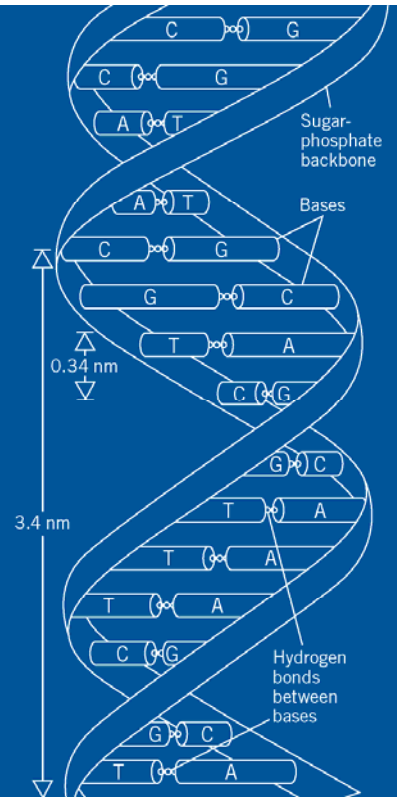

Developmental Immunotoxicology with Rituximab

ILSI/HESI DIT Workshop
Washington, DC

Anu Vaidyanathan, PhD, DABT

May 3-4, 2010

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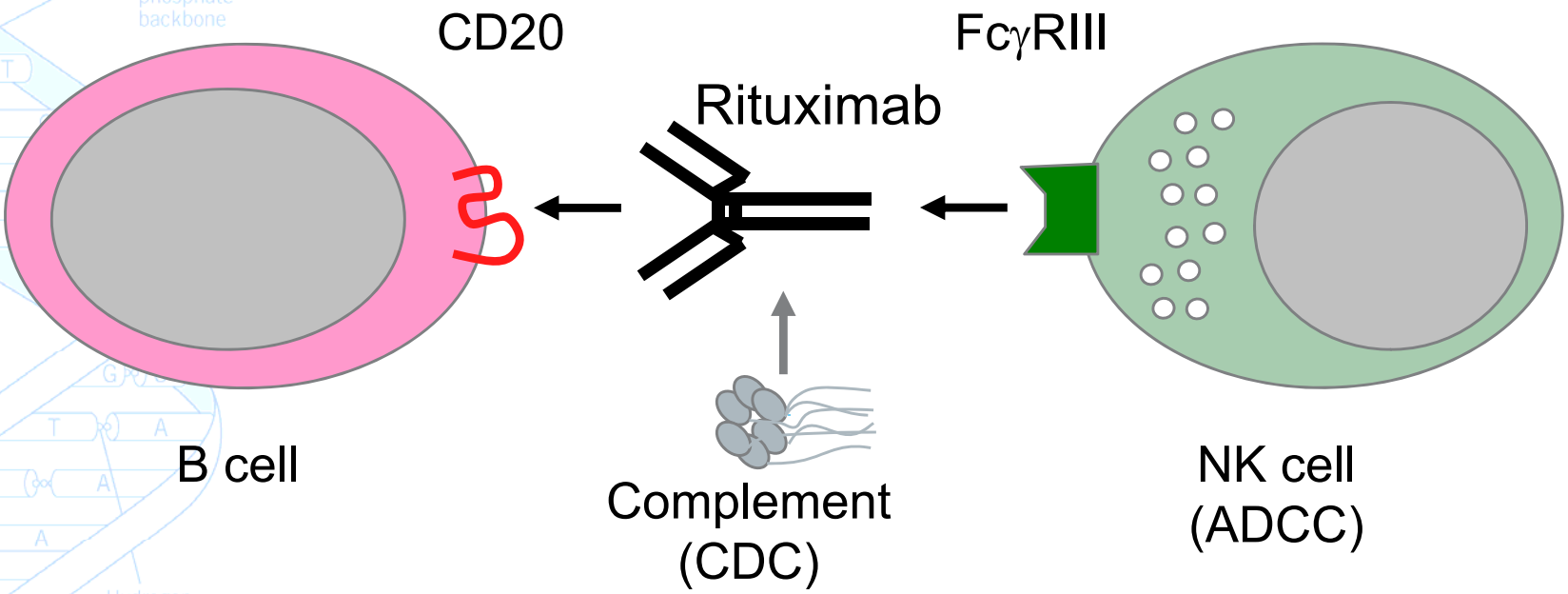
Agenda

- Mechanism of action of rituximab
- Design & results of EFD study
- Special design considerations for PPND study
- Results of PPND study
- Summary & Conclusions of Reproductive/Developmental Toxicology Evaluation
- Lessons learned

EFD: Embryofetal Development
PPND: Pre- and postnatal development

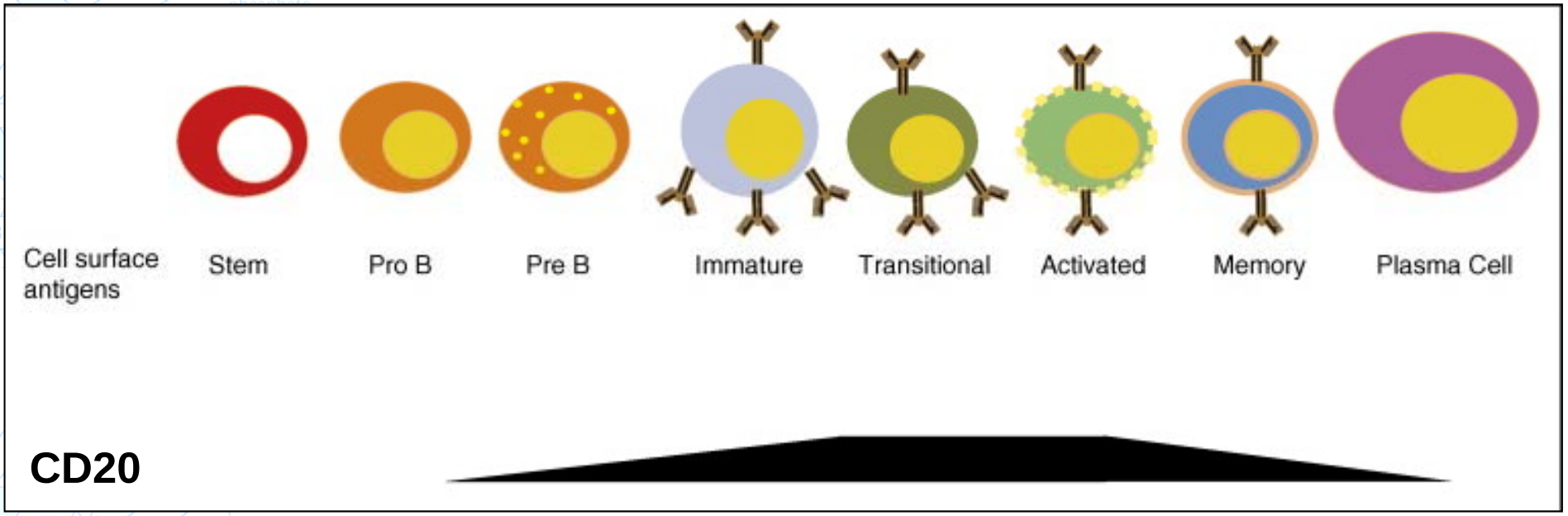
Possible Mechanisms of Action of Anti-CD20

- ADCC
- CDC
- Apoptosis
- Modulation of Ca^{2+} flux
- Other?
- Combination of one or more of the above?



CD20 expression during B-cell development

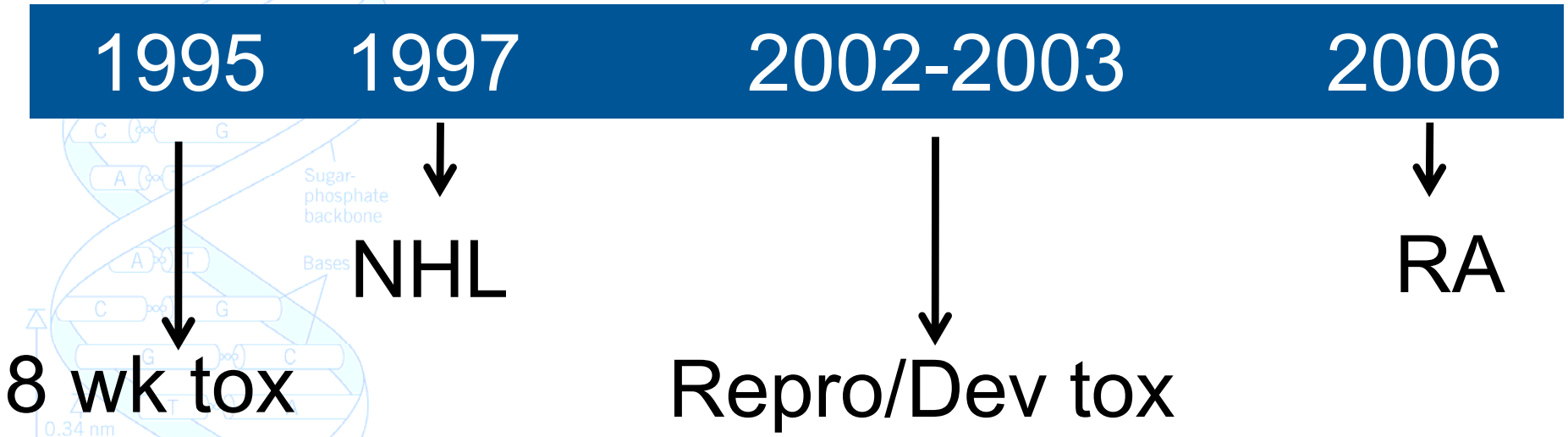
- CD20 antigen expressed at pre-B cell, immature, and mature through memory B-cell stage
- Not expressed on stem, pro-B cells or plasma cells
- NHP used as tox species based on pharmacologic activity of rituximab
- Chimeric IgG1 (murine/human)



Silverman G. *Arthritis Rheum 2006;54:2356–67*

Product Development Timeline

Rituximab



Approvals: Non-Hodgkins Lymphoma (NHL) & Rheumatoid Arthritis (RA)

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Design of EFD Study

Days of Gestation (GD)

Confirm pregnancy

TK

Ultrasound Examination

Fetal Observations

- **Skeletal & Visceral Exam**
- **TK**
- **Developmental Immunotox**
  - **Histopathology & IHC**

Birth

Timeline of Immune Development Cynomolgus Monkey

Thymic organogenesis

T & B-cell development

Splenic demarcation

Loading/Study dose: 15/20, 38/50 & 75/100 mg/kg

Buse, 2005

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Placental Transfer: Comparison of Maternal and Fetal Rituximab Serum Concentrations on Day 100

Group	Rituxan (mg/kg)	Maternal Serum (µg/mL)	Fetal Serum (µg/mL)	% of Maternal Concentration
2	20	8.85 ± 6.0	3.05 ± 2.4	35%
3	50	7.51 ± 3.8	5.59 ± 3.6	74%
4	100	18.5 ± 15	13.6 ± 9.7	74%

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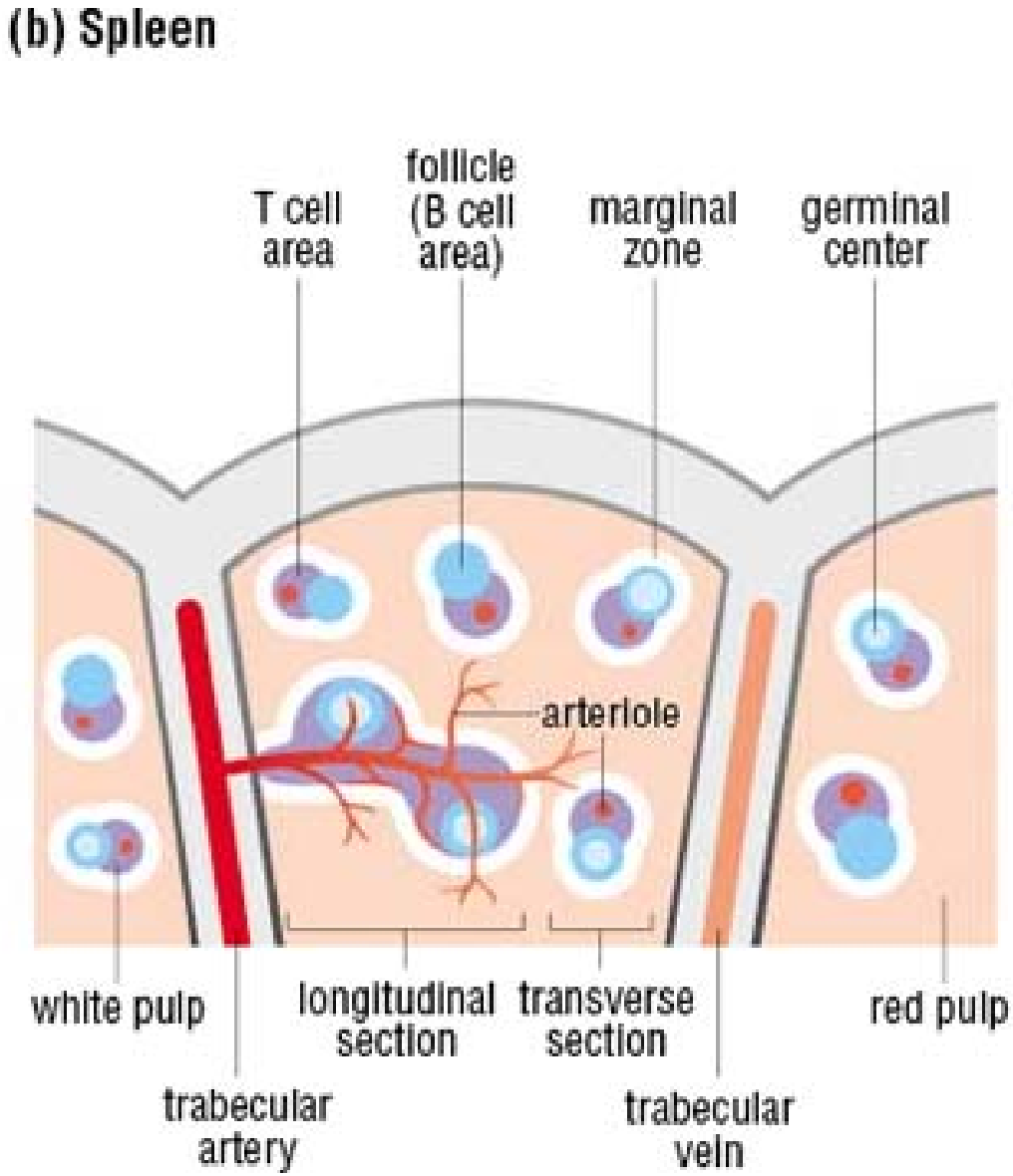
Anti-therapeutic antibody (ATA) Development

- **Previous toxicology studies with Rituximab resulted in ~40% of the cynos developing ATA**

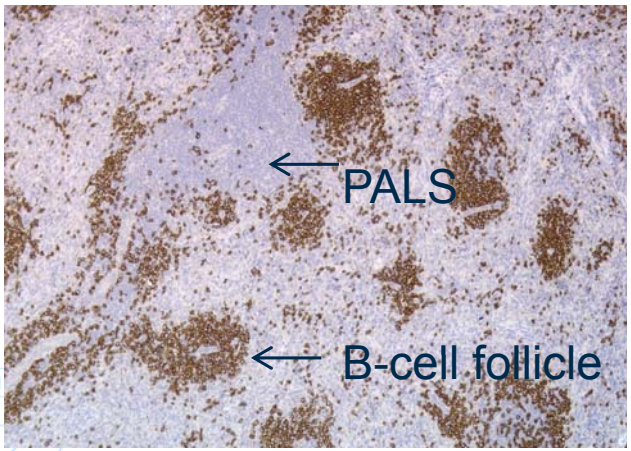
- A few maternal animals developed ATAs in EFD study
  - 2/12 from 20 mg/kg group
  - 0/12 from 50 mg/kg group
  - 1/12 from 100 mg/kg group
- ATAs first appeared between GD80 and GD100
- When maternal ATAs were present, ATAs were also detected in the fetus
- ATAs appeared to affect maternal and fetal blood concentrations

Immunohistochemistry (IHC)

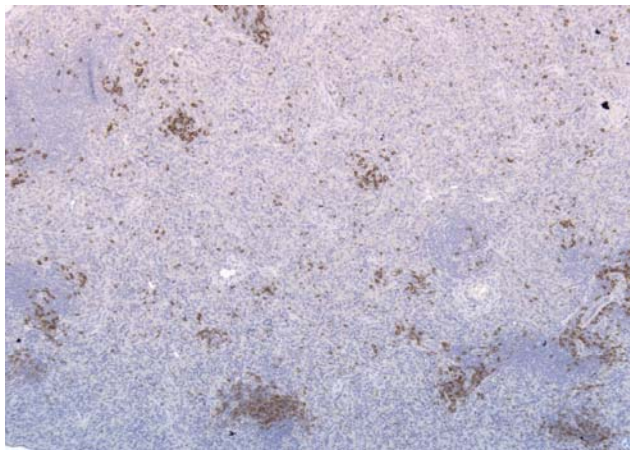
- Fetal tissues collected: mandibular and mesenteric lymph nodes, and spleen



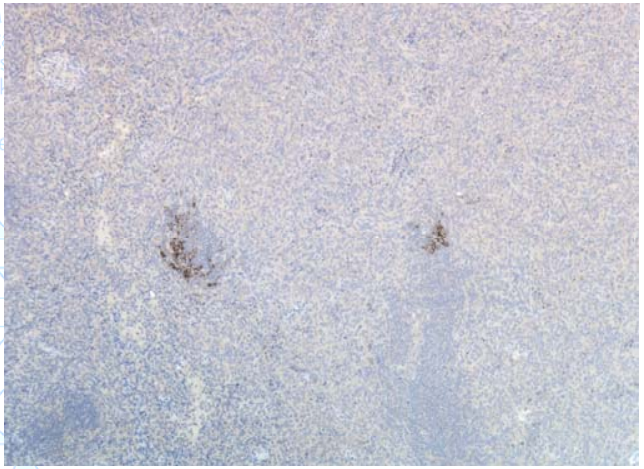
Immunohistochemistry of Fetal Spleen: CD20 + B-cells



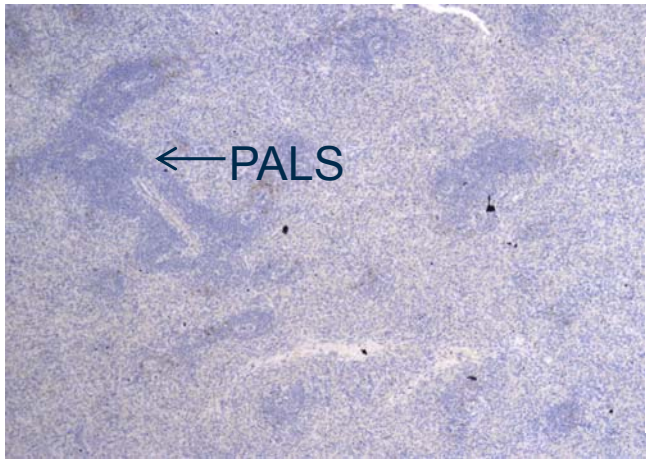
Vehicle Control



20 mg/kg



50 mg/kg



100 mg/kg

- Fewer CD20+ B cells and B-cell follicles (dose-related) in spleen, mandibular and mesenteric lymph nodes

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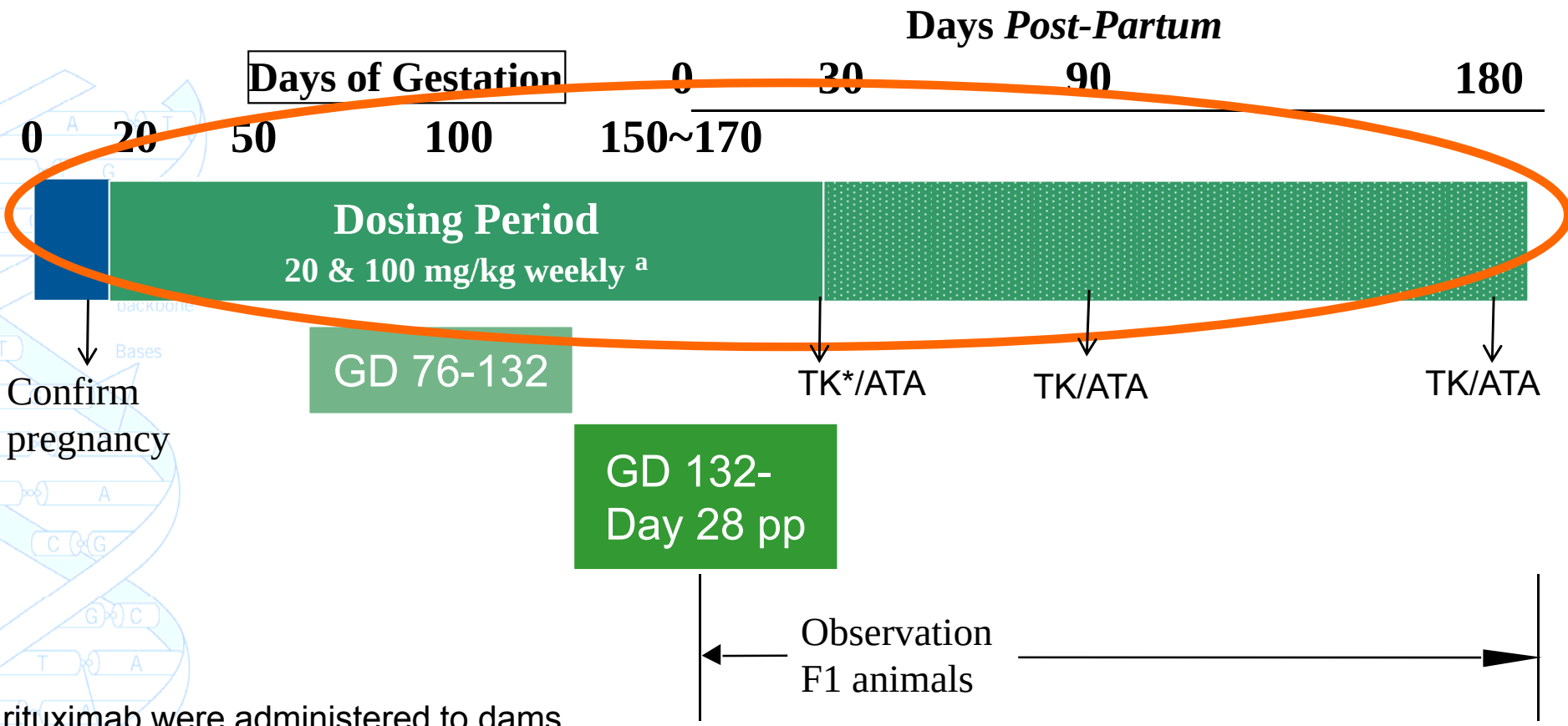
Summary & Conclusions of EFD Study

- Rituximab-related effects limited to expected pharmacology
  - Dams and fetuses demonstrated B-cell depletion in either peripheral blood (dams; as judged by lymphocyte counts) or lymphoid tissues (fetuses)
- Demonstration of placental transfer of rituximab to fetus during second trimester of gestation
  - Fetal exposures were up to 74% of maternal exposures at GD 100
  - Pharmacodynamic effect of B-cell depletion in fetuses at GD 100

❖ ***Rituximab, at doses up to 100 mg/kg weekly during organogenesis, was well-tolerated and did not elicit maternal toxicity, embryotoxicity or teratogenicity in cynomolgus monkeys***

Design of PPND Study

1. **Extended dosing period (similar to ePPND study) to capture B-cell depletion noted in EFD Study**
2. **Immunogenicity concerns**



[a] 5 doses of rituximab were administered to dams during the post partum period

* Infant & Maternal milk

Loading/Study dose: 15/20, 75/100 mg/kg

Immunophenotyping at 30, 90 and 180 days;
Immune cell function analysis at 90 & 180 days

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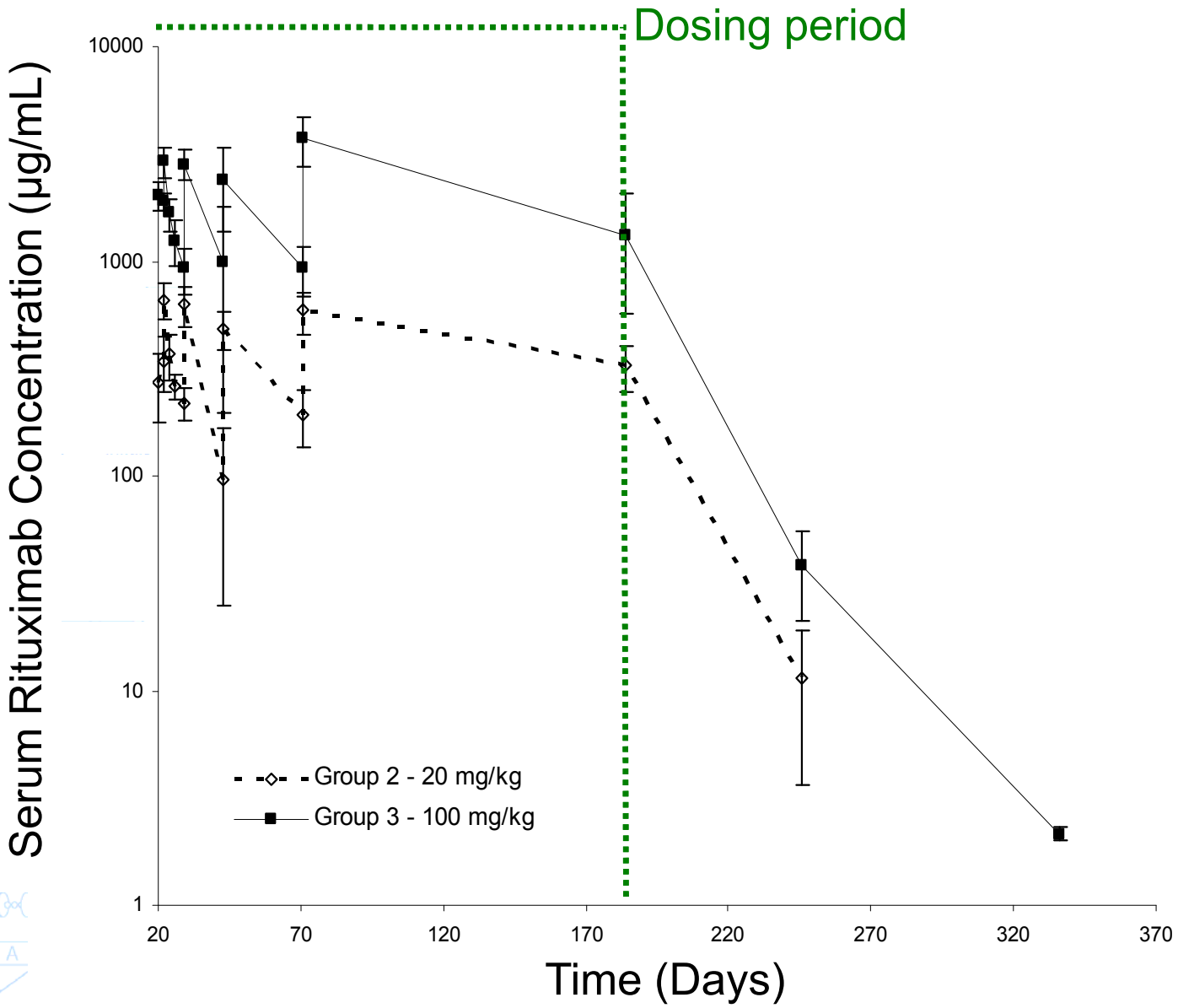
Immune System Evaluation in Infants

- Immunophenotyping (CD3, CD21 & CD40)
  - Dams: throughout treatment and through post-partum (pp) day 180
  - Infants: Days 28, 90 &180 pp
- Antigenic challenge
  - KLH: Day 90 & 180 (primary and secondary response)
  - Daptacel™ [diphtheria, tetanus (TT) & pertussis]: Day 180
  - Collection of serum
    - Anti-KLH (IgG) and anti-TT (IgG & IgM) specific antibodies by ELISA
- Total immunoglobulin levels (IgG &IgM): Days 89 & 179 pp
- T-cell function evaluation Day 90 &180 pp
  - *Ex-vivo* function & proliferation assays (mitogen and IL-2 responsiveness using PBMCs)
- Histopathology: spleen, thymus, lymph nodes, Peyer's Patches, bone marrow
- IHC of lymphoid tissue

Maternal Serum Rituximab Concentration-Time Profiles



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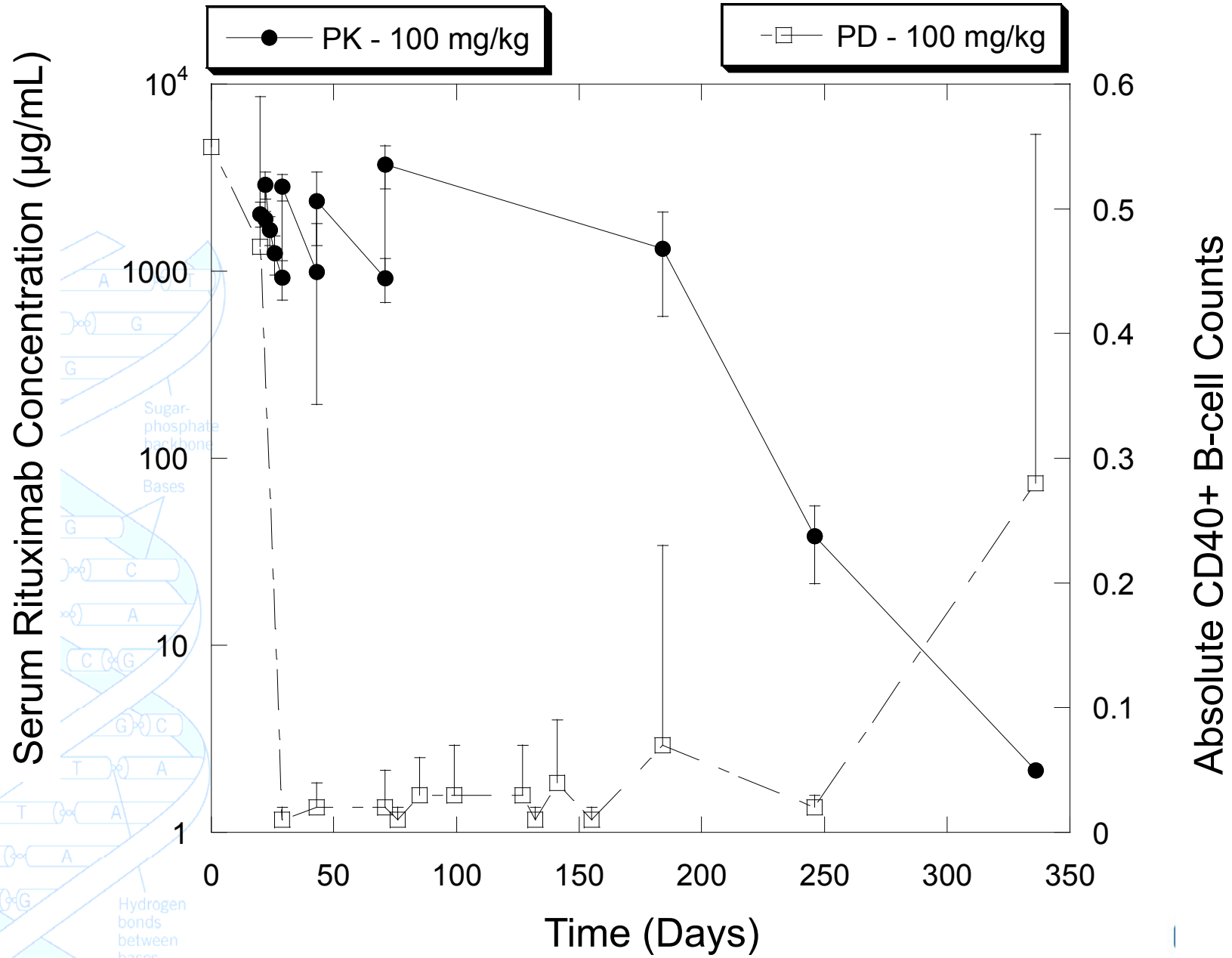
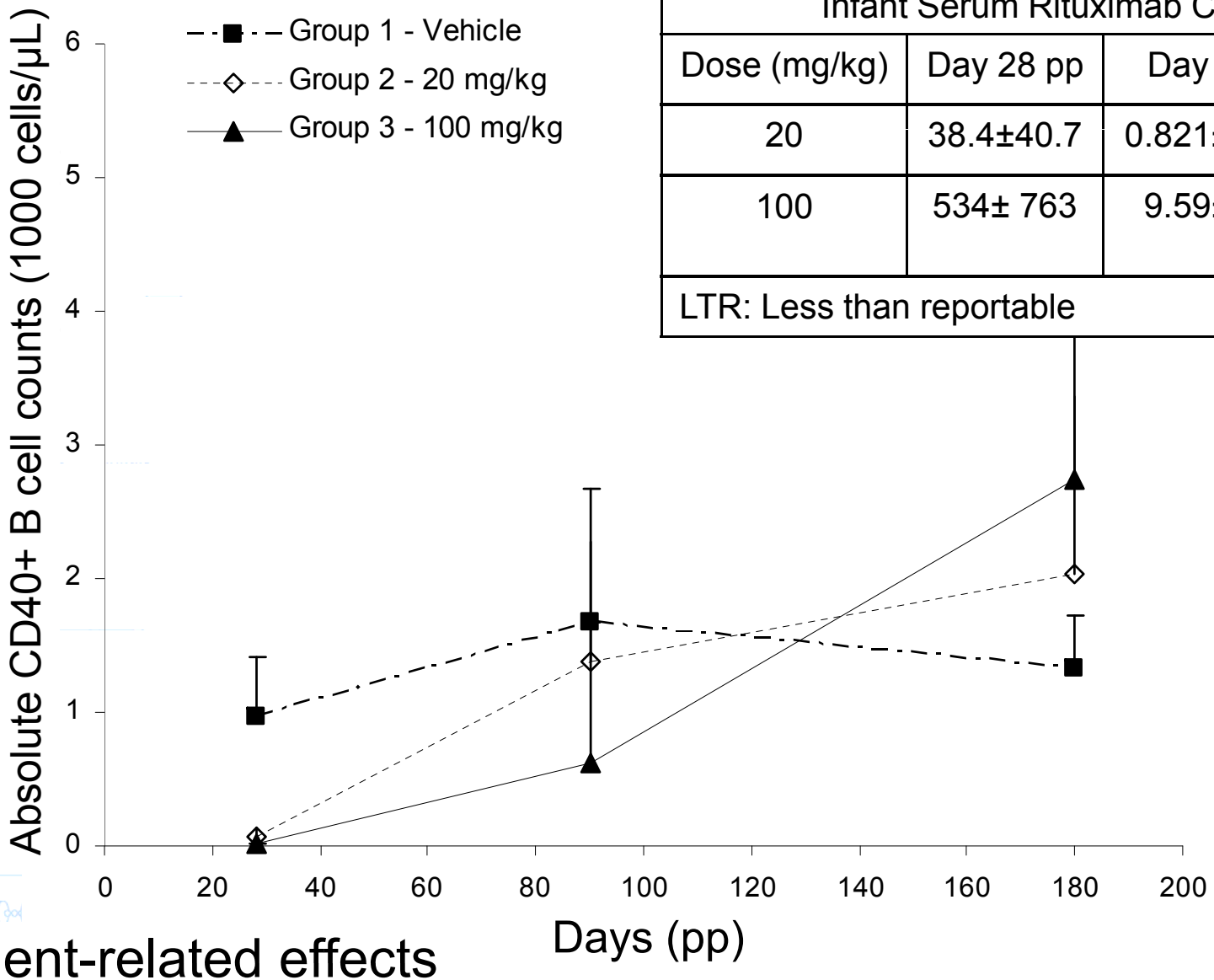
PK/PD Relationship (Maternal)



Infant B-cell Counts

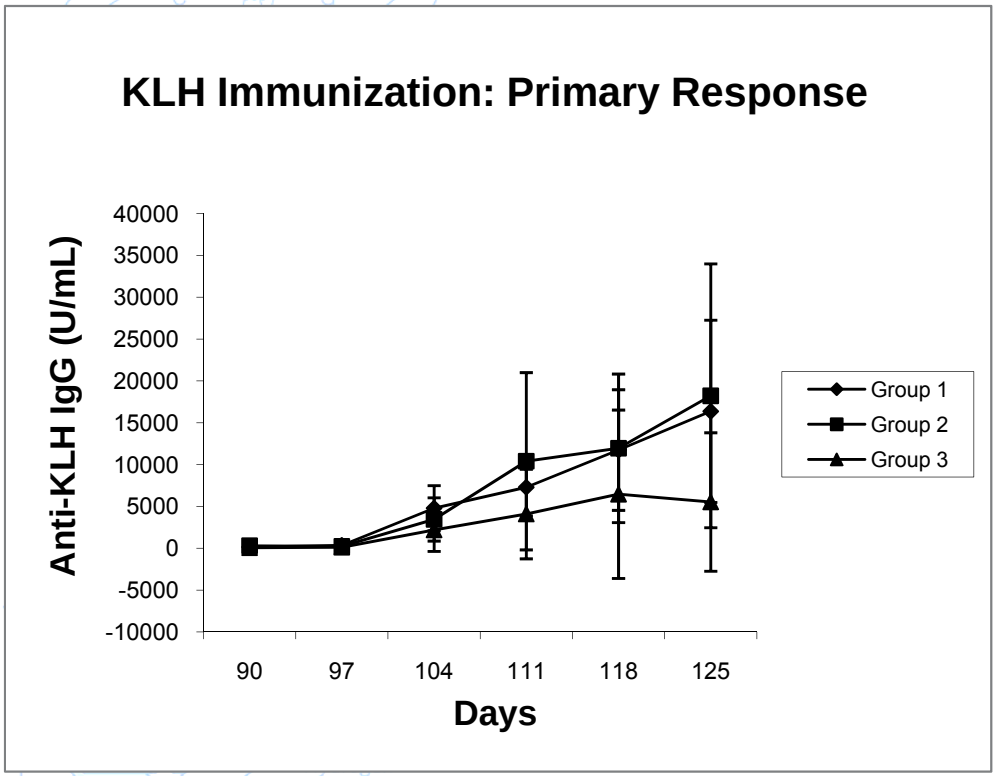
Infant Serum Rituximab Conc (µg/mL)			
Dose (mg/kg)	Day 28 pp	Day 90 pp	Day 180 pp
20	38.4±40.7	0.821± 0.476	LTR
100	534± 763	9.59± 8.44	LTR
LTR: Less than reportable			

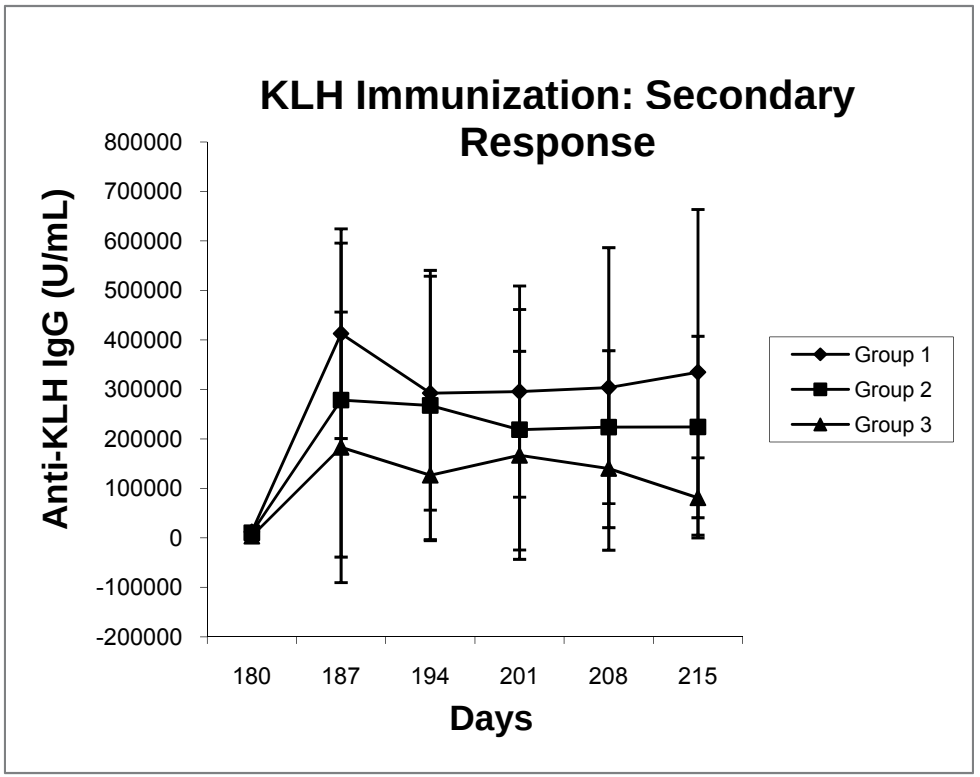


- Treatment-related effects
  -Flow cytometry: marked, dose-responsive B-cell depletion; B-cell repletion in all groups by Day 180 pp

Antigen Challenge in Infants

- Antigenic response to KLH (Days 90 & 180 pp): slight decrease in high dose groups, but within normal range of study control
- Initial administration of antigens at the time of PK/PD presence, could affect primary immune response resulting in a subsequently absent or muted secondary anamnestic response





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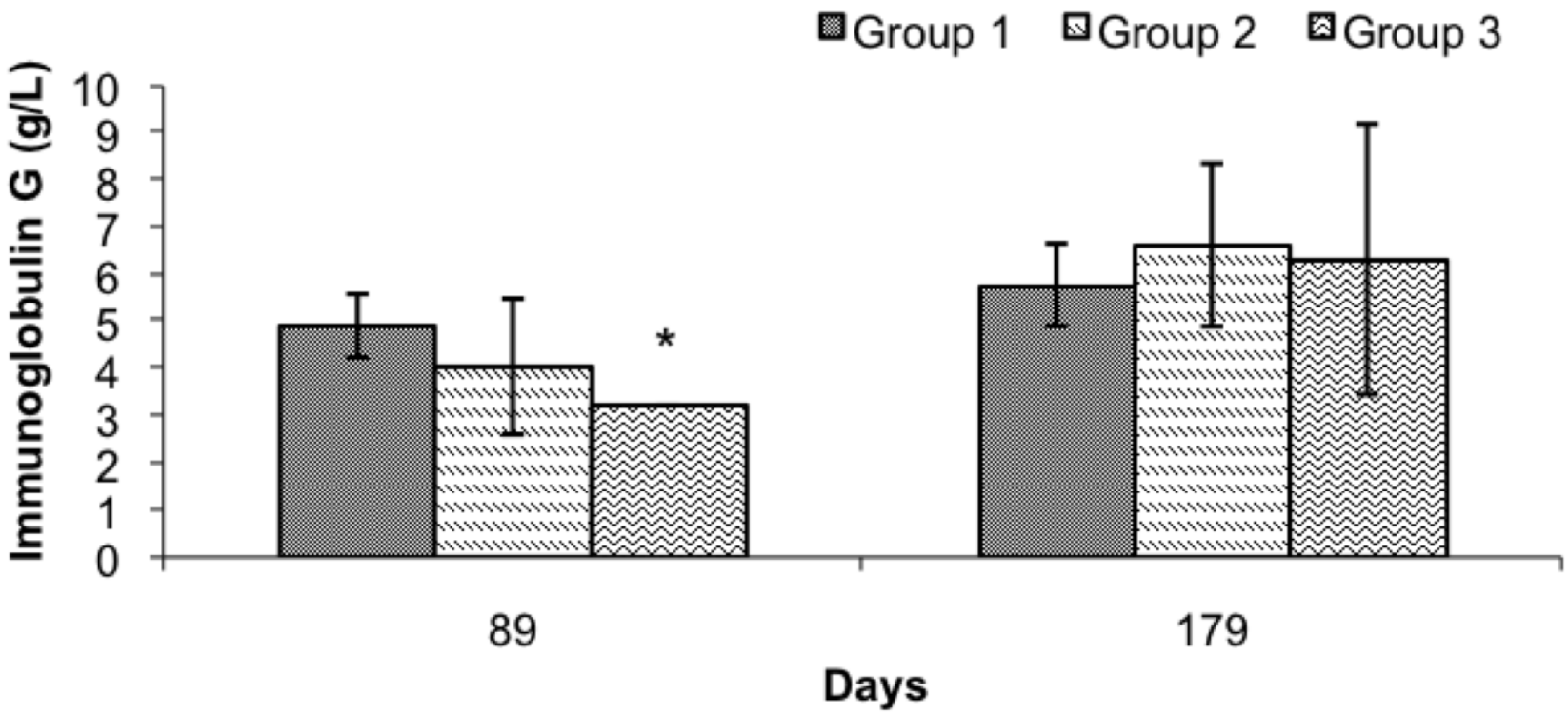
Infant IgG levels



* Statistically significant ($p<0.01$) to study controls

- Infant IgG levels significantly lower than control at Day 89 vs Day 179

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Summary of PPND Study

Noteworthy Findings: Maternal animals

- Effects limited to primary pharmacology of B-cell depletion
  - Complete repletion of B-cells by day 180 pp (except high dose group, trending toward repletion)
- Limited incidence of ATA

Noteworthy Findings: Infants

- Effects limited to primary pharmacology of B-cell depletion
  - B-cells lower than study control @ day 28 and 90 pp
  - **B-cells in all rituximab groups return to normal levels by day 179 pp**
- TK confirmed exposures (maternal and infant)
- Lactation transfer demonstrated
  - Rituximab levels in milk generally low (0.38-6.26 µg/mL)
- ATAs did not affect serum concentration
- Decreased IgG levels day 89 pp
  - Consequence of B-cell depletion
  - Normal levels by Day 179 pp (as compared to study control on Day 179)
- No organ wt changes; no IHC changes in CD markers
- No macroscopic or microscopic evidence of target organ toxicity

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Summary: Infant Immune Function Analysis

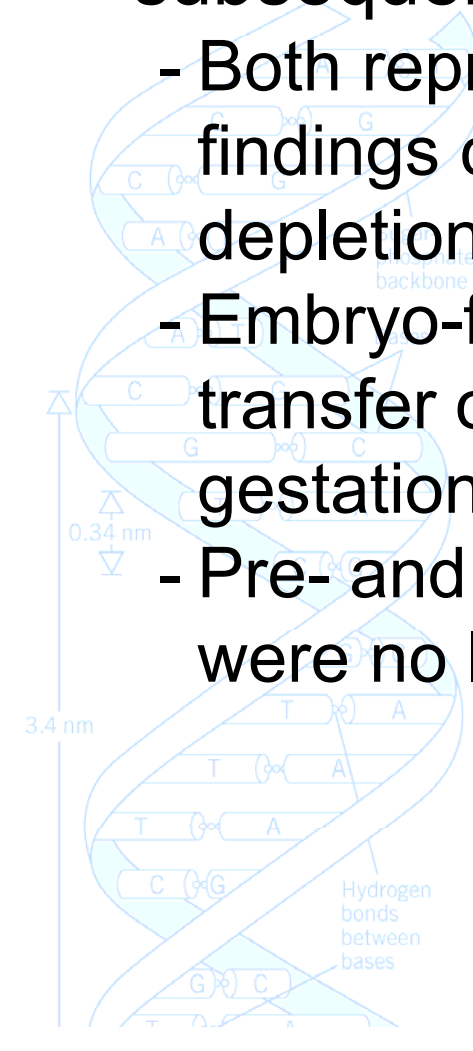
- Antigenic challenge
  - KLH
    - Antigenic response to KLH (Days 90 & 180 pp): slight decrease in high dose groups (measured by anti-KLH IgG responses), but within normal range
  - Daptacel™ immunization (Day 180)
    - No effect on humoral immune function, measured by anti-TT IgG and anti-TT IgM response (day 187-215)
    - No effect on T-cells based on T-cell proliferation assays
  - IL-2 and mitogen responsiveness

Conclusions

- Administration of rituximab at doses of 20 or 100 mg/kg throughout pregnancy & lactation produced the expected pharmacologic effect without evidence of overt maternal toxicity.
- Infants:
  - Findings consistent with expected pharmacology of B-cell depletion
  - No treatment-related adverse effects were observed on growth or development
  - B-cell depletion was apparent at birth, yet recovered to normal within 6 months
  - Immune function (KLH response) was mildly affected in the high dose treatment group, but recovered to normal within 6 months

Summary of Rituximab Reproductive/Developmental Studies

- Performance of the EFD study informed design of subsequent PPND study
  - Both reproductive/developmental toxicology studies had findings consistent with expected pharmacology of B-cell depletion.
  - Embryo-fetal development study demonstrated placental transfer of rituximab to fetus during the second trimester of gestation
  - Pre- and post-natal development study showed that there were no lasting effects on neonatal immune function



Predictivity of Nonclinical Data?

- Developmental immunotox data from rituximab nonclinical studies in cynomolgus monkeys match those from case reports in humans

	Diagnosis & treatment period	B cell recovery in infants	IgG, IgM, IgA	Vaccination status in infants
Case rpt #1 (*Lancet Oncol 2006; 7: 693–94)*	• Diagnosed at 15wks of pregnancy • 6 cycles of R-CHOP (q14days) • CR	• 1% of normal at birth • Normal by 12 wks	• Normal	• Normal at 8 mos • tetanus, diphtheria, pertussis, and hemophilus influenza
Case rpt #2 (*Haematologica 2006;91:1426-1427)*	• Diagnosed at 15wks of pregnancy • Rituxan weekly x4 & then R-CHOP (q3wks) until 37th wk of preg • CR	• Absent at birth • Normal by 18 weeks	• Normal	• Normal at 20 mos • tetanus, diphtheria, hepatitis B, measles, mumps and rubella
Cynomolgus Monkeys	• Rituxan weekly throughout pregnancy and through 1st month of lactation (~25 wks)	• Absent at birth • Normal by 3-6 mos	• Normal	• Normal at 6 mos • TT and KLH

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Lessons learned for future developmental immunotox studies with biologics

❖ The nonclinical studies are concordant with clinical outcome

- Immunogenicity may not be as great of a concern in pregnant animals
- Be selective about immune parameters and when to evaluate
  - KLH and Daptacel™ immunization
    - Consider MoA and half-life of mAb when administering antigen challenges
  - Immunophenotyping is critical
  - Rely on CROs to provide well validated methods for measuring TDAR responses
    - Some antigens do not stimulate as well as others
- Need large enough group sizes to account for a high spontaneous abortion/embryonic death rate
  - n=12 or greater pregnancies
  - Spontaneous abortions typically occur during early gestation

Acknowledgement

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- Noel Dybdal
- Tom Gelzleichter
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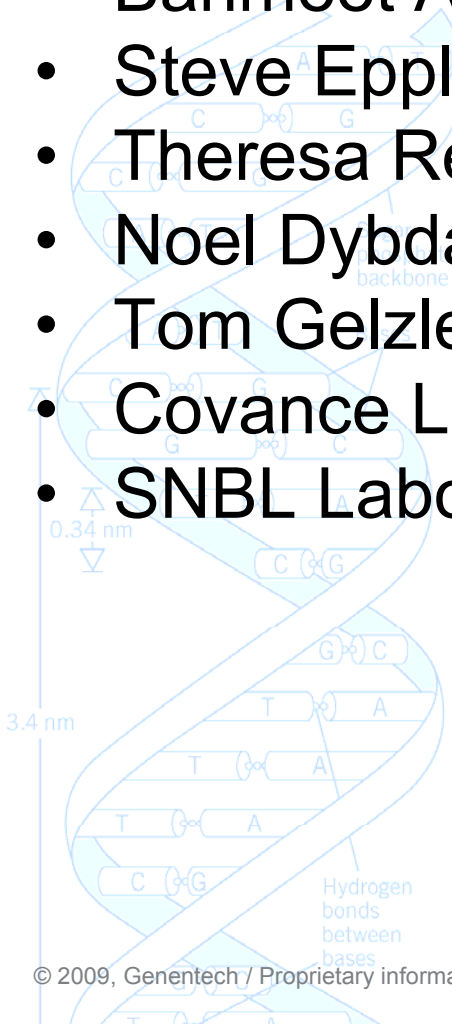
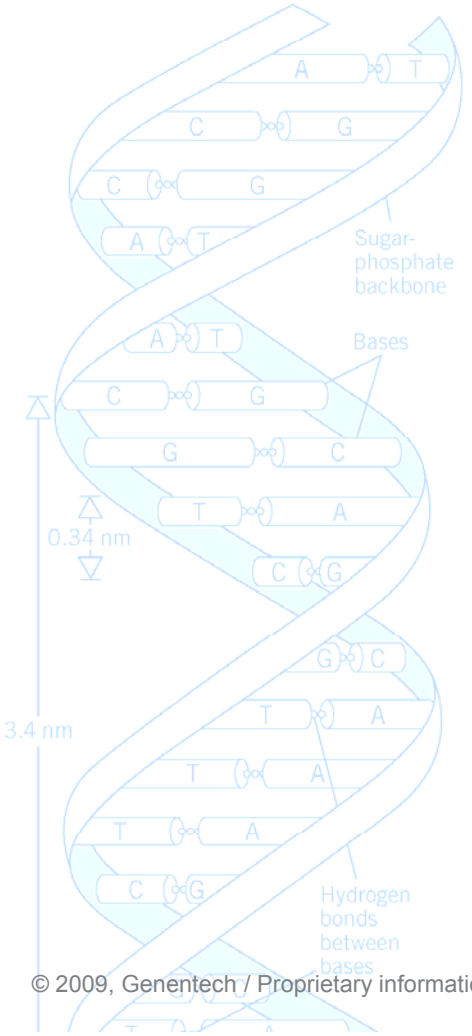
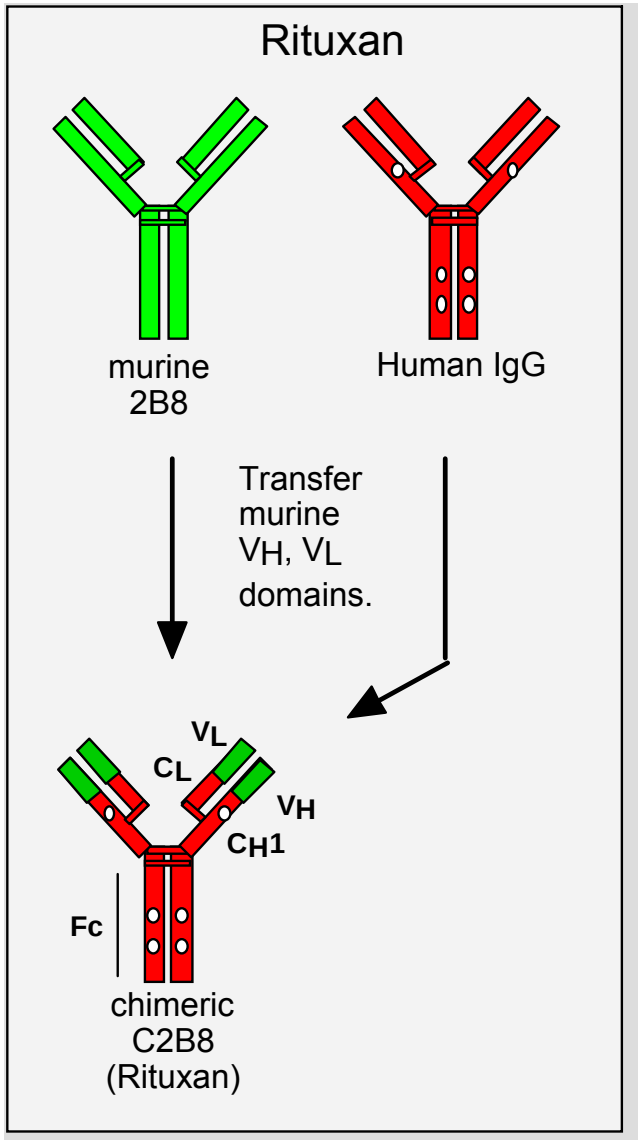
- SNBL Laboratories, Japan



Appendix



Antibody structure of chimeric rituximab

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