Non-Clinical Safety Assessment in Drug Development – and the 3R’s

Prof. Friedlieb Pfannkuch, MD
Introductory Remarks

*Personal views*

- There is a **public interest** to …
  - allow only products in the market with a well characterized safety profile
  - reduce / replace animal testing wherever feasible due to ethical reasons

- There is a generic **interest of Pharmaceutical Industry** to perform the requested / necessary animal studies in order to profile the safety of drug candidates to avoid …
  - harm to volunteers and patients
  - liabilities

- Industry’s activities are driven by (national, regional, global) regulatory requirements
REPLACEMENT – Current Constraints

Scientific – Regulatory

• Scientific reasons
  – Cells or *in vitro* systems cannot really provide a reliable picture on a complete biological organism (interactions and functions)
  – There are no alternative methods available which allow responsible safety / risk assessment on endpoints of repeated dosing:
    • Subchronic and chronic toxicity,
    • Reproduction toxicity or
    • Oncogenicity
  – Wherever feasible, alternatives are in place, e.g. for phototoxicity testing, local irritation, pyrogen testing

• Regulatory requirements
  – (Global) regulation request a fixed setting of animal studies for risk assessment and marketing authorization
Phase 0 / I: “Entry-into-human enabling”

Regulatory Toxicity Studies

- General Toxicology
  - 2- / 4-week toxicity study in rodent and non-rodent animal species, including toxicokinetics and recovery
  - ‘Acute’ (single-dose) toxicology study in rodents
    - For transportation classification - Material Safety Data Sheet; MSDS
      - Fixed Dose Procedure; Up and Down Procedure; Toxic Class Method
    - Local tolerance studies - for parenteral formulations

- Genotoxicity
  - Ames test
  - Mouse lymphoma test / Human Chromosome Aberration

- Safety Pharmacology
  - Core battery for CNS, cardiovascular and respiratory effects
Phase I / II: Early Clinical Development

Regulatory Toxicity Studies

• General Toxicology
  – 13-week toxicity study in rodent and non-rodent animal species, including toxicokinetics & recovery
  – 6-month in rodent and 9-month toxicity study in non-rodent animal species

• Genotoxicity
  – in vivo Micronucleus test in rats / mice

• Reproduction Toxicology
  – Embryo-fetal toxicity (Pilot and definitive study Segment II) in rats
  – Dose-range finding study and Embryo-fetal toxicity (Pilot Segment II) in rabbits
  – Fertility (Segment I) in rats

• Special studies
  – (Sensitization / phototoxicity in guinea pigs)
Phase III: Entry into ‘life-cycle management’

*Regulatory Toxicity Studies*

- Reproduction toxicity studies
  - Perinatal Development (Segment III)
- Carcinogenicity studies
  - In 2 rodent species or
  - In 1 rodent species and “alternative” test
- Environmental risk assessment
### Repeated-dose Toxicity Studies

#### Animal numbers

<table>
<thead>
<tr>
<th>Study type</th>
<th>OECD Guideline</th>
<th>Duration</th>
<th>Dose Groups</th>
<th>Animals / Group</th>
<th>Groups</th>
<th>Animals / Group</th>
<th>Total No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Main study</td>
<td>Toxicokinetics and/or Recovery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rodent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range-Finding</td>
<td>407</td>
<td>14-day</td>
<td>0, 1, 2, 3</td>
<td>4m / 4f</td>
<td>0, 1, 2, 3</td>
<td>2m / 2f</td>
<td>48</td>
</tr>
<tr>
<td>Subchronic Toxicity</td>
<td>407</td>
<td>28-day</td>
<td>0, 1, 2, 3</td>
<td>10m / 10f</td>
<td>0, 1, 2, 3</td>
<td>4m / 4f</td>
<td>112</td>
</tr>
<tr>
<td>Subchronic Toxicity</td>
<td>408</td>
<td>13-week</td>
<td>0, 1, 2, 3</td>
<td>10m / 10f</td>
<td>0, 1, 2, 3</td>
<td>6m / 6f</td>
<td>128</td>
</tr>
<tr>
<td>Chronic Toxicity</td>
<td>(452)</td>
<td>6-month</td>
<td>0, 1, 2, 3</td>
<td>20m / 20f / 5m / 5f</td>
<td>0, 1, 2, 3</td>
<td>5m / 5f</td>
<td>200</td>
</tr>
<tr>
<td>Non-Rodent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range-Finding</td>
<td></td>
<td>14-day</td>
<td>0, 1, 2, 3, 4</td>
<td>1m / 1f</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Subchronic Toxicity</td>
<td></td>
<td>28-day</td>
<td>0, 1, 2, 3</td>
<td>3m / 3f</td>
<td>0, 3</td>
<td>2m / 2f</td>
<td>32</td>
</tr>
<tr>
<td>Subchronic Toxicity</td>
<td></td>
<td>13-week</td>
<td>0, 1, 2, 3</td>
<td>3m / 3f</td>
<td>0, 3</td>
<td>2m / 2f</td>
<td>32</td>
</tr>
<tr>
<td>Chronic Toxicity</td>
<td>(452)</td>
<td>9-month</td>
<td>0, 1, 2, 3</td>
<td>4m / 4f</td>
<td>0, 1, 2, 3</td>
<td>2m / 2f</td>
<td>48</td>
</tr>
</tbody>
</table>

Dose groups: 0 = Control, 1 = low, 2 = mid, 3 = high
Repeated-dose Toxicity Studies

Animal numbers

Estimated numbers of animals for full development

- 600 - 800 Mice
- 1000 - 1700 Rats
- 100 - 140 Rabbits
- 150 - 250 Non-rodents
Oncogenicity Studies

Classical approach and Alternatives

- There is poor correlation of tumor incidences in rodents and humans, and predictability of human tumors is not enhanced by rodent data

- Classical approach
  - 2 rodent species (rat; mouse) - 3 dose- and one control group
  - 50 animals / sex / group (400 - 500 animals / study)

- Alternatives
  - Transgenic mice are not overly sensitive, more subject to false negatives than false positives
    - P53+/-: if clearly or equivocally genotoxic
    - Tg.AC: for dermally applied products
    - TgRasH2: for genotoxic or non-genotoxic products
    - Neonatal: if clearly or equivocally genotoxic
  - 15-25 animals / sex / group (210 – 350 animals / study)
REDUCTION – Opportunities / State of the Art
Scientific – Regulatory

- Scientific
  - Early (predictive) safety studies
  - Acute Toxicity Studies
    - For transportation classification - Material Safety Data Sheet (MSDS)
    - Fixed Dose Procedure; Up and Down Procedure; Toxic Class Method
  - Dose-range finding studies
  - Alternative oncogencity studies (mentioned before)

- Regulatory
  - International Conference on Harmonization (ICH) Guidelines
  - New approaches of earlier Entry-Into-Human
    - Exploratory clinical studies (e-IND; microdosing procedures)
Discovery Research Process

*Use of animals for proof of concept and safety*

- **Target selection**
  - *In silico* assessment of likely fit to target; Molecular modeling
  - (High throughput) Screening; “drugness properties”

- **Target validation / efficacy models**
  - *in vitro* Pharmacology,
    - interpretable structure-activity-relationship (SAR)
    - selectivity /specificity screening (molecular / cellular assays)

- **Lead development and optimization → Drug candidate**
  - Pharmacological profiling / evaluation of potency, selectivity and DMPK
    - Whole animal Pharmacology (normal animals)
    - Whole animal disease models
  - Early (“predictive”) safety studies
### Discovery: Clinical Candidate Selection (CSS)
#### Early (‘Predictive’) Safety Studies

<table>
<thead>
<tr>
<th>In silico tools</th>
<th>(see: subsequent slide)</th>
<th>(see: subsequent slide)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ames microsuspension</td>
<td>Genotoxicity</td>
<td>Salmonella typhimurium</td>
</tr>
<tr>
<td>Micronucleus test in vitro</td>
<td>Clastogenicity</td>
<td>Lymphoma cell lines or human lymphocytes</td>
</tr>
<tr>
<td>Embryonic Stem Cell Test</td>
<td>Teratogenicity</td>
<td>Mouse embryonic stem cell line</td>
</tr>
<tr>
<td>hERG inhibition in vitro</td>
<td>Cardiotoxicity</td>
<td>CHO-transfected cells</td>
</tr>
<tr>
<td>Phototoxicity in silico / in vitro</td>
<td></td>
<td>3T3 murine fibroblast cell line</td>
</tr>
<tr>
<td>Phospholipidosis in silico / in vitro</td>
<td></td>
<td>Bovine corneal fibroblast, primary cells</td>
</tr>
<tr>
<td>under evaluation:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxicogenomics in vitro</td>
<td>Hepatotoxicity</td>
<td>Primary hepatocytes and several hepatic cell lines</td>
</tr>
<tr>
<td>Primary cell cultures for organ toxicity</td>
<td></td>
<td>Hepatocytes, kidney cells, cardiomyocytes; BM</td>
</tr>
</tbody>
</table>


Discovery: Clinical Candidate Selection (CSS)

Early DMPK Evaluation

- P450 interaction
- Time dependent P450 interaction
- Reactive metabolites
- Microsomal (hepatocyte) stability
- Stability in plasma (first assessment)
- Absorbability, e.g. CaCo-2-cell monolayer
- Protein binding
- Transporters, e.g. P-glycoprotein
in silico Toxicology
...Great help in tailoring safety testing strategy

Paradigm: Structural properties may shed light on mechanism of metabolic / toxicological action of a compound

• Search for chemically related compounds and associates information (e.g. Scifinder)
• Predictive model expert systems for (Quantitative) Structure Activity Relationship - (Q)SAR
  – DEREK
    • for Genotoxicity, Skin sensitization, Irritation, Phototoxicity
  – VITIC database (LHASA; ILSI/HESI, 2004)
    • for Genotoxicity, Carcinogenicity, hERG, Hepatotoxicity, Skin sensitization
  – Multi-CASE
    • for Carcinogenicity, Teratogenicity, Hepatotoxicity in humans
  – Local (Q)SAR tools
    • tailored systems
    • applicable to a certain biological activity, e.g. Phospholipidosis or Phototoxicity
Pilot Toxicity Study in the Dog

Current Approaches

- Ascending dose study
  - Ascending single dose for e.g. 5 days
  - 1 m / 1 f animal

- Fix dose study
  - 14-day
  - No recovery period
  - 1m / 1f animal per control- or dose-group at 3 dose levels (total of 8 animals)

- Age at study commencement: animals not younger than 9 months

- Mode of administration: preferred oral gavage / gelatine capsule or according to clinical program
ICH meeting Nov-2007 in Yokohama (J)

Progress in the right direction …

• ICH S2: Guidance on Genotoxicity Testing
  – No longer require concurrent positive controls in every in vivo assay
  – Integration of genotoxicity into toxicology assays
  – Reduction in “non-relevant” in vitro results will reduce number of follow-up in vivo assays
  – Advice on choice of second in vivo genotoxicity endpoint includes Comet assay, (decreases emphasis on UDS assay)

• ICH M3: Timing of Pre-clinical Studies in Relation to Clinical Trials
  – 9-month non-rodent studies in almost all cases in all regions
    • 12-month studies only to be used to support replacement of chronic non-rodent and juvenile toxicology study where primary population is pediatric
    • 6-month acceptable in EU
  – Consensus reached on two microdose approaches and sub-therapeutic approach for clinical trials
  – Acute Toxicity Testing vs. Dose-range finding approaches
REFINEMENT – Our current focus

Animal Welfare - Scientific

- Animal welfare
  - Improve conditions of laboratory animal husbandry, care, handling, e.g. reduce impact on test animals (degree of severity) through
  - Hygiene, aseptical techniques (not only antiseptic)
  - Minimize pain and stress (anesthesia, analgesic, euthanasia, humane endpoints)
  - Techniques (injection, surgery, sutures)
  - (Non-invasive) methods such as CT, MRI; Pet; animal (disease) models

- Use of latest technologies (‘omics) –> EU FP6 “PredTox”
  - Toxicogenomics:
    - Gene expression profiling, genome-wide screening of expressed mRNA in a tissues or cell culture; good prediction after single dose studies and data used for mechanistic understanding
  - Proteomics
    - Evaluation of all proteins in a biological sample (e.g. tissue, urine)
  - Metabonomics
    - Metabolic profiling in body fluids (e.g. urine, plasma)

- Development of new, sensitive and specific biomarkers –> “IMI”; C-Path (US)
Animal Welfare Legislation
Adherence without compromises

• Introduction of a wide variety of measures to improve conditions of animals breeding and safety testing, e.g. humane criteria for euthanasia,

• Reduction of stress / burden
  – Figure on severity grades (retrograde judgement) of animals studies in Switzerland
New Dimension in Industry Collaboration

Joint efforts are mandatory

• European Framework Programme 6 - Innovative Medicines for Europe – InnoMed
  – Integrated Project: Predictive Toxicology -> www.innomed-predtox.com
    • Ultimate aim: Design of multiplex assays to rapidly and sensitively detect nephro- and hepatotoxicity

• Innovative Medicines Initiative -> www.imi.europa.eu/
  – Topics for a “1st. IMI Call” (2008)
    • Improved Predictivity of Immunogenicity
    • Non-genotoxic carcinogenesis
    • Expert system for in silico toxicity prediction
    • Qualification of translational safety biomarkers
    • Improved Predictivity of Non-clinical Safety Evaluation
  – General aim and challenge: Closer co-operation; information exchange
The Primate Issue

How to overcome conflicting requests?

• Some figures
  – 60% of the 12 million experimental animals in Europe are used in biomedical research
  – Only 0.1% are non-human primates, and 67% of those are used in studies requested for regulatory purposes
  – Great apes (chimpanzee, gorilla, orang-utans) and other endangered species are not used in Europe in drug development

• New compound classes, e.g. therapeutic humanized monoclonal antibodies are currently causing an increased use of primates
  – Relevance of safety results in non-primates questionable
  – Is the production of the respective antibodies and testing in mice really the solution?
  – Use of in vitro techniques, human micro-dosing, and imaging techniques, such as MRI or PET scans to be considered
Conclusions

Replacement - Reduction - Refinement

- Pharmaceutical Industry seriously involved and interested in the 3R’s
  - A wide variety of alternative methods are already in place / in use
  - For several relevant sectors of safety evaluation alternatives to animal testing not yet available

- (Global) Regulatory acceptance is of key importance
  - ICH process is the most appropriate platform for 3R challenges
  - Use of alternative methods as an “add-on” is inappropriate
  - Further develop the basis of clinical trials with less animal data needed

- Development of the scientific base of safety testing
  - Reliability of extrapolation from animals to humans has to be relevantly improved
  - Collaborative approaches of industry and academia to be strengthened (EU Research Framework Programmes, e.g. FP6 “PredTox”; Innovative Medicines Initiative – IMI)

- Contribution to public risk awareness and acceptance by improved information policy
Acknowledgement

• Dr. Hans-Juergen Ahr, Bayer-Schering-Pharma

Roche colleagues:
• Dr. Franziska Boess (3R Platform Switzerland)
• Dr. Edilio Borroni
• Dr. Guenther Fischer
• Dr. Wolfgang Muster
• Dr. Markus Stephan-Gueldner
• Dr. Laura Suter-Dick

Thank you very much for your attention!