Non-clinical development of biologics
Requirements, challenges and case studies

Sigrid Messemer vet. med. – M4 Seminar March 10\textsuperscript{th} 2014
Aurigon - your full service CRO

• Non-clinical full service CRO based in Germany
• Approx. 170 qualified professionals
• 30 years of experience in non-clinical R&D
• Track record of 5,000 non-clinical studies
• Services: Pharmacology (Immunology & Oncology)
  Toxicology
  Bioanalytics
• Experts in non clinical evaluation of biologics since 2001
• GLP / GMP sites

Optimized & tailor-made drug development
Non-clinical challenges for biologics

- General requirements and guidelines
- Specific considerations for biologics
- Case studies

"Looks like you've got all the data—what's the holdup?"
Key questions of safety assessment

Non-clinical assessment goal: **determination of the MHRSD for clinical studies**

- Pharmacological dose (MABEL)
- Clinically relevant biomarkers
- NOAEL / MTD / toxic dose
- Target organs for toxicity
- Local reactions
- Acute life threatening risks for vital organ systems (safety pharmacology)

**Investigate expected and unexpected risks**
Guidelines for non-clinical assessment

Regulatory framework

- German Medicine Act
- German Animal welfare law
- EMA Guidelines (www.ema.europa.eu)
- ICH Guidelines (www.ich.org)

Note:
EMA guidelines only help you to set up the strategy but don’t tell you how to realize it.
Diversity of biologics

Your biologics

- Proteins & Antibodies
- Biosimilars & Biobetters
- Vaccines
- DNA & RNA
- Peptides
- Cell based therapies (ATMPs)
Guidelines for non-clinical assessment

Compound specific guidelines, e.g.

- **Biologics**…………………
  - ICH S6 (1997) biotechnology-derived products
  - ICH S6 (A) biotechnology-derived pharmaceuticals
- **Gene therapy products**…..
  - EMA / CHMP / GTWP / 125459 / 2006
- **DNA vaccines**…………..
  - EMA / CHMP / 308136 / 2007 concept paper
- **Cell-based**……………….
  - EMA / CHMP / BWP / 271475 / 06
  - EMA / CHMP / 410869 / 06 (human CBMPs)
- **Biosimilars**………………
  - EMEA / CHMP / BMWP / 4283 / 2 / 2005 rev (draft)
  - > monoclonal antibodies, follicle stimulating hormone,
  - > erythropoietin, interferon alpha
Guidelines for non-clinical assessment

Regulatory framework

- European Pharmacopoeia
- OECD guidelines ([www.oecd-ilibrary.org](http://www.oecd-ilibrary.org))

For example:

- OECD Guideline For The Testing Of Chemicals (407) "Repeated Dose 28-day Oral Toxicity Study in Rodents"
- OECD Guideline For The Testing Of Chemicals (409) "Repeated Dose 90-day Oral Toxicity Study in non-Rodents"

**Note:**
OECD Guidelines tell you how to design the study.
**But:**
The minimal designs do not meet the requirements for biologics!
Example for a non-clinical standard program for a NBE

- Pharmacodynamic / POC (non-GLP)
- Bioanalytics (non-GLP)
  - ADME-PK / species selection (non-GLP)
- Short term toxicity – rodents & non rodents (GLP)
  - Bioanalytics (GLP)
  - Local tolerance (GLP)
  - Safety pharmacology (GLP)

Phase I
Specific considerations for biologics – formulation and analytics

Compound
• Is the quality sufficient (GMP batch) ?
• Is activity assay established ?
• Do we have enough compound ?

Formulation
• Is drug substance in the final formulation used for clinical trials ?
• Is stability in formulation and each dilution confirmed ?

Formulation- and bioanalytics
• Is appropriate method with sufficient specificity and sensitivity established ?
• Is method validated (EMEA/CHMP/EWP/192217/2009) ?

Note: Physico-chemical and pharmacological properties often depends on concentration and formulation. Dosing will be made by varying the volume.
Specific considerations for biologics – species selection

Species selection

Key question: In what rodent and non-rodent species does my compound show biological activity?

Testing:
- In vitro receptor binding/blocking activity
- In vitro cell proliferation/apoptosis assay
- In vitro tissue cross reaction (antibodies)
- In vivo pharmacology studies

Answer: The rodent and non-rodent species that are most similar to human are identified.

But if not: Humanized mouse or surrogate compound required?

PK / PD

- Are relevant biomarkers for clinical trial identified?
- What is the minimum anticipated biological effect level (MABEL)?
Specific considerations for biologics – toxicity testing

General toxicity

- Duration of recovery period?
- Which doses to select for repeated dose toxicity?

Note: Low doses may result in a volume below the minimum applicable volume. High doses may result in a volume above the maximum applicable volume.

Immunotoxicity

- What should be evaluated?
  - Immunogenicity
  - Induction of autoimmunity
  - Unintended immuno-stimulation / -suppression
Non-clinical challenges for biologics

Case studies
Case study 1 – small peptide

Analytics

• Challenge: - Strong adherence to surfaces

• Solution: - Chemical modification of the formulation solution before analysis
  - Qualification of all materials used for formulation and application
  - Use of special column for bioanalytics

Toxicity

• Challenge: - No toxicity observed in mouse efficacy models, but peak toxicity at doses below intended human dose

• Solution: - Permanent infusion (24h) enables 100fold increase for daily dose
Specific risks

- Hemocompatibility (hemolysis, coagulation)
- Immunotoxicity (complement activation, cytokine storm)

Tests for hemocompatibility

- In vitro hemolysis in human whole blood
- In vitro coagulation in human whole blood

Tests for immunotoxicity

- Complement activation in human serum
- In vitro cytokine release in human PBMCs

Pathways of interest

- Th0: IL-2, IL-6, IL-13, IFN-g, TNF-alpha
- Th1: IL-2, IL-3, IFN-g, TNF-alpha
- Th2: IL-4, IL-5, IL-6, IL-10, IL-13
- Th17: IL-17
Case study 3 – somatic cell therapy (CBMP)

Specific concerns:
- Biodistribution (non-GLP)
- Tumorigenicity (GLP)
- Toxicity (GLP)

Challenge:
- Survival and traceability of a human clinical product in animals
- Appropriate tissue selection for biodistribution
- Contamination during tissue sampling, processing and analysis
- Mimic anticipated clinical route
- Combination of studies possible?

Detection method:
- PCR of marker gene or of human gene (e.g. on Chr.17, 450 pb)
- Quantitative
- Sensitivity (Aurigon: 5-6 cells per mg tissue)
- Validation of the method, how far?
Diversity & specificity ➔ a strategy and study designs for testing that fits all biologics

Combine:
- scientific expertise
- technical experience
- regulatory understanding

to:
- identify relevant animal models for *in vivo* efficacy and safety data
- consider country specific requirements
- evaluate specific risk for specific patient populations

Submission of the safety assessment strategy and study designs to regulatory authorities *prior* starting the regulatory safety animal studies
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Thank you for your attention!