3Rs in Safety Assessment in Pharmaceutical Industry

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Introductory Remarks

Personal views – no Company statement

• 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
- 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**

<table>
<thead>
<tr>
<th>• General Toxicology</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 2- / 4-week toxicity study in rodent and non-rodent animal species, including toxicokinetics and recovery</td>
</tr>
<tr>
<td>- ‘Acute’ (single-dose) toxicology study in rodents</td>
</tr>
<tr>
<td>• For transportation classification – Material Safety Data Sheet; MSDS</td>
</tr>
<tr>
<td>- Local tolerance studies – for parenteral formulations</td>
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<tr>
<td>• Genotoxicity</td>
</tr>
<tr>
<td>- Ames test</td>
</tr>
<tr>
<td>- Mouse lymphoma test / Human Chromosome Aberration</td>
</tr>
<tr>
<td>• Safety Pharmacology</td>
</tr>
<tr>
<td>- Core battery for CNS, cardiovascular and respiratory effects</td>
</tr>
</tbody>
</table>
### Phase I / II: Early Clinical Development

#### Regulatory Toxicity Studies

- **General Toxicology**
  - 13-week toxicity study in rodent and non-rodent animal species, including toxicokinetics and 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 Segment II) in rats
  - Embryo-fetal toxicity (Segment II) in rats
  - Dose-range finding study in rabbits
  - Embryo-fetal toxicity (Pilot Segment II) in rabbits
  - Fertility (Segment I) in rats

- **Special studies**
  - (Sensitization / phototoxicity in guinea pigs)
  - (Mechanistic toxicity studies)
### 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>Main study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rodent</strong></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>
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<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>
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<tr>
<td>Chronic Toxicity</td>
<td>(452)</td>
<td>6-month</td>
<td>0, 1, 2, 3, TK</td>
<td>20m /20f 5m / 5f</td>
<td>0, 1, 2, 3</td>
<td>5m / 5f</td>
<td>200</td>
</tr>
<tr>
<td><strong>Non-Rodent</strong></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
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 nongenotoxic 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
  - Dose-range finding studies
  - Alternative oncogencity studies (mentioned before)

- Regulatory
  - International Conference on Harmonization (ICH) Guidelines Revisions
  - New approaches of earlier Entry–Into–Human
    - Exploratory clinical studies (e–IND; microdosing procedures)
**Discovery: Clinical Candidate Selection (CSS)**

**Early (‘Predictive’) Safety Studies**

<table>
<thead>
<tr>
<th>In silico tools</th>
<th>Genotoxicity</th>
<th>Salmonella typhimurium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ames microsuspension</td>
<td></td>
<td></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>Embryotoxicity</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>3T3 murine fibroblast cell line</td>
<td></td>
</tr>
<tr>
<td>Phospholipidosis in silico / in vitro</td>
<td>Bovine corneal fibroblast, primary cells</td>
<td></td>
</tr>
</tbody>
</table>

**under evaluation:**

<table>
<thead>
<tr>
<th>Toxicogenomics in vitro</th>
<th>Hepatotoxicity</th>
<th>Primary hepatocytes and several hepatic cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary cell cultures for organ toxicity</td>
<td>Hepatotoxicity</td>
<td>Hepatocytes, kidney cells, cardiomyocytes</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; PAMPA
- Protein binding
- Transporters
  - 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 offered together with a small program (on the internet) applicable to a certain biological activity, e.g.
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 legislation
  - Improve conditions of animals breeding and safety testing
  - E.g. reduce impact on test animals (degree of severity)
- Use of latest technologies (‘omics) → “PredTox”
  - Toxicogenomics:
    - Gene expression profiling, genome-wide screening of expressed mRNA in 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](http://www.innomed-predtox.com)
    - More informed decision making earlier in preclinical safety evaluation by combining results from ‘omics technologies together with conventional toxicology methods.
    - Ultimate aim: Design of multiplex assays to rapidly and sensitively detect nephro- and hepatotoxicity

- **Innovative Medicines Initiative -> [www.imi-europe.org](http://www.imi-europe.org)**
  - Topics for a “1st. IMI Call” (2008)
    - Predictive Toxicology – PredTox II
    - Qualification of translational biomarkers from non-clinical to early clinical studies
    - Immunogenicity
    - Non-genotoxic carcinogens
    - Development of expert system for in silico toxicity prediction
The FP6 “PredTox” Consortium

Nycomed (former: Altana)
Bayer Schering Pharma (former: Bayer)
Boehringer-Ingelheim
Johnson & Johnson
Lilly (B) (Facility closed) - no EU funds
Merck-Serono (former: Merck KGaA)
Novartis
Novo-Nordisk
Organon (by end-2007: Schering-Plough)
Roche
Sanofi-Aventis (D)
Sanofi-Aventis (F)
Bayer Schering Pharma (former: Schering)
Merck-Serono (former: Serono)
Servier
University of Wuerzburg
Univ. College Dublin
University Hacettepe
Genedata
Bio-Rad (former: Ciphergen) - no EU funds
The Primate Issue

How to overcome conflicting requests?

- 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 request of production of the respective mouse antibodies really the solution?

Discussion ongoing
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
  - Performance of alternative methods as an “add-on” is inappropriate
  - Further develop the basis of clinical trials with less animal data
- 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...
Acknowledgement

- Dr. Hans-Juergen Ahr, Bayer Healthcare
- Roche colleagues:
- Dr. Franziska Boess (3R Platform Switzerland)
- Dr. Guenther Fischer
- Dr. Wolfgang Muster
- Dr. Markus Stephan-Guedner
- Dr. Laura Suter-Dick

Thank you very much for your attention!