Minipigs or Primates in Safety Assessment of Biopharmaceuticals?

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Question?

The Minipig or Primate: That is the Question
Outline of Presentation

• General introduction into biologicals
• The minipig vs non-human primate
• The minipig in safety assessment
• Some conclusions
Regulatory Guidance

 Guidance for Industry ICH S6 Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals

- The ICHS6 (R1) covers a varied range of products:
  - monoclonal antibodies and antibody fragments
  - plasma or tissue derived proteins
  - recombinant DNA protein vaccines
  - recombinant peptides and proteins
  - oligonucleotide drugs
Small Molecule vs Biological

**Small Molecule**
- Chemically synthesised
- Low molecular weight
- May be metabolised to toxic intermediate
- Usually not immunogenic
- Can interact with multiple cells or organs
- Generally active in many species

**Biological**
- Derived from living cells
- High molecular weight
- Degraded to non-toxic amino acids or peptides
- May initiate immune response to “foreign” protein
- Usually highly targeted to specific cellular receptors
- Activity often limited to species possessing relevant receptors/mechanism of action
Regulatory Expectations for Biologicals

For small molecules and biological products the principles remain the same

- **Safety of the volunteer or patient**

• Use the non-clinical data to inform clinician on:
  - Safe starting dose and regimen
  - Dose escalation scheme
    - Estimate margin of safety between clinical and toxic dose
  - Target organs for toxicity
  - Human endpoints for clinical monitoring
  - Toxicity onset, reversal, and duration of monitoring
  - Predict pharmacokinetic and pharmacodynamic parameters
Regulatory Expectations for Biologicals

• ICH S6 (R1) guideline recognises that conventional approaches to toxicity studies used for small molecule drugs are often **NOT** appropriate for biologicals

• Does **NOT** provide a “one size fits all” or “cookbook” approach to toxicology study design
  
  – provides a framework for design of animal studies to address safety of biotechnology derived products, based on characteristics of the product and intended clinical use
How to Select the Appropriate Species for Non-clinical Safety Assessment?

- ICH S6 (R1) Addendum

A number of factors should be taken into account when determining species relevancy. Comparisons of target sequence homology between species can be an appropriate starting point, followed by in vitro assays to make qualitative and quantitative cross-species comparisons of relative target binding affinities and receptor/ligand occupancy and kinetics.

Assessments of functional activity are also recommended. Functional activity can be demonstrated in species-specific cell-based systems and/or in vivo pharmacology or toxicology studies. Modulation of a known biologic response or of a pharmacodynamic (PD) marker can provide evidence for functional activity to support species relevance.

Consideration of species differences in target binding and functional activity in the context of the intended dosing regime should provide confidence that a model is capable of demonstrating potentially adverse consequences of target modulation. When the target is expressed at very low levels in typical healthy preclinical species (e.g., inflammatory cytokines or tumor antigens), binding affinity and activity in cell-based systems can be sufficient to guide species selection.'
Species Selection

Non-human primate (NHP)

- Genetic and pharmacological similarity to humans
- Practicability/availability reasons cynomolgus monkey preferred primate species
- Marmoset and rhesus monkey occasionally used
- Pharmacological activity of biologicals in monkeys often resembles that in humans than dogs, rabbits and rodents
- Primate immune system is similar to humans
- Human/humanised monoclonal antibodies (mAbs) are less immunogenic following chronic dosing in primates than lower species

NHP identified as most suitable and relevant toxicology non-rodent species for biologicals
The Non-Human Primate

Relevance for safety assessment of human therapeutic antibodies in NHPs?

- Increasing public pressure to explore advance approaches to reduce NHP numbers
- Amgen reported differences in IgG and FcγR between human and cynomolgus monkey (Jacobsen et al., 2011)
  - Are there additional differences between NHPs and humans in terms of FcR expression, binding affinity, immune complexes of drug and anti-drug Abs, which can cause a multitude of effects ranging from accelerated drug clearance and reduced efficacy to more severe pathologic effects?
- Recently questioned why NHP used when pharmacological mediated adverse effects of mAbs are highly predictive from in vitro studies (Van Meer et al., 2013)
- NC3Rs initiatives to minimise the increase NHP use
  - How often can rodent models support biologic development?
  - How and when recovery animals should be included?
Ethical Concern

- NHP use will not be solved by introduction of minipig as an alternative non-rodent species
- Minipig’s capacity for pain and suffering is same as for NHP
- The minipig as a more acceptable experimental animal than NHP is rejected
  - Their use may prove less offensive to some groups within society at large
- Species selection must be made on a case-by-case basis
  - Benefits are assessed by weighing the scientific evidence
    - Predictability of the animal model against the harm that may accrue to the animals both from the test procedures and their lifetime experience within the laboratory environment

Webster et al., J Pharmacol Toxicol Methods 2010
What Are Pharmaceutical Companies Doing?

Key drivers of comparative biology

• Tissue and species specificity of target pathway biology
  – Target specificity, potency and off-target potential
  – Characterising PK/PD
  – Predicting safety liabilities
  – Opportunity for new indications
Comparative Biology at Molecular, Biochemical, Cellular and Tissue Levels

Integration of gene sequence, gene expression (e.g. splice variants, post translational modifications, sub-cellular localisation) and endogenous protein interactions & functions (e.g. cofactors, enzyme activity) at molecular/biochemical/cellular/tissue levels

(A adapted from J Moggs, Novartis)
In Silico Target/Epitope Sequence Comparison

Status Minipig at Roche

- The genome size/content of minipig is comparable to primates
- Tissue gene expression profiles in minipigs and humans are highly similar
- Variability of gene expression levels is comparable to other animal models

Figure from S Kronenberg, Roche
General Points

- Minipigs are readily available from reputable suppliers
- Health status and quality of minipigs
- Defined genetic and history background
# Minipigs in Biomedical Research

<table>
<thead>
<tr>
<th>Target Organs/ System</th>
<th>Indications</th>
<th>Drug Classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical (dermal)</td>
<td>• Acne vulgaris</td>
<td>• Antibiotics, lincosamides</td>
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<td></td>
<td>• Dermatitis, atopic</td>
<td>• Anti-infectives, topical</td>
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<td></td>
<td>• Hirsutism</td>
<td>• Anti-inflammatory</td>
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<td></td>
<td>• Hypopigmentation, facial</td>
<td>• Corticosteroids, topical</td>
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<td></td>
<td>• Impetigo</td>
<td>• Depigmenting agents</td>
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<td></td>
<td>• Keratosis, actinic</td>
<td>• Depilatory agents</td>
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<tr>
<td></td>
<td>• Lentigines, senile &amp; solar</td>
<td>• Dermatologic</td>
</tr>
<tr>
<td></td>
<td>• Melasma</td>
<td>• Immunosuppressant</td>
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<td></td>
<td>• Prevention of sunburn due to sun exposure</td>
<td>• Keratolytics</td>
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<td></td>
<td>• Psoriasis</td>
<td>• Photosensitizers</td>
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<td></td>
<td></td>
<td>• Retinoids</td>
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<tr>
<td></td>
<td></td>
<td>• Sunscreen products</td>
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<tr>
<td>Ocular</td>
<td>• Choroidal neovascularization</td>
<td>• Ophthalmics</td>
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<tr>
<td></td>
<td>• Histoplasmosis, ocular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Inflammation, ophthalmic, postoperative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Macular degeneration</td>
<td></td>
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<tr>
<td></td>
<td>• Myopia, pathologic</td>
<td></td>
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<tr>
<td></td>
<td>• Pain, ophthalmic</td>
<td></td>
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<tr>
<td></td>
<td>• Photophobia, postoperative</td>
<td></td>
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<tr>
<td>Target Organs/System</td>
<td>Indications</td>
<td>Drug Classes</td>
</tr>
<tr>
<td>------------------------------</td>
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</tbody>
</table>
| Cardiovascular               | • Arrhythmia, ventricular  
• Fibrillation, ventricular  
• Heart failure, congestive  
• Tachycardia, ventricular | • Antiadrenergics, beta blocking  
• Antiarrhythmics, class III | |
| Anaesthesia / analgesia      | • Anaesthesia (infiltration, local, regional)  
• Pain, mild to moderate  
• Dysmenorrhoea            | • Analgesics, non-narcotic  
• Anaesthetics, local amide-type | |
| Bone (calcium metabolism)    | • Hypercalcaemia  
• Osteoporosis  
• Paget’s disease       | • Bisphosphonates  
• Hormone/hormone modifiers | |
| Arthritides                  | • Ankylosing spondylitis  
• Arthritis, osteoarthritis & rheumatoid | • Analgesics, non-narcotic  
• NSAIDS | |
| Central nervous system       | • Parkinson’s disease, early-stage idiopathic | • Antiparkinson agents  
• Dopaminergics | |
| Transplantation              | • Rejection, heart, liver, renal transplant, prophylaxis | • Immunosuppressives | |
| Other                        | • Hypertension, essential  
• Imaging, cardiac  
• Infection, human immunodeficiency virus  
• Infection, Trypanosoma brucei gambiense | • Antiprotozoals  
• Antivirals  
• Diagnostics, radiopharmaceuticals  
• Fusion inhibitors  
• Vasodilators |
## Minipigs in Biomedical Research

<table>
<thead>
<tr>
<th>Market drug (Active ingredient)</th>
<th>Indication</th>
<th>Manufacturer</th>
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<tbody>
<tr>
<td>Actonel (Risedronate sodium)</td>
<td>Osteoporosis</td>
<td>Warner Chilcott</td>
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<tr>
<td>Altabax (Retapamulin)</td>
<td>Impetigo</td>
<td>GSK</td>
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<tr>
<td>Clobex (Clobetasol propionate)</td>
<td>Psoriasis</td>
<td>Galderma</td>
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<tr>
<td>Cordarone (Amiodarone HCl)</td>
<td>Arrhythmias</td>
<td>Sanofi Aventis</td>
</tr>
<tr>
<td>Dovonex (Calcipotriol)</td>
<td>Psoriasis</td>
<td>LEO</td>
</tr>
<tr>
<td>Elidel (Pimecrolimus)</td>
<td>Atopic dermatitis</td>
<td>Novartis</td>
</tr>
<tr>
<td>Mirapex (Pramipexole dihydrochloride)</td>
<td>Parkinson’s Disease</td>
<td>Boehringer Ingelheim</td>
</tr>
<tr>
<td>Mobic (Meloxicam)</td>
<td>NSAIDs</td>
<td>Boehringer Ingelheim</td>
</tr>
<tr>
<td>Tazorac (Tazarotene)</td>
<td>Psoriasis/acet</td>
<td>Allergan</td>
</tr>
<tr>
<td>Temovate (Clobetasol propionate)</td>
<td>Eczema &amp; psoriasis</td>
<td>GSK</td>
</tr>
<tr>
<td>Vaniqa (Eflornithine HCl)</td>
<td>Excessive facial hair growth</td>
<td>Shire</td>
</tr>
</tbody>
</table>
Minipig – The Alternative Non-Rodent Species

**Small molecule** - pharmaceuticals
- Increasingly used
- Well accepted for dermal products

**Biopharmaceuticals**
- Used for several therapeutic proteins/peptides not for monoclonal antibodies
- Potential reasons:
  - Test item demand and lack of experience in this species
  - Perceived associated scientific and regulator risk

Adapted from S Kronenberg, Roche
Göttingen Minipig Bodyweight Profile

I maybe cute but I demand more test compound than the NHP!!

- Male sexually mature at 3-4 m
- Female sexually mature at 4-5 m
- Skeletal development completed
- Adult weight around 35 kg
Cynomolgus Monkey Bodyweight Profile

Ribeiro Andrade et al., Mem Inst Oswaldo Cruz, Rio de Janeiro, 2004
Minipig – The Alternative Non-Rodent Species

What are the similarities to humans?

- Gastro-intestinal tract
- Cardiovascular system
- Structure of the skin
- Kidney
- Reproductive system
- Endocrine glands
- Liver
- Eye
- Respiratory tract
- Immune system
- Bones and joints

Images from Google
Swine Immune System

• Subject of substantial research efforts driven by economic importance in agriculture
• Well studied and is probably better characterised than that of the dog or monkey
• Multiple databases of pig immunology resources available (Bode et al., J Pharmacol Tox Methods 2010)
Pig immune system shows analogous structure and function to human immune system

- Some immunological species-specific features
  - Pigs have an inverted lymph node structure and an unusual route for lymphocyte circulation
  - Relatively high numbers of extra-thymic CD4/CD8 double positive T-cells and resting T-cells expressing *Swine Leukocyte Antigen* class II molecules
  - Pigs can have high numbers of natural killer cells and γδ cells
  - Harbour an unusual diversity of B-cell and antibody repertoire development
  - Highly heritable variation in immune cell parameters

Differences do not appear to represent biological impact on the overall functioning of the immune system

*Dawson et al., BMC Genomics 2013*
Swine Lymph Node Structure

- Lymph node is inverted compared with other animals
- Typical composition of cortex and medulla are reversed with the germinal centers in the middle of the gland
- The outer zone contains lymphocytes, macrophages and plasma cells
- The germinal centres have a lymphatic corona, and a paracortex with typical high endothelial venules
Swine Immunoglobulins

- Four different isotypes of immunoglobulins in pigs: IgM, IgG, IgA and IgE
- Porcine IgD not found
- None of these immunoglobulins are able to pass through the placenta
- Immunoglobulin transference from the mother to the foetus only takes place through the colostrum
- Piglets absorb immunoglobulins in their intestine and later on these immunoglobulins reach the serum

### Porcine immunoglobulin concentrations

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
<th>IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>17-29</td>
<td>1-5</td>
<td>0.5-5</td>
<td>-</td>
</tr>
<tr>
<td>Milk</td>
<td>1-3</td>
<td>0.3-0.9</td>
<td>3-7</td>
<td>-</td>
</tr>
<tr>
<td>Colostrum</td>
<td>30-70</td>
<td>2.5-3.2</td>
<td>9.5-10.5</td>
<td>-</td>
</tr>
</tbody>
</table>

Bode et al., J Pharmacol Tox Methods 2010
Swine Immunity – Transfer of Immunity

- Receptor-mediated transport of IgG occurs
  - Group I via placenta
  - Group III via mammary gland
- Differences reflect in immunoglobulin constituency of colostrum
- Uptake of suckling neonate differs among mammals
  - rodents mediated by FcRn transports IgG
  - pig is receptor independent

Placental transfer of human IgG/role of FcRn not known for Minipig – Work in progress

Butler et al., Vet Immun Immunopath 2009
Swine Placenta Structure

Lack of placental transfer of macromolecules due to tight placental barrier in minipig may limit their role in developmental toxicity testing of mAbs

Figure from S Kronenberg, Roche (from DeSesso et al., Crit Rev Toxicol. 2012)
Swine Immune System

- Lymphocyte subsets defined and reagents available for principal porcine lymphocyte populations (AbD Serotec®)
  - Anti porcine CD4a, CD8a and CD25 and anti human cross-reactive CD3 for porcine T cell research
  - Antibodies for porcine dendritic cell research against CD163, CD172a (SIRP-alpha), SLA Class II DQ and DR
  - Anti porcine CD31 (PECAM-1) and clone MIL11 for endothelial cell research
  - Anti porcine CD11R3, CD14 and CD16 for monocyte and macrophage research
  - Anti porcine CD5 and CD79a, B cell markers

- Luminex multiplex swine cytokine
  - ProcartaPlex Porcine Cytokine & Chemokine Panel
  - 9 cytokines detected
    - IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p40, IFN-α, TNF-α, IFN-γ
Swine Immune System

Gaps in our knowledge

- Role and characterisation of effector function
  - What is the affinity of human IgG to swine FcγR and/or different expression levels of FcγR immune cell population?
- Sensitivity for cytokine release in the minipig
- Lack of some immuno-monitoring tools
Pharmacokinetics

- Binding to the neonatal Fc receptor (FcRn) protects circulating IgG from systemic elimination by recycling.

- Altering the binding affinity of IgG to FcRn can impact PK of mAb.
Pharmacokinetics

**Work at Genentech and Roche**

- Several human mAbs after intravenous and subcutaneous administration in minipig showed IgG like PK properties (low clearance, long half-life and low volume distribution)
- Binding affinities of human IgG molecules tested were similar across 3 species

*Provides additional rationale for minipig and cynomolgus monkey use as translatable species for mAb PK*

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Figure from S Kronenberg, Roche
Physiological consequences of Complement activation-related pseudoallergy (CARPA)

- Minipigs show acute cardio-pulmonary reactions to intravenous polymer excipients in biological formulations due to complement activation (Szebeni et al., Adv Drug Deliver Rev 2012)
- Not reproduced in human
- No pseudoallergic reaction to 0.1% Polysorbate 20 and 80 or Poloxamer 188 (Festag et al., MRF 2013)

Supports use of minipigs using typical mAb formulations
Minipig in Safety Assessment

Routes of Administration

• Injection
  – Intramuscular or subcutaneous

• Intravenous
  – Bolus (use ear veins for single dosing and repeated intravenous dosing; surgical intravenous access is recommended)
  – Continuous infusion
The Minipig in Safety Assessment

Routine observations

- Clinical signs
- Body weights
- Food and water consumption
- Ophthalmoscopy
- Integration of safety pharmacology end points
  - Electrocardiography (use axial lead system “Nebh-Spörri” - more precise and consistent measurements)
  - Jacketed telemetry
  - Blood pressure
- Organ weights

All endpoints in minipig as in NHP
The Minipig in Safety Assessment

- Clinical Pathology
  - Haematology, coagulation and clinical chemistry parameters
  - Background data available at CROs
  - Automated standard methods
  - Standard biomarkers available

- Pathology/Histopathology
  - Background data at CROs

- Standard clinical pathology established
- Historical background pathology data available
But still a lack of published experience with biologics
In conclusion

- Minipig recognised as suitable non-rodent species associated with efficacy and safety assessment of small molecules and certain types of biologicals
- Minipig is largely physiologically similar to primates
- There is sufficient background data now available to allow studies to be interpreted
- Unanswered questions regarding IgG placental transfer, cytokine release and effector function of IgGs
- Body weight issues leading to more test item than NHPs
In conclusion

• Limited experience on risk of immunogenicity for human proteins
• Technical and logistical practicalities of intravenous dosing
• Improvement of immunological tools (e.g. better marker for B cell determination)
• No published regulatory studies with mAbs using the minipig
• If more studies/projects published probably have a different impression of minipigs in biological testing
• Despite above challenges the minipig could serve in future as a potential alternative species where NHP is less appropriate
Acknowledgement

Sven Kronenberg and colleagues at F. Hoffmann-La Roche Limited, Basel, Switzerland
Question?

OR

The Primate: That is the answer for now!
"That's all folks!"