Juvenile toxicity studies with biopharmaceuticals: considerations and current practices

Shaun Maguire
Toxicologist, Biologics Safety Assessment, MedImmune, Cambridge, UK
maguires@medimmune.com

AGAH Workshop: Critical aspects of integrated drug development – expect the unexpected
Munich 29 Nov 2014
Considerations impacting the need and design of juvenile animal toxicity studies

Juvenile Toxicity Testing of Biologics
Biopharmaceuticals are different: Impact on approach to evaluation of biopharmaceuticals in juvenile animals

NHP: Enhanced PPND Study Design Study
NHP: Juvenile Toxicology Studies
Considerations……
Some key questions to address when designing juvenile animal studies

- The relevance of the animal model
- The sensitivity of the animal model (to the drug, drug class or a particular toxicity)
- The ability of animal model to produce reliable and reproducible results
- Overall feasibility of using the animal model in a nonclinical safety evaluation study
- Understanding of developmental stages of the target organ(s) in the animal model as it relates to the paediatric population
  - Ensure a comparison can be made with relevant paediatric age groups
Key considerations impacting the need and design of juvenile animal toxicity studies

- Age of population in clinical paediatric program
- Duration of treatment (Acute vs Chronic)
- Pharmacology (mode of action)
- Class history of effects on developing systems
- Known adult target organs in adult clinical program
- Known adult targets organs in adult toxicity assessments
- Identified reproductive / developmental toxicity
- Pharmacokinetic and metabolism data in adult animals and human
- Route of administration
- Unique formulation requirements / novel excipients

→ Develop rationale regarding the need for a study
Do we need to do a Juvenile Study to support the clinical plan??

Justify need for a non clinical study!

Prepare rationale for regulatory interactions
What is the likelihood of conducting a nonclinical Juvenile toxicity study?

Class history of effects on developing systems

Target organs are late developing

Age ≤ 11 years

Age dependant metabolism

Age ≤ 2 years

Age ≥ 12 years

Chronic therapy

Acute therapy

Subchronic therapy

Age ≤ 4 years
What is the likelihood of conducting a nonclinical Juvenile toxicity study?

Class history of effects on developing systems
Age ≤ 2 years
Target organs are late developing
Age ≤ 4 years
Age dependant metabolism
Chronic therapy
Age ≤ 11 years
Subchronic therapy
Acute therapy
Age ≥ 12 years
Juvenile Toxicity Testing of Biologics
### Box 1 | Range of biotech-product classes

- **Hormones**
  - Growth hormone, insulin (analogaues) and erythropoietin

- **Blood products**
  - Albumin, thrombolytics, fibrinolytics and clotting factors

- **Cytokines and growth factors**
  - Interferons, interleukins and colony-stimulating factors

- **Antagonists/inhibitors**
  - Soluble receptors

- **Monoclonal antibodies and related products**
  - Mouse, chimeric or humanized; whole molecule or fragment; single chain or bispecific; and naked or conjugated

- **Modified human proteins**
  - Fusion (IgFc), polyethylene glycol (PEG)ylation, liposome encapsulation and drug-toxin conjugate

- **Vaccines**
  - Recombinant proteins or peptides, DNA plasmid and anti-idiotypic

- **Gene-transfer products**
  - Viral and non-viral vector-delivery systems and DNA–RNA chimaeras

- **Cell-based therapies**
  - Autologous, allogeneic and xenogeneic

- **Tissue-engineered products**
  - Cells, tissues, naturally occurring/synthetic biomaterials, extracorporeal and long-term implants

- **Wide range of different molecular entities**
## Some General Differences Between Small Molecule Drugs and Biologics

<table>
<thead>
<tr>
<th>Small molecule drugs</th>
<th>Biologics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species ‘independent’</td>
<td>Species specific</td>
</tr>
<tr>
<td>Non-immunogenic</td>
<td>Immunogenic</td>
</tr>
<tr>
<td>Metabolised</td>
<td>Degraded/catabolised</td>
</tr>
<tr>
<td>Short acting</td>
<td>Long acting</td>
</tr>
<tr>
<td>Chronic daily dosing</td>
<td>Intermittent dosing</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Exaggerated pharmacology</td>
</tr>
<tr>
<td>Linear dose response</td>
<td>Linear/bell-shaped dose response</td>
</tr>
<tr>
<td>Direct effects</td>
<td>Complex temporal effects</td>
</tr>
<tr>
<td>Complex formulations</td>
<td>Simple formulations</td>
</tr>
<tr>
<td>Typically oral route</td>
<td>Parenteral routes</td>
</tr>
</tbody>
</table>

Biologics toxicity usually understood: exaggerated pharmacology, not off-target effects

Adapted from [Cavagnaro, 2002](Cavagnaro.2002)
Biopharmaceuticals are different: Impact on approach to evaluation of biopharmaceuticals in juvenile animals

- Species Selection Cross-Reactivity
- Dosage Selection
- Immunogenicity
- PK/TK Distribution and Elimination
- Pharmacodynamics
ICH S6(R1): Guiding Principles for Species Selection for Nonclinical Safety Studies with Biologics

- 2 species toxicology is required - 1 rodent + 1 non-rodent
  - Only pharmacologically relevant species should be used

- Pharmacological relevance based on:
  - Target sequence homology/identity, expression of receptor or epitope
  - *In vitro* binding affinity, receptor occupancy, on/off rate vs human
  - *In vitro* potency/bioactivity vs human
  - Pharmacological or pharmacodynamic activity *in vivo*

- Single species toxicology program is acceptable if only 1 relevant species can be identified

⇒ Key principle is that species relevance or irrelevance needs to be formally demonstrated
Species Selection / Cross-reactivity

- Need to demonstrate pharmacologic relevance
  - Small molecules key concern is ADME
  - Even in the absence of pharmacologic activity a selected species could provide an assessment of toxicity (off target).
- Owing to high target specificity (esp mAbs) often restricted in their pharmacologic activity
- Lack of concern for off target activity driven acceptability of single species – if pharmacologically active across multiple species then testing required in two species (rodent/non-rodent)

- For Juvenile studies same rationale
Dose Selection

- Dose selection differ from biopharmaceutical vs small molecule
  - Small molecules: MTD or 2g/kg
- Owing to high target specificity, toxicity is a function of exaggerated Pharmacology and is often limited
- High dose usually selected to achieve multiple of the highest projected clinical dose or max feasible dose
- Allows assessment of exaggerated pharmacology without confounding nonspecific tox due to high amounts of protein/antibody

- Juvenile animal dose selection can be an issue for small molecules (differing regulatory expectations)
- For biopharmaceuticals a similar philosophy as adult chronic tox assessment should be used
Immunogenicity

- Anti Drug Antibody (ADA) can impact assessment of toxicity
- Number factors influence ability to elicit ADA
  - Route / dose levels and interval

- ADA response can result in decreased exposure through enhanced clearance via ADA-complex or diminished pharmacologic effect (neutralising ADA)
- ADA response may limit the value of assessments conducted
- Other toxicity-related issues with ADA
  - Hypersensitivity reactions, nephrotoxicity

- **For Juvenile studies** as adult toxicity studies, an ADA response may impact interpretation.
- For studies in very young animals mature pattern of immune response may not exist
PK/TK Distribution and Elimination

- For small molecules ontogeny of metabolising and transport systems (eg p450) key role in understanding toxicity and efficacy
- Age affects expression and function of these systems which can lead to alterations in PK and elimination
- For mAbs number of factors can influence PK including:
  - antigen properties (soluble vs membrane bound)
  - mAb format and protein engineering.
  - ADA can also affect kinetics
- IgG homeostatis neonatal FcRn
Pharmacodynamics

- Need to establish species relevance including a pharmacodynamic response

- An added consideration in testing of biopharmaceuticals in Juvenile animals is age
  - At what age in development is this a pharmacologically relevant species?
  - How does this compare to Human? Would assume the clinical plan designed around the appropriate age group

- Testing of a biopharmaceutical in juvenile animal where the target is only expressed in older animals may be irrelevant?
Strategy for assessing the preclinical safety of biopharmaceuticals in juvenile animals
Strategy for assessing the preclinical safety of juvenile animals

- Goal of the strategy is to design a preclinical development plan that adequately addresses safety concerns for the intended paediatric population.

- Guidelines (EU/US/Japan) for Juvenile testing

Guidance for Industry
Nonclinical Safety Evaluation of Pediatric Drug Products

London, 24 January 2008
Doc. Ref. EMEA/CHMP/SWP/169215/2005

COMMITTEE FOR HUMAN MEDICINAL PRODUCTS (CHMP)

GUIDELINE ON THE NEED FOR NON-CLINICAL TESTING IN JUVENILE ANIMALS OF PHARMACEUTICALS FOR PAEDIATRIC INDICATIONS

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
February 2006
Pharmacology and Toxicology
ICH guideline M3(R2) on non-clinical safety studies for the
conduct of human clinical trials and marketing
authorisation for pharmaceuticals

The appropriateness of juvenile animal toxicity studies should be considered only when previous
animal data and human safety data are judged to be insufficient to support pediatric studies. One
rodent species is generally considered adequate, although studies in non-rodent species can be
appropriate when justified. If a juvenile animal study is considered important for conduct of a
specific trial, it should be available prior to initiation of that pediatric clinical trial.

PRECLINICAL SAFETY EVALUATION OF
BIOTECHNOLOGY-DERIVED PHARMACEUTICALS
S6(R1)
Strategy for assessing the preclinical safety of biopharmaceuticals in juvenile animals

1. Does the current Toxicology data package adequately address concerns for the intended pediatric population (age, target organ development)
   - General Tox package
   - Reproductive Tox package

2. Is additional animal testing warranted
   - Yes
   - No additional studies conducted
   - No
   - Is additional animal testing warranted

3. Is a RODENT or NON-RODENT Species more relevant due to biology, species-specific toxicology etc.

Adapted from Coogan et al. 2011
Is a RODENT or NON-RODENT Species more relevant due to biology, species-specific toxicology etc.

Rodent

Does the molecule have rodent cross reactivity

Yes

Conduct RODENT Juvenile Study

No

Is a rodent surrogate available

Yes

Consider appropriateness of testing surrogate; IF acceptable conduct juvenile RODENT study with surrogate

No

NHP

Is it feasible/appropriate to conduct a juvenile NON-RODENT study

No

Yes

• Consider developing surrogate for testing in Juvenile Rodent
• If rodent not appropriate species no additional studies feasible

Conduct NON-RODENT Juvenile Animal Study

• Consider developing surrogate for testing in Juvenile Rodent
• Consider NON-RODENT pathway if applicable
  • Investigate cross reactivity with non traditional species (e.g. minipig, rabbit)
Enhanced PPND Study Design
Adapted from slides by Gerhard Weinbauer & Jane Stewart

Postnatal duration & endpoints designed to address specific mAb concerns, e.g. ontogeny of immune system, target organ histopath, duration of PD effect etc
Transport of Antibodies During Gestation: Role of the Neonatal Fc Receptor (FcRn)

Maternal blood pH 7.4

Uptake

Endosome, pH 6.0
IgG binds FcRn

Recycling

Release

Unbound IgG degraded in lysosome

Release

Fetal blood

IgG

FcRn
Non Rodent Juvenile Toxicology Studies
Non Human Primate (NHP)
## Macaque/Human Age Equivalent

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Cynomolgus</th>
<th>Rhesus</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>24-hr postnatal</td>
<td>24-hr postnatal</td>
<td>At term</td>
</tr>
<tr>
<td>Neonate</td>
<td>0-4 months</td>
<td>0-1 months</td>
<td>0-1 months</td>
</tr>
<tr>
<td>Infant</td>
<td>Up to 8 months</td>
<td>1-12 months</td>
<td>1-24 months</td>
</tr>
<tr>
<td>Juvenile</td>
<td>Up to 36 months</td>
<td>12-24 months</td>
<td>Not defined</td>
</tr>
<tr>
<td>Adolescent</td>
<td>3-5 years</td>
<td>2-4 years</td>
<td>12-16/18 years</td>
</tr>
<tr>
<td>Adult/Young Adult</td>
<td>&gt;5 years</td>
<td>&gt;4 years</td>
<td>&gt;16/18-20 years</td>
</tr>
</tbody>
</table>

NHP : Juvenile Toxicity Testing

◆ Study design developed case by case based on consideration of the properties of the test article and clinical plan

◆ **Practical** and **ethical** challenges exist
  – Animal Numbers : challenge to have number of relevant age (breeding on site)
  – CRO capabilities

◆ Lead times

◆ Cost
Blood Volumes: Everyone wants blood.....PK, PD, ADA, clinical pathology, TDAR........

**Blood collection volume and frequency**

- Maximum blood collection volume usually within 1% of body weight in two weeks.

<table>
<thead>
<tr>
<th>Age (Months)</th>
<th>Average Body Weight (g)</th>
<th>Maximum Blood Collection Volume in 2 Weeks (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (one week)</td>
<td>350</td>
<td>~3</td>
</tr>
<tr>
<td>1</td>
<td>420</td>
<td>~4</td>
</tr>
<tr>
<td>3</td>
<td>700</td>
<td>~7</td>
</tr>
<tr>
<td>6</td>
<td>1100</td>
<td>~11</td>
</tr>
<tr>
<td>9</td>
<td>1400</td>
<td>~14</td>
</tr>
<tr>
<td>12</td>
<td>1650</td>
<td>~16</td>
</tr>
</tbody>
</table>
NHP : Juvenile Toxicity Testing : Model

- NHP can provide an effective model for Juvenile toxicity testing
- Similarities in late developing organ systems
  - Immune System, bone, nervous system

Source: Modified from Weidauer et al.
Example Design for a 13 Week Toxicity Study in Juvenile Cynomologus Monkeys with Recovery

<table>
<thead>
<tr>
<th>Animal Age</th>
<th>12–18 mo (most common)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosing regimen</td>
<td>Daily, weekly, or as appropriate</td>
</tr>
<tr>
<td>Routes of administration</td>
<td>All standard routes possible (PO, IV, SC)</td>
</tr>
<tr>
<td>Test system</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>Dose level (mg/kg)</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>Low</td>
</tr>
<tr>
<td>3</td>
<td>Mid</td>
</tr>
<tr>
<td>4</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Total population</td>
<td>n = 40 (24 main study and 16 recovery)</td>
</tr>
<tr>
<td>Duration of pretreatment/dosing/recovery periods</td>
<td>1–4 wk/13 wk/4–13 wk</td>
</tr>
<tr>
<td>Clinical signs</td>
<td>1–2 × daily, including 1 wk prestudy</td>
</tr>
<tr>
<td>Body weight</td>
<td>Prestudy, weekly thereafter</td>
</tr>
<tr>
<td>Food consumption</td>
<td>Daily, including 1 wk prestudy</td>
</tr>
<tr>
<td>Clinical pathology parameters</td>
<td>Hematology, serum chemistry, coagulation, and/or urinalysis; prestudy and at end of dosing and recovery periods</td>
</tr>
<tr>
<td>Special assessments (as applicable)</td>
<td>Toxicokinetics: after first and last dose and/or recovery (biologics)</td>
</tr>
<tr>
<td></td>
<td>Ophthalmology: slit lamp biomicroscopy and indirect ophthalmoscopy</td>
</tr>
<tr>
<td></td>
<td>Cardiovascular: heart rate, blood pressure, and/or ECGs. Immunology: flow cytometry, immunoglobulins, TDAR (e.g., KLH) assay, NK cell assay, cytokines, lymphocyte proliferation</td>
</tr>
<tr>
<td></td>
<td>Skeletal growth evaluation: radiographic evaluation of long bone prestudy and at end of dosing and recovery periods. Can also conduct quantitative measures of bone density (DXA, pQCT)</td>
</tr>
<tr>
<td></td>
<td>Neurobehavioral testing: if applicable (based on test article pharmacology)</td>
</tr>
<tr>
<td>Terminal procedures</td>
<td>Complete necropsy of all animals, including gross pathology and organ weights</td>
</tr>
<tr>
<td></td>
<td>Full tissue collection and histopathologic evaluation</td>
</tr>
<tr>
<td></td>
<td>Immunohistochemistry possible</td>
</tr>
</tbody>
</table>

*Abbreviations: TDAR, T-cell-dependent antibody response; NK, natural killer cell; KLH, keyhole limpet hemocyanin; DXA, dual-energy X-ray absorptiometry; pQCT, peripheral quantitative computed tomography, PO = oral, IV = intravenous, SC = subcutaneous, ECG = electrocardiogram.*
Feedback from a submission to PDCO

Completed tox program including a NHP chronic study and a ePPND study with follow-up in offspring until ~6 months of age so that functional immune assessments could be performed (no adverse effects noted).

In the Day 30/60 comments, PDCO had asked for the NCWG to comment - PDCO raised a concern to NCWG about the potential need for juvenile studies with this molecule especially to be done with focus on the immature immune system.

The NCWG said the strategy was fine and no juvenile studies need be conducted (rationale in PIP why we considered a stand alone Juvenile study is not needed – waiver <5yr)

PDCO disagreed with the NCWG opinion and have asked us to perform juvenile NHP study. The concern was they felt we did not have adequate exposure for the duration of the 6 month follow-up on infants in the PPND study. We’ve drafted our justification on why our position still remains against performing juvenile NHP studies for this molecule, but have not yet submitted to PDCO for comment/review (were a number of key clinical comments from PDCO to address as well).

No US request for Juvenile Study
Conclusions

Juvenile toxicity studies with biopharmaceuticals: considerations and current practices

- Biopharmaceutical drug development differs from small molecule drug development with regard to specific challenges related to the type of molecule and how we approach preclinical safety assessment.
- Study design case by case.
- Regulatory interaction and agreement important.

- With the increased interest in developing biopharmaceuticals for paediatric use comes the challenge of designing an appropriate strategy to assess the preclinical safety of biopharmaceuticals in juvenile animals – thus supporting safe testing in paediatric clinical trials.