General toxicity study designs

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General Principle on Guidelines

- Guidelines are purposed to help development of pharmaceuticals
- Guidelines are not written to stop development.
- If there are good scientific reasons not to follow a guideline, do it, and justify it explicitly
- In case of doubt ask scientific advice

Risk assessment process

- **Hazard identification**
  - depends on data quality and relevance of the animal model
- **Hazard characterisation**
  - find sensitive period and relevant dose metric
  - biomarkers
  - mechanistic basis for interspecies extrapolation
- **Dose-response assessment**
  - quantitative relationships, not just administered dose
- **Human exposure assessment**
  - subpopulations may differ
- **Risk characterisation- integration of above**

Toxicity

- Repeated dose toxicity

  To detect

  – target organ toxicity

  – at a relevant exposure (toxicokinetics)

  – histopathololological screening of fertility

    - **(in the EU and US 14 days is sufficient)**

  – local tolerance might be included

Duration of toxicity studies

- Dependent on intended duration of treatment

e.g. contrasting agents (1 day)

anaesthetics (2-3 days?)

antibiotics (7 days)

antidepressants (chronic)

antirheumatics (chronic)

Duration of the Repeated Dose Toxicity Studies (M3)

Duration of clinical trials	Minimum duration of Repeated Dose Toxicity studies	
	Rodents	Non-rodents
Single Dose	2-4 weeks**	2 weeks
Up to 2 weeks	2-4 weeks**	2 weeks
Up to 1 month	1 month	1 month
Up to 3 Months	3 month	3 months
Up to 6 months	6 months	6 months***
> 6 months	6 months	Chronic***

Repeated Dose Toxicity Studies

Choice of species

Generally: two species (convential products/small molecules)

Rodents: Rats, mice, if needed: hamster

Non-rodents: Dogs, Non-Human Primates, Minipigs

(rabbits uncommon in repeated dose)

Criteria for choice: pattern and level of metabolites

if appropriate: special toxicity pattern.

Repeated Dose Toxicity Studies

Study design

Rodents:

Number of animals: 8-10 per dose per sex (3 dosages)

Other approaches: more dosages but smaller groups for more precise determination of sensitivity (more precise dose-response relationships)(not usual with pharmaceuticals)

Disclaimer: exceptions are possible if justifiable

Repeated Dose Toxicity Studies

Study design

Non-Rodents:

Number of animals: 4 animals per dose per sex (+ 2 -3 recovery animals, only in pivotal studies)

In general both sexes, only in case of sexual hormones is the use of one gender acceptable.

Disclaimer: exceptions are possible if justifiable

Repeated Dose Toxicity Studies

Dose selection:

High dose:	maximum tolerable dose
	X multiple in case of low-toxic drug
	(limit dose M3, 2000 mg/kg)
Mid dose:	if needed replacing high dose
	(in case of too much toxicity)
Low dose:	intended to be NOAEL, but at least show intended pharmacodyn. effect.
	Is NOEL possible/important?

Repeated Dose Toxicity Studies

Study design

Toxicokinetics: See ICH 3A

NOT: precise pharmacokinetics, but just control of exposure

THINK about most relevant time points

e.g. near Tmax (TOP) and/or

just before administration next dose (TROUGH)

Issue:

Check for Contamination of control group.

Important for the interpretation of the study

Repeated Dose Toxicity Studies

Study design

Maximum duration

Rodents: 6 months (see carcinogenicity)

Non-rodents: 9 months See ICH S4a

(FDA might still require 12 months, depending on the division)

Repeated Dose Toxicity Studies

Biotechnology-derived pharmaceuticals

Non-rodents: 6 months is sufficient (Clarke et al 2007)

(see next slide)

For monoclonal antibodies enhancing the dose prolongs the effect, not enhances it. Stop at 10 (?) fold human exposure.

Repeated Dose Toxicity Studies

Biotechnology-derived pharmaceuticals

Review by Clarke et al (2008)

Toxicity related to exaggerated pharmacodynamics

All 6 months tox studies sufficient to signal toxicity

Maximum Tolerable Dose not relevant.

Reproductive toxicity Studies

Biotechnology-derived pharmaceuticals

High molecular weight proteins

e.g. protein hormones, enzymes, monoclonal antibodies

- insulin, cytokines, metabolic enzymes
- rituximab, infliximab
- etanercept, abatacept

Reproductive toxicity Studies

Purpose of the study

Hazard identification of exposure to proteins during gestation (organogenesis and development)

Study design:

Fertility and early developmental study (FEED)

Embryo-fetal developmental toxicity study (EFD)

Peri-Postnatal developmental study (PPND)

Reprotox of biotech proteins (1)

- Search at EPARs database March – May 2008
- In total, 82 recombinant biotechnology-derived products determined (the number of compounds with reproductive toxicity studies/the total number of products in a category):
  - blood coagulations factors (1/5)
  - erythropoietins (4/8)
  - hormones (5/8)
  - insulins (8/11)
  - interferons (4/6)
  - metabolic enzymes (6/8)
  - monoclonal antibodies (15/20)
  - others (11/16)
- Commonly used study design types: FEED and EFD
- Most often used species: rats (FEED, EFD, PPND) and rabbits (EFD). Macaques in studies with interferons.
- The information level for reproductive toxicity studies in EPARs highly variable

Reprotox of biotech proteins (2)

Findings by category:

- **Blood coagulation factors**

RT studies not performed due to the therapeutic indication → haemophilia (X-linked recessive disease and affects mostly men)

- **Erytropoietins**

4/8 developed as biosimilars to Eprex/Erypo (epoetin alfa), reproductive toxicity studies not required

For other products, studies on rats and rabbits, no significant reproductive toxicity was observed

- **Hormones**

Reproductive hormones – contra-indicated in pregnancy

Parathyroidhormones – indicated for treatment of post-menopausal women, no need for reproductive toxicity studies

Studies done for most compounds in rat/rabbit → reduced fertility index and fetal viability, increased abortion rates, lower fetal body weight

Reprotox of biotech proteins (3)

- Insulins

Studies on rats/rabbits → effects characteristic to the treatment induced secondary hypoglycaemia

- Interferones

Studies on primates → abortifacient abilities
Contra-indicated in pregnancy

- Metabolic enzymes

Studies on rats → no reproductive toxicity
Data scarce, potential risk for human unknown

- Others

Antithrombic agents - studies on rats/rabbits → vaginal bleeding, reduced viability of fetuses, increased abortion rates

Reprotox of biotech proteins (4)

Granulocyte stimulating factors – studies on rabbits → highly increased abortion rates in high dose group. Effect for human unknown, products should not be used during pregnancy

Growth hormones and growth factors – contra-indicated during pregnancy. Reduced fetal viability and female/male reproducive performance observed in rat/rabbit studies
Poor reproductive toxicity study profile for growth hormones

IL-receptor antagonist – studies on rats/rabbits → no risk in pregnancy

TNFα- no reproductive toxicity studies due to oncology indication

Reprotox of biotech proteins (5)

Monoclonal antibodies

- From EPARs – 18 mAbs and 2 fusion proteins
- Commonly used species –
  Cynomolgus monkey (9rt+4rdt/20)*
  Transgenic mice (4/20)
- For 17/20 products – reproductive toxicity studies performed
- Study designs – FEED and EFD (Cynomolgus)

* Rt – reproductive toxicity studies

Rdt – repeated dose toxicity studies

Reproductive toxicity Studies

Purpose of the study

Hazard identification of exposure to proteins during gestation (organogenesis and development)

Question: Is there placental transfer?

Placental transfer of antibodies

- Placental antibody transfer in human:
  - Only IgG activily transported across placenta
  - Transfer period: IgG transport to fetus increases exponentially, starting from gestation week (WG) 13-18 and exceeds maternal levels at term.
  - Transfer rate of IgG subclasses: IgG1>IgG4>IgG3>IgG2
  - Transfer mechanisms:

    1) across two placental barriers – maternal syncytiotrophoblast and fetal capillary endothelium (>WG30)

    FcRn receptors on syncytiotrophoblast (pH<6.5)

    FcγRIIb receptors on endothelium

    2) across fetal small intestine – fetal swallowing of amniotic fluid (WG15-25)

    FcRn receptors on intestinal epithelium

Reproductive toxicity Studies

Purpose of the study

Hazard identification of exposure to proteins during gestation (organogenesis and development)

Question: Is there placental transfer of antibodies?

Conclusion: Mainly (only?) during the last part of pregnancy in monkeys.

Reproductive toxicity Studies

Conclusion:

Guidance on reproductive toxicity studies for Biotech-derived proteins is currently under discussion in international fora.

SMEs should, therefore, seek advice on proposed studies for these products.