes, splenic organization, and potentially organization of mucosal environments
  - Adult: homeostatic regulation of lymphoid microenvironments, *e.g.* maintenance of stromal elements and cell positioning
  - Dendritic cell (DC) biology and T-cell activation
  - Adult: T-DC or T-T cell signaling
EFD Study Species Selection

Assumptions

• Human drug was not active in rodents
  – Based on *in vitro* affinity data
    • 30X less affinity for rodent receptor

• Immunogenicity to the human fusion protein would preclude repeat dosing in rodents

• Safety studies with clinical candidate were conducted in cynomolgus monkeys
  – Sub chronic & chronic tox (6 months)
  – Pilot non GLP EFD in cynos (conducted prior to Phase 2a)

• Murine surrogate:
  – 28 Day GLP Study
  – Non GLP Developmental Toxicity Study in mice
    • Conducted by Research
Preclinical Data

Preclinical Repeat Dose Toxicology Studies

- No AEs, no MTD at doses up to 50 mg/kg
  28 Day, 3 month & 6-month chronic cyno

- Results consistent with expected Pharmacology
  - Effects on LN architecture
  - Effects on lymphocyte trafficking
Preclinical Data

Data to suggest a risk of developmental effects associated with inhibition of the pathway

- KO mice: Lack central and peripheral LN’s
- Research study conducted early in preclinical development
  - Administration of a murine surrogate to pregnant mice altered normal fetal LN development
    - Effects on brachial, inguinal, and popliteal LN’s depending on time of treatment
    - No effects on mesenteric LNs
    - LNs effected dependent on time during gestation drug was administered
    - Effect was dependent on Fc-mediated placental IgG transfer
Pilot Non GLP EFD Study in Cynomolgus Monkeys

- Conducted prior to Phase 2a
- Drug administered IV to pregnant monkeys once per week from GD 20 to 48 (5 doses)
- C-sections performed between GD 135 and GD 151
- Standard fetal examinations, and LN’s were collected and processed for histopath evaluation

Results:
- No apparent adverse effects on pregnant animals
- No treatment-related fetal malformations in external, visceral, or skeletal examinations
- Fetal LN’s appeared normal on macroscopic examination
- Microscopic examination: selected lymph nodes absent or poorly represented in all groups, including controls
  - Missing lymph nodes may have resulted from technical limitations due to small size of fetal LNs
- Possibility of a treatment-related effect could not be excluded
Pivotal EFD Study in Cynos

Considerations

• Technical limitations with harvesting fetal LNs

• LN development in cynomolgus monkeys

• Placental transfer of drug
Lymph Node Development

Irreversible Disruption of NHP or Human Lymph Node Development Will Depend on the Dose During Gestation

Ig Based Drugs: Delivery to the Fetus

Pre FcRN, i.e. Leakage only
[mother] = 100-1000x [fetus]

With FcRN Transport
[mother] = 10-1x [fetus]

<table>
<thead>
<tr>
<th>Dose</th>
<th>Maternal Conc (ug/ml)</th>
<th>Est. Range Fetal Conc (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/kg</td>
<td>10-40</td>
<td>0.01-0.4</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>100</td>
<td>0.1-1</td>
</tr>
<tr>
<td>15 mg/kg</td>
<td>500-800</td>
<td>0.5-8</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>1000-2000</td>
<td>1-20</td>
</tr>
</tbody>
</table>

Based on mouse studies, it was estimated that as little as 1 ug/ml of drug in the fetal circulation would block LN development.

If LN development initiated, then filling may be slowed in the presence of drug, but no irreversible events should occur.
Lymph Node Development

Relationship between lymph node development and the onset of efficient maternal-fetal immunoglobulin transport

Week (Gestation)

4  8  12  16  20  24  28  32  36

Man

Macaque

LN Development 8-11 wks

FcRN Mediated Placental Transport

Birth

Major Organogenesis

biogen idec
Microscopic Examination of Fetal LN’s from Normal Untreated Animals

Technical limitations with harvesting fetal LNs

- LN’s were collected from 5 untreated animals between GD120 and 149 and evaluated microscopically
- All but one sample (Peyer’s Patch) were present and in good condition
- Demonstrated technical feasibility of collecting fetal lymph nodes

| Animal No. | Dose (mg/kg) | Axillary H&E | Cervical H&E | Mandibular H&E | Mesenteric H&E | Inguinal H&E | Peyer’s Patch
<table>
<thead>
<tr>
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<tbody>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A</td>
</tr>
</tbody>
</table>

Frequency of missing nodes:

- 0/5, 0/5, 0/5, 0/5, 0/5, 1/5

+ = lymph node present; A = lymph node absent in all evaluated sections or insufficient lymphoid tissue to identify as lymph node;  "ileum"
Cyno EFD Study Design
Conducted prior to Phase 2b

Pre-dose
GD 20

Dosing
3, 15, 50 mg/kg qw
GD 90

End of Organogenesis

C-Sections
GD 138 - 143

Dosed to GD 90 to cover lymphogenesis

Cynomolgus fetal immune system development

GD 35
Primordial LNs

GD 70-85
Mesenchymal Lacunae

T & B-Cell Development

1° LNs

Splenic demarcation

GD 99-105

GD 125

GD 148-154

LNs Developed
No Germinal Centers

Haberman et. al.
Buse et. al.
Cyno EFD Exposure Data

Maternal Exposure

• Exposure maintained throughout study period
  – With exception of animals in low dose group (3 mg/kg) with Ab titers >21870

• Changes in drug disposition GD 20 vs GD 90
  – ↑ Absorption, AUC, Cmax
  – ↓ Tmax, T1/2, Vz/F
  – No change in CL/F

• Maternal & Fetal Exposure (@ C-section, ≈ 50 days post final dose on GD 90)
  – Most animals fetal > maternal
  – 3 mg/kg  6/15 Maternal & Fetal; Fetal/Maternal Ratio: 2.5 to 8.9
  – 15 mg/kg 13/14 Maternal & 14/14 Fetal; Fetal/Maternal Ratio: 0.5 to 11.6
  – 50 mg/kg 15/15 Maternal & Fetal, Fetal/Maternal Ratio: 0.3 to 4.4
Immunogenicity: Maternal & Fetal Anti-Drug Ab

Anti-Drug Ab generally detected after GD55

- Most cases if dam Ab+, respective fetus also Ab+
  - 15/15 Ab+ 3 mg/kg
  - 6/14 Ab+ 15 mg/kg
  - 1/15 Ab+ 50 mg/kg

- Drug interfered with Ab assay when Ab:Drug molar ratio of ≥1:1

- Exposure affected when Ab titers ≥ 21870
Cyno EFD Results: Maternal

No adverse effects:

- Clin obs
- Maintenance of pregnancy or any pregnancy parameters
- Coagulation
- Clinical Chemistry
- Hematology

Only drug-related effect:

- Expected pharmacology observed in all cyno studies
  - Lymphocytosis
EFD Cyno Study Results: Fetal

No drug-related effects:
• Fetal survival
• Fetal weights
• Placental weights
• Fetal external measurements
• Fetal external or skeletal development

Drug-related effect:
• Gross decrease in LN size: mandibular, cervical, axillary, inguinal and/or mesenteric

• Dose dependent incidence of lack of microscopically identifiable LNs and hypocellularity of LN cortex

• Both macroscopic & microscopic findings correlated with gross decrease in LN size

• Flow & IHC on LNs not conducted (Fetal)
### Summary Incidence of small to very small lymph nodes* (%)

<table>
<thead>
<tr>
<th>Lymph node:</th>
<th>Group 1 0 mg/kg (n=12)</th>
<th>Group 2 3 mg/kg (n=15)</th>
<th>Group 3 15 mg/kg (n=14)</th>
<th>Group 4 50 mg/kg (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesenteric</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>10 (71.4)</td>
<td>14 (93.3)</td>
</tr>
<tr>
<td>Mandibular</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>13 (92.9)</td>
<td>15 (100)</td>
</tr>
<tr>
<td>Cervical</td>
<td>0 (0)</td>
<td>1 (6.7)</td>
<td>13 (92.9)</td>
<td>15 (100)</td>
</tr>
<tr>
<td>Axillary</td>
<td>0 (0)</td>
<td>1 (6.7)</td>
<td>4 (28.6)</td>
<td>13 (86.7)</td>
</tr>
<tr>
<td>Inguinal</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>11 (78.6)</td>
<td>15 (100)</td>
</tr>
</tbody>
</table>

N = Number of animals/group  
* Bilaterally or unilaterally in any individual fetus
Cyno EFD Study Results: Fetal LN Histology

Incidence of Lack of Identifiable Lymph Nodes

<table>
<thead>
<tr>
<th>Dose Group (mg/kg)</th>
<th>Incidence of Lack of Histologically Identifiable Lymph Nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 – 8%</td>
</tr>
<tr>
<td>3</td>
<td>0 - 13%</td>
</tr>
<tr>
<td>15</td>
<td>7 - 86%</td>
</tr>
<tr>
<td>50</td>
<td>57 – 100%</td>
</tr>
</tbody>
</table>

Incidence similar in control and 3 mg/kg dose group
EFD Study in Cynos: Conclusion

- Drug well tolerated in pregnant cynos
- No maternal adverse effects
- Maternal NOEL < 3 mg/kg
  - Expected pharmacological effects
- Maternal NOAEL 50 mg/kg

- Fetal NOEL < 3 mg/kg hypocellularity of LN cortex

- Fetal NOAEL 3 mg/kg
  - Drug-related fetal adverse effects: decreased LN development at ≥ 15 mg/kg (decreased gross size and lack of histologically identifiable lymph nodes)
Drug-related adverse fetal effects on LN development

• Was it considered teratogenic?
  – Malformation: not reversible
  – LN effects in older cynos:
    • **Reversible** lymphoid follicular atrophy characterized by reduction in size or number of lymphoid germinal centers and/or decrease in lymphoid follicular hyperplasia
  – Reversibility of fetal LN effects not known
  – If LN effects were reversible after birth, drug-related LN effects would **not be considered** teratogenic

• Teratogenicity inconclusive from this study
Species Selection & Challenging Company Dogma

Time Line in preclinical development
- Completed chronic and EFD studies in cynos

General consensus in research: Dosing in rodents would not be feasible
- Receptor affinity
- Immunogenicity would preclude repeat dosing

Binding affinities: Apparent Kd

<table>
<thead>
<tr>
<th>Drug</th>
<th>Human R1</th>
<th>Human R2</th>
<th>Mouse R1</th>
<th>Mouse R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Drug</td>
<td>1-2 nM</td>
<td>0.5 – 1nM</td>
<td>20 – 30 nM</td>
<td>2 –5 nM</td>
</tr>
</tbody>
</table>

No data to indicate that dosing of clinical candidate was not feasible in rodents
- Similar Pathway: mouse and human
- Similar mouse and human receptor distribution
- Binds mouse receptor (weak)
A New Twist in Preclinical Development: Activity in Rodents?

Carcinogenicity Strategy: Rodent as a Tox Species

- During course of carcinogenicity strategy assessment
  - Demonstrated clinical candidate pharmacologically active in rodents
  - Findings in rodents similar to those in cyno

- Immunogenicity did not preclude repeat dosing
  - Immunogenicity “similar” in rodents and cynos
  - ADA effects on exposure similar in rodents and cynos

Species Selection for PPN Study

- Rodent PPN Study preferable
  - Incorporation of more immunological endpoints
  - Power group size for more meaningful data
Rat EFD Study Design

- **Pre-dose**
  - GD 5

- **Dosing GDs 5, 12, 19 10, 30, 100 mg/kg**

- **C-Section GD 21**

- **Dosing LDs 2, 9, 16 Control & 100 mg/kg**

- **LD 1**

- **LD 21**

**Fetal TK, ADA**

**LN, Thymus, Spleen (gross & histo)**

**Standard rodent EFD Assessments**

**Fetal TK & ADA**

**Subset of animals (Control & 100 mg/kg) dosed through lactation**

- Technical issues harvesting fetal LNs GD 21
- Rodent LN development continues post birth
Rat EFD Study: Exposure

- Exposure confirmed
  - F0 dams gestation through lactation
  - F1 offspring gestation through lactation

- F1 Exposure
  - F1 < F0 across all dose groups
    - Maternal:Fetal ratios ≈ 2-9
    - GD 21 all dose groups
    - LD 21 100 mg/kg dose group

  - Note: this is in contrast to cyno study where F1>F0
    - Fetal:Maternal ratio ≈ 0.3-12
Rat EFD Study: Maternal Results

• Unexpected adverse maternal effects in 100 mg/kg dose dams
  – 6/44 dams
  – Apparent after 2 doses

• No adverse pregnancy or fetal parameters on surviving dams

• Exclusive of the unexpected maternal adverse effects @ 100 mg/kg dose, no drug-related effects on any C-Section or litter parameters

• No drug-related effects on natural delivery parameters (100 mg/kg dose)

• Maternal MTD 30 mg/kg
Rat EFD Study: Fetal Results

• Exclusive of F1 pups from 100 mg/kg dose dams where drug-related adverse effects observed\(^1\)
  
  – No drug-related effects (GD21)
    • Gross external findings
    • Soft tissue or skeletal

  – No differences in LN, spleen, thymus development in F1 pups from 100 mg/kg treated dams vs. controls (LD21)

\(^1\) F1 effects considered 2\(^o\) to maternal tox
Species Selection PPND Study

- **LN developmental effects**
  - KO mice
  - Pregnant mice treated with murine surrogate
  - Pregnant cyno
  - Why not pregnant rats (under conditions of the study)?

- **Rat would not be appropriate species for PPND Study**
  - Species specific fetal effects
    - Species specific placental transfer differences
    - Species specific differences in LN development
    - Threshold (exposure) for rodent LN developmental effects

- **Basis for the unexpected maternal toxicity?**
  - Species specific maternal effects
  - Under evaluation
Lack of LN Effects in Rat EFD: Exposure?

- Limited by maternal toxicity in rats for > exposure

- Cynos @ C-section (GD140)
  - NOEL LN Effects 1,400 ng/ml

- Rat EFD Exposure
  - C-Section GD 21 (pooled F1, 10 litters)
    - 10 mg/kg 5,576 + 3703 ng/ml (895 – 12,100)
    - 30 mg/kg 21,820 + 8638 ng/ml
    - 100 mg/kg 36,270 + 8683 ng/ml

  - During lactation
    - LD 17 100 mg/kg F1 2,200 - 92,800 ng/ml
    - LD 21 100 mg/kg F1 7,070 – 81,700 ng/ml

- Based on mouse studies, it was estimated that as little as 1 ug/ml of drug in the fetal circulation would block LN development.

- Species difference with respect to exposure threshold for effect on LN development?
Lack of LN Effects in Rat EFD: Exposure?

Max exposure (mean) in F1 animals during gestation:
• 5-10 X below where decreased number/size germinal centers in GALT of ileum and jejunum observed in repeat dose tox studies
• 10-20 X below where decreased number/size germinal centers observed for mandibular and/or mesenteric lymph nodes in repeat dose tox studies
  – There were splenic effects observed in F1
    • Decreased thickness of splenic marginal zone

Why no LN effects in rat EFD?
• Exposure threshold
• Species specific placental transport
• Species specific immune system development
Conclusions

• Species Selection
  – If studies had first been conducted in rodent, would we have identified potential fetal toxicity?

• If we had conducted the ePPND would we have known to incorporate extensive immunotox testing?

• Given the adverse effects on fetal LN development, what immunological parameters should be evaluated in the PPND Study?

• Duration of cyno PPND Study?
  – Adult animals recovery of LN effects:
    • > 3 months following 28 days dosing
    • 6 months following 6 months dosing
Acknowledgements

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Matt Stagray

SNBL, USA
Everett, WA
Satoru Oneda, DVM, Ph.D., DJTS
BACKUP SLIDES
Pilot Developmental Toxicity Study of in Cynomolgus Monkeys

Frequency of Missing Lymph Nodes

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Axillary</th>
<th>Cervical</th>
<th>Mandibular</th>
<th>Mesenteric</th>
<th>Inguinal</th>
<th>Peyer’s(^1) Patch</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1/5</td>
<td>2/5</td>
<td>1/5</td>
<td>0/5</td>
<td>1/5</td>
<td>3/5</td>
</tr>
<tr>
<td>1</td>
<td>1/5</td>
<td>0/5</td>
<td>1/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>10</td>
<td>1/5</td>
<td>4/5</td>
<td>2/5</td>
<td>0/5</td>
<td>2/5</td>
<td>0/4</td>
</tr>
</tbody>
</table>

\(^1\)ileum

Conclusion

• Microscopic evaluations of Day 135 to 151 fetal lymph nodes not routine
• Missing lymph nodes may reflect technical limitations imposed by small size of the fetal LN
• Develop technical ability to harvest fetal LNs (pilot study)
• A larger follow-up study needed to clarify the significance of the data
## Cyno EFD Study Design

- Doses administered weekly between GD20 and GD90 (11 doses)
  - Note Seg II studies in cynos typically dose through end of organogenesis GD 48
- C-sections performed between GD 138 and GD 143

<table>
<thead>
<tr>
<th>Group</th>
<th>Test or Control Article</th>
<th>Dose Level (mg/kg)</th>
<th>Dose Conc (mg/mL)</th>
<th>Dose Vol</th>
<th>Number of Pregnant Animals*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>BG9924</td>
<td>3</td>
<td>6</td>
<td>0.5</td>
<td>15</td>
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<tr>
<td>3</td>
<td>BG9924</td>
<td>15</td>
<td>30</td>
<td>0.5</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>BG9924</td>
<td>50</td>
<td>100</td>
<td>0.5</td>
<td>15</td>
</tr>
</tbody>
</table>

*Intent to treat: 16/dose group
# Cyno EFD Study Results: Fetal

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-Paired Lymph Node:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesenteric</td>
<td>(n=12)</td>
<td>(n=15)</td>
<td>(n=14)</td>
<td>(n=15)</td>
</tr>
<tr>
<td>Hypocellularity</td>
<td>0/11 (0%)</td>
<td>0/14 (0%)</td>
<td>7/9 (78%)</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>Tissue Not Present</td>
<td>1/12 (8%)</td>
<td>0/15 (0%)</td>
<td>5/14 (36%)</td>
<td>12/15 (80%)</td>
</tr>
<tr>
<td>Insufficient</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Paired Lymph Node:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandibular</td>
<td>(n=12X2)</td>
<td>(n=15X2)</td>
<td>(n=14X2)</td>
<td>(n=15X2)</td>
</tr>
<tr>
<td>Hypocellularity</td>
<td>0/24 (0%)</td>
<td>6/25 (24%)</td>
<td>6/6 (100%)</td>
<td>NA</td>
</tr>
<tr>
<td>Tissue Not Present</td>
<td>0/24 (0%)</td>
<td>4/30 (13%)</td>
<td>22/28 (79%)</td>
<td>30/30 (100%)</td>
</tr>
<tr>
<td>Insufficient</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cervical</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypocellularity</td>
<td>0/23 (0%)</td>
<td>0/28 (0%)</td>
<td>0/4 (0%)</td>
<td>NA</td>
</tr>
<tr>
<td>Tissue Not Present</td>
<td>0/24 (0%)</td>
<td>2/30 (7%)</td>
<td>24/28 (86%)</td>
<td>30/30 (100%)</td>
</tr>
<tr>
<td>Insufficient</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Axillary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypocellularity</td>
<td>0/22 (0%)</td>
<td>9/29 (31%)</td>
<td>18/24 (75%)</td>
<td>12/12 (100%)</td>
</tr>
<tr>
<td>Tissue Not Present</td>
<td>1/24 (4%)</td>
<td>0/30 (0%)</td>
<td>2/28 (7%)</td>
<td>17/30 (57%)</td>
</tr>
<tr>
<td>Insufficient</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Inguinal</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypocellularity</td>
<td>0/24 (0%)</td>
<td>6/26 (23%)</td>
<td>8/10 (80%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td>Tissue Not Present</td>
<td>0/24 (0%)</td>
<td>0/30 (0%)</td>
<td>18/28 (64%)</td>
<td>29/30 (97%)</td>
</tr>
<tr>
<td>Insufficient</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**a** The incidence of hypocellularity only involved animals with sufficient lymph node tissue in the section for evaluation of architecture/cellularity (individuals from the Insufficient and Tissue Not Present categories were excluded)

**b** Tissue Not Present in the section examined

**c** Insufficient tissue for evaluation of architecture/cellularity in the section examined
EFD Study in Rats

• Dosed drug @ 0, 10, 30, & 100 mg/kg on gestation days 5, 12, 19
• C-section GD 21
  – Standard Seg II parameters

• Subset of Control & 100 mg/kg F0 animals, continued dosing through lactation to weaning (LD 21) to allow evaluation of fetal LNs
  – Dosed Lactation Days (LD) 2, 9, & 16
  – F1 LNs, spleen, thymus evaluated macro and microscopically on LD Day 21 (weaning)
    • LN maturation continues post birth in rodents
    • Technical difficulties in harvesting LNs from GD21 rat fetuses due to maturation stage
  – Fetal and maternal drug levels
    • GD 21 (at time of C-Section)
    • LD 21 (at necropsy)
### Summary Incidence of Histopathologic: Rat Seg EFD

<table>
<thead>
<tr>
<th>Tissue/Finding</th>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (mg/kg/dose)</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Number of animals</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

#### Paired Lymph Nodes

<table>
<thead>
<tr>
<th>Lymph node axillary</th>
<th>Number of samples examined</th>
<th>16</th>
<th>18</th>
<th>18</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lymph nodes present</td>
<td></td>
<td>14 (87%)</td>
<td>16 (89%)</td>
<td>13 (72%)</td>
<td>14 (78%)</td>
</tr>
<tr>
<td>Present: bilateral</td>
<td></td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Present: unilateral</td>
<td></td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

#### Lymph node cervical

<table>
<thead>
<tr>
<th>Number of samples examined</th>
<th>16</th>
<th>18</th>
<th>18</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lymph nodes present</td>
<td>9 (56%)</td>
<td>17 (94%)</td>
<td>9 (50%)</td>
<td>14 (78%)</td>
</tr>
<tr>
<td>Present: bilateral</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Present: unilateral</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

#### Lymph node inguinal

<table>
<thead>
<tr>
<th>Number of samples examined</th>
<th>16</th>
<th>18</th>
<th>18</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lymph nodes present</td>
<td>12 (75%)</td>
<td>17 (94%)</td>
<td>16 (89%)</td>
<td>18 (100%)</td>
</tr>
<tr>
<td>Present: bilateral</td>
<td>5</td>
<td>8</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Present: unilateral</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

#### Lymph node mandibular

<table>
<thead>
<tr>
<th>Number of samples examined</th>
<th>16</th>
<th>18</th>
<th>18</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lymph nodes present</td>
<td>15 (94%)</td>
<td>17 (94%)</td>
<td>16 (89%)</td>
<td>16 (89%)</td>
</tr>
<tr>
<td>Present: bilateral</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Present: unilateral</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

#### Non-Paired Lymph Node

<table>
<thead>
<tr>
<th>Lymph node mesenteric</th>
<th>Number of samples examined</th>
<th>8</th>
<th>9</th>
<th>9</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lymph nodes present</td>
<td></td>
<td>8 (100%)</td>
<td>9(100%)</td>
<td>9 (100%)</td>
<td>9 (100%)</td>
</tr>
<tr>
<td>Present</td>
<td></td>
<td>8</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

#### Spleen

<table>
<thead>
<tr>
<th>Decreased thickness: marginal zone</th>
<th>Number of animals affected</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

| Minimal                           |                            | 0    | 5      | 1      | 5      |