




Hollie S. Skaggs, PhD, DABT
Janssen Research and Development

Dorethey Gorham, *Day of the Armada*  Dorethey is a joyful, self-taught artist living with arthritis, general anxiety syndrome, and diabetes.



Introduction and Issue Statement

- Increased interest and need in developing this model for immunotoxicology testing during drug development
- Pig immunology is quite well defined.
 - Importance in food production.
 - Swine flu
 - Xenotransplantation
- The CONFIRM initiative is intended to trigger immunological safety research in Göttingen Minipigs, to assist and synergize fundamental, translational and regulatory investigative efforts relevant to the immunological safety evaluation of pharmaceuticals and biologics, and to spread current knowledge and new findings to the scientific and regulatory toxicology community.



History

- 2006 – RETHINK Project
- 2010 – 8 papers resulting from RETHINK Project
- 2012 – CsA/DEX Minipig Studies w/ ICH S8 Endpoints
(Penninks and van Mierlo, 2012)
- 2012 – Immunogenicity Testing w/IL-1 antag
(Van Mierlo et al 2012)
- 2013 - Immunogenicity Testing w/TNF α blocker
- 2013 – Refinement of the TDAR assay
(Peachee et al 2013)
- 2016 – “Minipigs in Translational Immunotoxicology: A Perspective”
(Rubic-Schneider et al 2016)
- 2018 – “Nonclinical Evaluation of Immunological Safety in Gottingen Minipigs: The CONFIRM Initiative”
(Descotes et al 2018)



Special Issue in February 2016

Examples of Registered Products that Used the Minipig During Development

Dermatology

- Altanax, Altargo® (GSK)

Cardiovascular

- Cordarone® (Wyeth Lab.)
- Coreg ® (GSK)

Osteoporosis/Arthritis

- Voltaren ®
- Fosamax ® (Merck & Co Inc.)
- Miacalcin ® (Novartis)
- Mobic ® (Boehringer Ingelheim)

Metabolic diseases

- Levemir ® (Novo Nordisk)

CNS

- Mirapex ® (Pharmacia & Upjohn)

Infectious Diseases

- Fuzeon® (Trimeris, Hoffmann La Roche)



Traditional Strategy to Assess Immunosafety

- Information from Standard Toxicology Studies
 - Hematology
 - Serum chemistry (e.g., globulins)
 - Lymphoid organ weight/histology
- Additional Assays
 - Non-functional assay (e.g., immunophenotyping, electrophoretic analysis of immunoglobulins)
 - Immune function (e.g., TDAR, NK cell activity, DTH, lymphocyte proliferation)
- Immunosafety Assessment of Biologics
 - The product itself
 - Allergic reactions
 - Immunogenicity
 - Autoimmunity
- Immunosafety Assessment of Vaccines



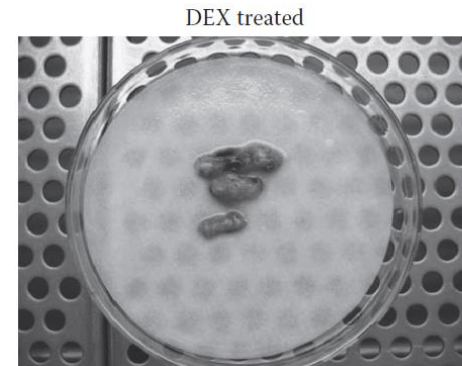
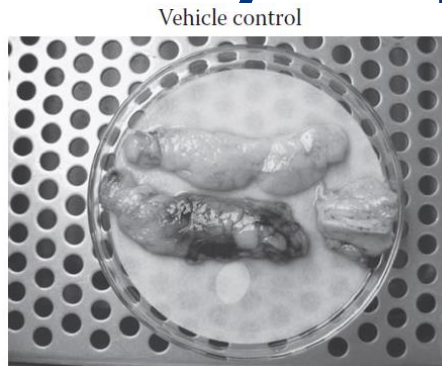
Studies Identified that Evaluated Immunotoxicity in the Minipig

- cyclosporine and dexamethasone
- adalimumab (TNF α antag), infliximab (TNF α antag)
- anakinra (IL-1R antag)
- *Small number of animals on test, fairly fundamental in their endpoints, no immune activators tested, limited cellular subpopulations explored*



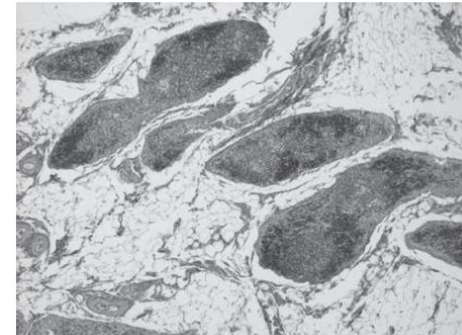
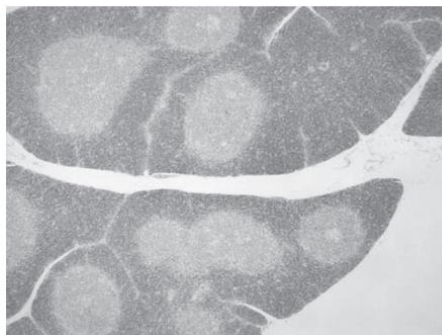
Standard Immunosafety Endpoints

Assay	CsA	DEX
Organ Weights		
Spleen weight	-	-
Thymus weight	↓	↓

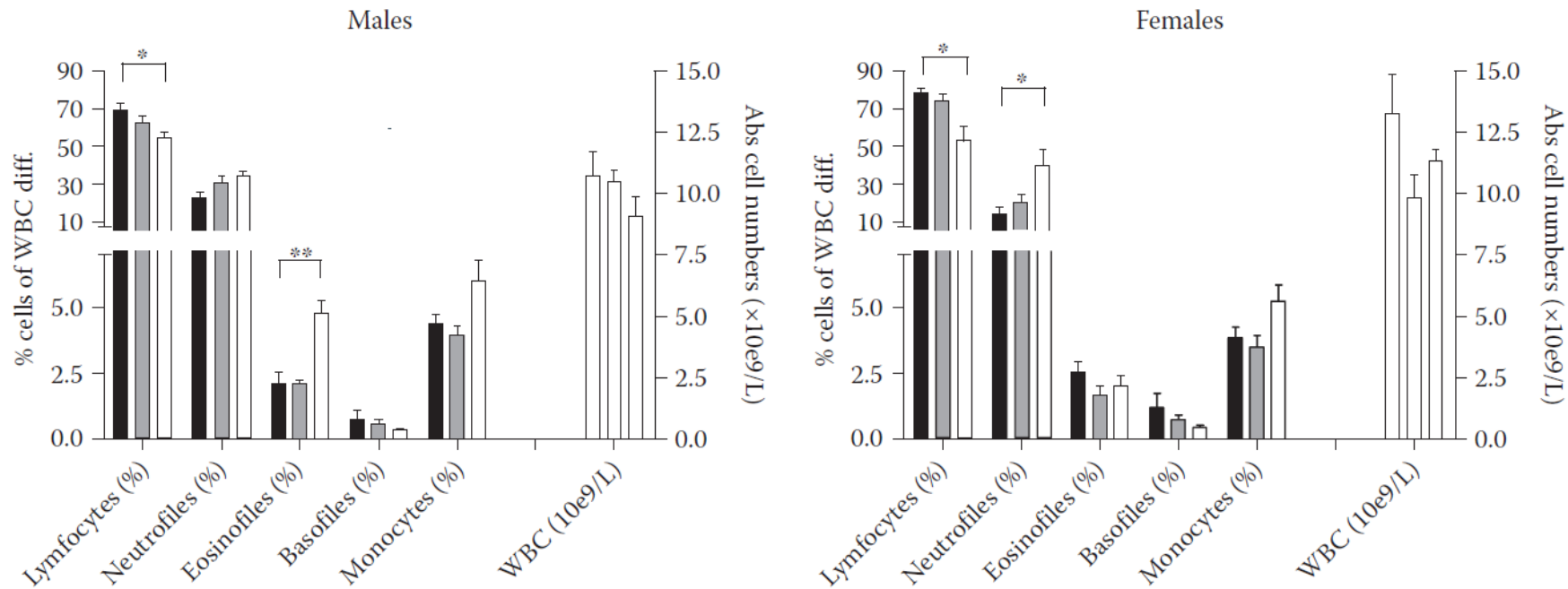


Histopathology

Marked decreased cortex to medulla ratio, due to a reduction in cortical thymocytes



Standard Immunosafety Endpoints



Black: Vehicle – Grey: CSA – White:Dex

Further Investigation: Non-functional Assays

- Immunophenotyping

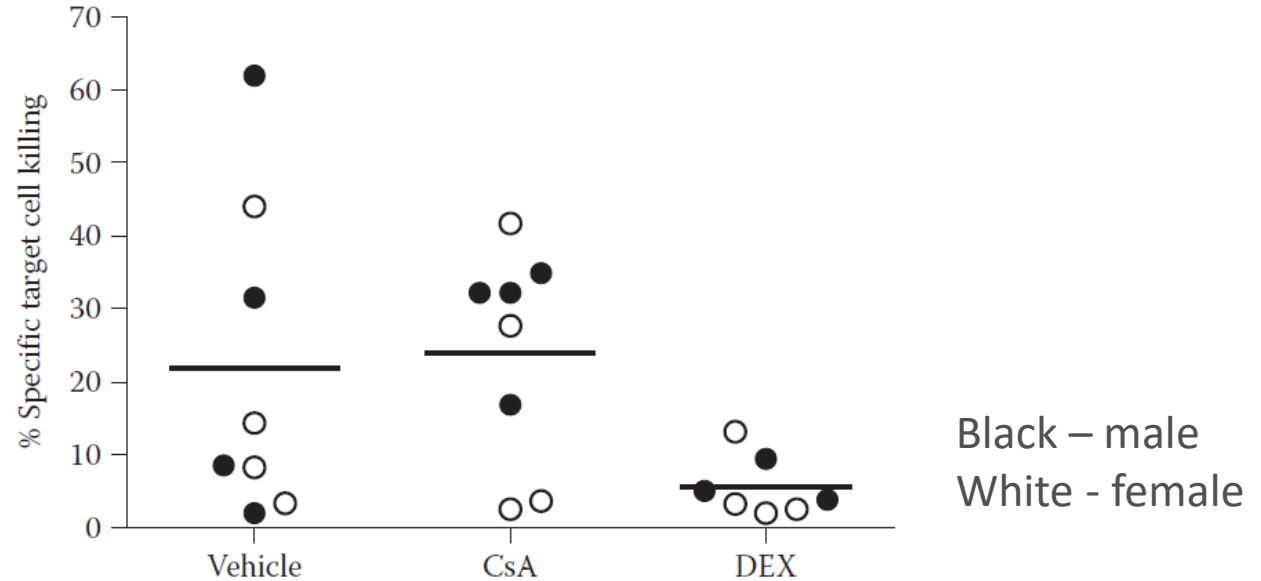
	CD3+(%)	CD3+/CD4+(%)	CD3+/CD8+(%)	CD4/CD8 Ratio ^a	CD4+/CD8+ (%)	B-Cells (%)
Day -1						
Vehicle	48.7 ± 13.7	14.0 ± 2.9	13.4 ± 5.8	1.2 ± 0.6	5.8 ± 5.8	5.5 ± 1.8
CsA	48.4 ± 7.3	14.0 ± 4.6	8.9 ± 3.9	1.7 ± 0.5	4.2 ± 4.1	5.5 ± 1.4
DEX	50.4 ± 7.1	13.1 ± 2.0	8.6 ± 2.7	1.6 ± 0.5	5.1 ± 2.0	6.9 ± 2.3
Day 27						
Vehicle	37.3 ± 12.2	7.1 ± 2.3	5.9 ± 2.8	1.3 ± 0.7	ND	18.8 ± 4.7
CsA	39.2 ± 8.7	11.8 ± 4.9	6.8 ± 3.5	1.4 ± 0.7	ND	23.7 ± 6.3
DEX	27.2 ± 5.5	8.7 ± 3.2	3.0 ± 1.7	1.8 ± 0.5	ND	19.3 ± 4.2

Note: Percentages of lymphocyte subsets in the blood on study days -1 and 27. Mean ± SD is shown. CsA, cyclosporin A; DEX, dexamethasone. ND: not determined (omitted to add anti-CD4).

^a Calculated from the CD4-PE and CD8-PE single staining.

Further Investigation: Functional Assays

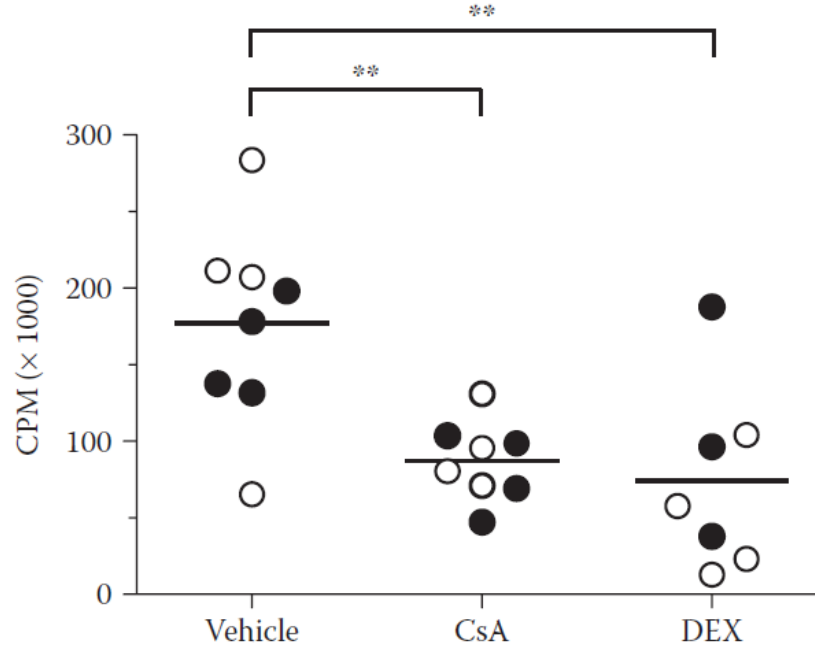
- NK cell assay



Penninks and van Mierlo, 2012, Peachee et al 2014

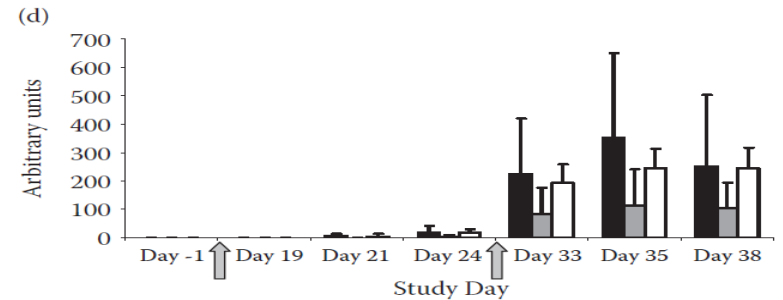
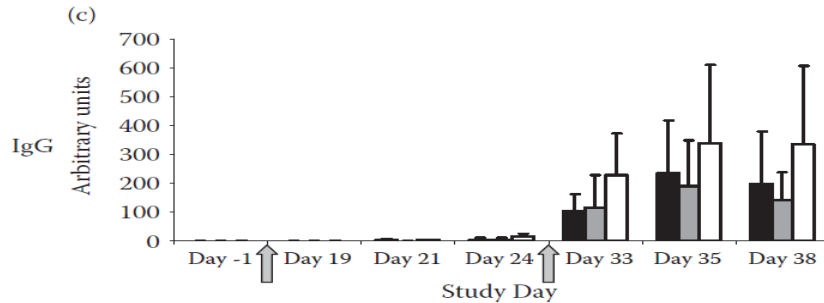
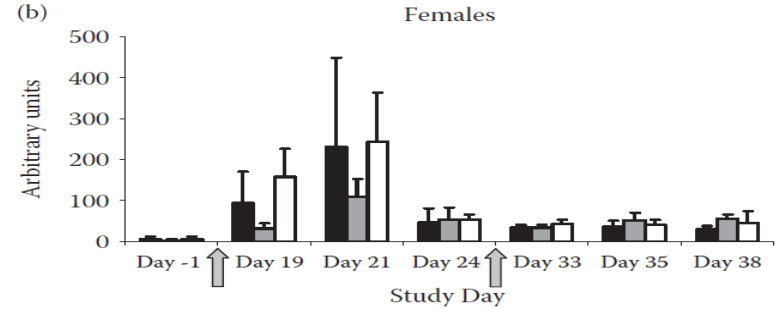
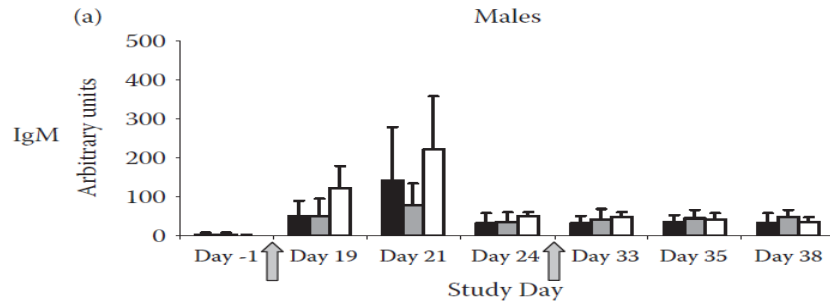
Further Investigation: Functional Assays

- Lymphocyte proliferation assay



Further Investigation: Functional Assays

- TDAR



Immunosafety of Biologics

- Anakinra (IL-1 receptor agonist)
 - No clinical or histological findings except injection site reaction, which were observed in both the vehicle and treatment group.
 - ADA detected at day 14.
 - Results were comparable to NHPs.
- Adalimumab and Infliximab (TNF α blocker)
 - No clinical or histological findings except injection site reaction.
 - Neutralizing ADA detected.
 - Results were comparable to NHPs.



Immunosafety of Vaccines

- Several studies demonstrating successful use of the minipig for the safety testing of vaccines.
 - HIV-1 vaccine with novel cationic adjuvant (2011)
 - Hep B vaccine for dermal vaccine delivery (2014)

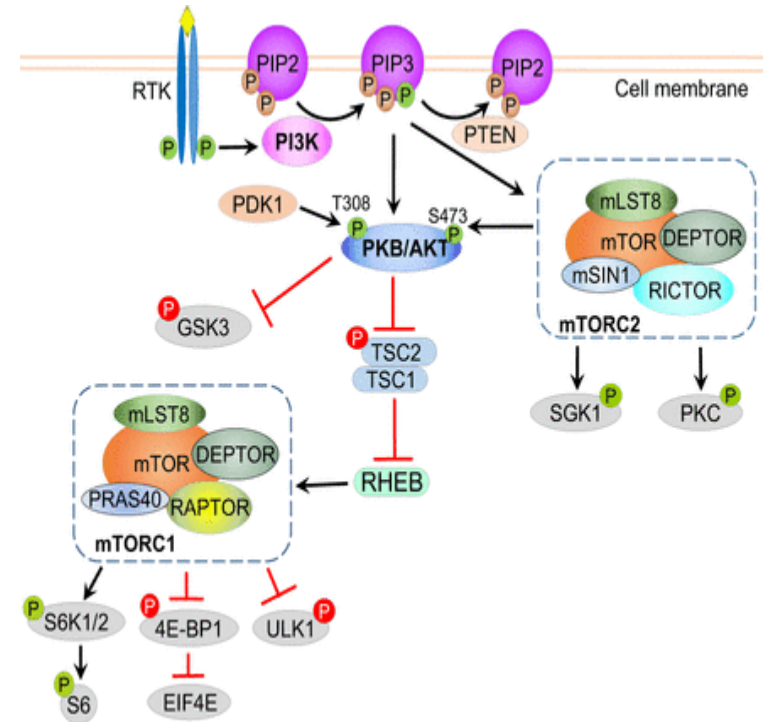


Summary

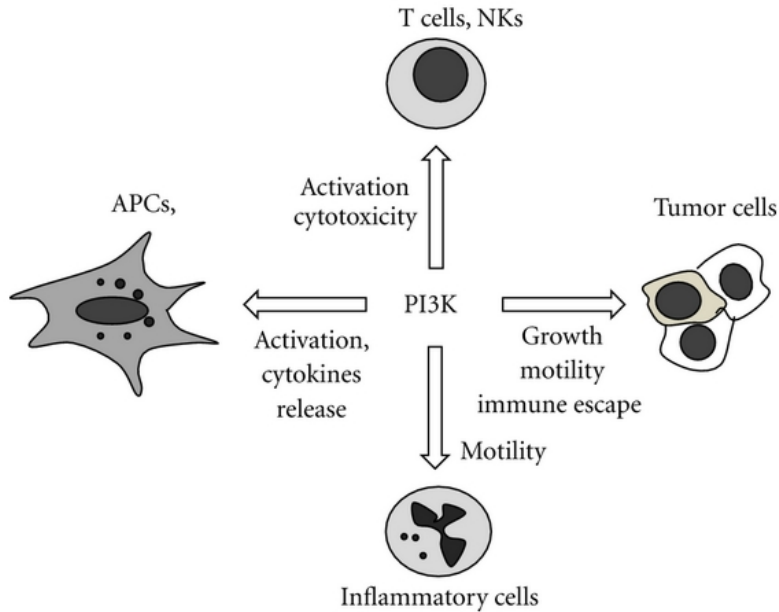
- Substantial knowledge of porcine immune system
- Need to confirm the findings and functionality in the minipig
- Initial evaluation of ICHS8 endpoints, in addition the immunosafety evaluation of biologics and vaccine, indicate the minipig is a potentially translationally relevant model of immunosafety evaluation

PI3Ks

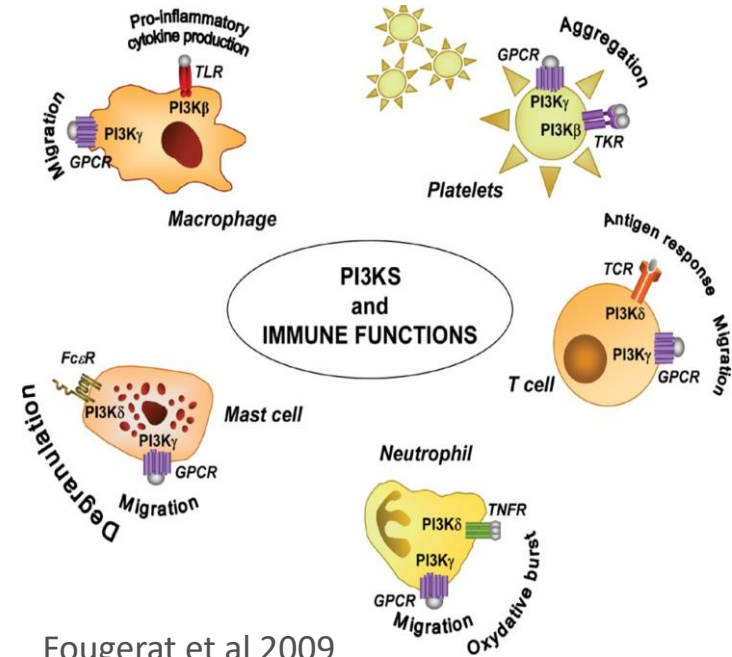
- Family of lipid kinases
- Phosphorylate the 3'-OH group of phosphatidylinositol lipids on the cell membranes
- Three classes (I, II, and III) based on the coding genes, structure and substrate preference.
 - Class IA – PI3K α , β , γ
 - Class IB - PI3K δ
- Idelalisib, Copanlisib, and JNJ-XYZ



PI3Ks in Immunity and Cancer Regulation



Dituri et al 2011



Fougerat et al 2009

Known Immunotoxicities with PI3K Inhibitors

Idelalisib (PI3K δ)

- Indications (US label): Chronic Lymphocytic Leukemia, Follicular B-cell non-Hodgkin Lymphoma, Small Lymphocytic Lymphoma
- Rat and Dog (SBA)
 - Inflammation in several tissues
 - Hematopoietic/lymphoid system
 - Skin - erythema, dryness, swelling, and redness
- Human (US label)
 - Anaphylaxis
 - Neutropenia
 - Fever
 - Rash

Known Immunotoxicities with PI3K Inhibitors

Copanlisib (pan PI3K)

- Indications (US label): relapsed follicular lymphoma
- Rat and dog (SBA)
 - Inflammation
 - Hematopoietic/lymphoid system
 - Skin – inflammation, reactive epidermal hyperplasia
- Human (US label)
 - Infections
 - Neutropenia
 - Rash

Case Study: JNJ-XYZ

- PI3K inhibitor for oncology (non-immunological)
- Need an appropriate large animal species to achieve appropriate exposure
- **Will the Gottingen minipig produce sufficient exposure to move forward as a large animal model?**
- **Does the Gottingen minipig produce similar immunotoxicological findings observed with other PI3K inhibitors?**

Case Study: JNJ-XYZ

2 male minipigs/group

Daily oral gavage for 6 days

Control and 2 dose groups (low and high)

Endpoints:

- In-life parameters (clin obs, BW, FC, BT)
- Clinical pathology (hematology, clinical chemistry, coagulation)
- Anatomic pathology (gross pathology, organ weights, and histopathology)

Case Study: JNJ-XYZ

- **Preterminally sacrificed HD male on Day 6**
 - Clinically: hypoactive, slight tremors, warm to touch, decreased weight and food consumption
 - Clinical Pathology:
 - Hematology
 - Increased WBC and neutrophils
 - Decreased retics, lymphocytes, eosinophils, basophils
 - Increased PT, APTT, and fibrinogen
 - Clin chem
 - Increased globulin
 - Decreased albumin
- **Toxicokinetics**
 - Same doses used for dog, NHP, and minipig
 - Day 0 values in minipig
 - Dog
 - $AUC_{0-24hr} - 10x$
 - $C_{max} - 5x$
 - NHP
 - $AUC_{0-24hr} - 3x$
 - $C_{max} - 6x$

Immunotoxicity Findings with JNJ-XYZ

	Mouse*	Minipig
Clinical		Warm to touch/increased body temperature Red discoloration of skin
Clinical Chemistry		Increased fibrin Decreased globulin
Hematological	Decreased lymphocytes	Increased WBCs and neutrophils Decreased lymphocytes
Histopathological	Decreased cellularity in marginal zone of spleen	Lymphoid depletion in the thymus, spleen, mesenteric lymph node, and Peyer's patches Multisystemic mixed cell infiltration Increased M/E ratio

*Exposure ~50% of minipig

Conclusion

- Minipig provided sufficient exposure as a large animal species.
- Standard immunotoxicological effects may have be target-related.
- Inflammatory reaction was consistent with other PI3K inhibitors in animals and in humans.
 - Project terminated prior to human studies.
- Fewer immunotoxicological effects in mice.

Overall Conclusions

- Minipigs are developing to be a translationally relevant immunosafety model
 - Additional work is needed
- The foundational work has been conducted in terms of standard immunosafety assessment.
- In a case study of a PI3K inhibitor, the minipig provided a more relevant model in terms of TK and toxicity.

