Non-Clinical Safety Evaluation in Drug Development

Prof. Friedlieb Pfannkuch M.D.
F. Hoffmann-La Roche Ltd
Pharma Research - Global Non-Clinical Drug Safety
CH-4070 Basel (Switzerland)
friedlieb.pfannkuch@roche.com
Outline

Non-clinical Drug Safety Evaluation

- Some Very Basic Considerations
- Targets of Toxicology
- Schedule of Toxicity Studies
- Early Safety Evaluation
- Safety Testing of Biopharmaceuticals
- Extrapolation to Man
- Safety Assessment in Transition
Some Very Basic Considerations

- Identify diseases with unmet medical needs
- “Blockbusterology”
- The medical product development process is no longer able to keep pace with basic scientific innovation
- Increasingly difficult regulatory environment and public risk perception
- Shift to “smarter money” through e.g.
  - Drug delivery systems
  - Personalized Health Care
- Health care economics
  - Health care costs in the US 16% of GDP vs. 8% in Norway
- Functional Food and Dietary Supplements
  - Nearly equals the prescription drug market!
  - Potential Drug-“Drug”-interactions
## Steps, Duration, Costs …

<table>
<thead>
<tr>
<th>Characterization</th>
<th>Duration Months</th>
<th>% of Expenditure*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1 Target selection</td>
<td>variable</td>
<td>24</td>
</tr>
<tr>
<td>Step 2 Target validation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 3 Lead finding / identification / development</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 4 Lead optimization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 0 Entry Into Human enabling (non-clinical)</td>
<td>6 - 12</td>
<td>6 (NCS)</td>
</tr>
<tr>
<td>Phase I Early clinical safety</td>
<td>6 - 9</td>
<td>37</td>
</tr>
<tr>
<td>Phase IIa Early clinical efficacy</td>
<td>9 - 12</td>
<td></td>
</tr>
<tr>
<td>Phase IIb Open clinical trials</td>
<td>12 - 16</td>
<td></td>
</tr>
<tr>
<td>Phase III Placebo controlled trials</td>
<td>14 - 20</td>
<td></td>
</tr>
<tr>
<td>Registration / Launch Dossier preparation; Submission ; Health authorities review</td>
<td>6 - 18</td>
<td>4</td>
</tr>
<tr>
<td>Post Launch Marketing; Pharmacovigilance</td>
<td></td>
<td>12</td>
</tr>
</tbody>
</table>

* adapted from Paraxel’s Pharmaceutical R&D Statistical Sourcebook 2005/06
Dose-response relation*

*Dosis facit venenum

…”was ist das nit gifft ist?
alle ding sind gift /
und nichts ohn gifft /
allein die dosis macht das
ein ding kein gifft ist…”
Tasks of Non-clinical Safety

Non-clinical Drug Safety Evaluation

■ Spectrum of toxicity
  - Testing of chemicals in selected laboratory animal species and description of the dose-effect relationship over a broad range of doses
    » Detection of secondary (harmful/unwanted) pharmacological effects
    » Detection of adverse (toxic) effects
  - Extrapolation and Prediction of adverse effects to man

■ Identification of:
  - Initial safe starting dose and subsequent dose escalation scheme in humans and setting exposure limits (ceiling), if required due to dose-limiting toxicity findings
  - Potential target organs of toxicity and reversibility of toxicity
  - Parameters for clinical monitoring (…are the adequate / appropriate methods available / in place?)

■ Elucidation of mechanisms of toxic / adverse effects
Target of Toxicology – The Biological System

Non-clinical Drug Safety Evaluation

Variety
Age
Gender
Race
Health
Lifestyle
Nutrition
Medication

one-size-fits-all?
Non-clinical Drug Safety Evaluation

Targets Systems of Toxicology

Thymus  Liver  Kidney  Bone marrow

Embryo  DNA

Eye  Heart  Lung

Brain
Myelosuppression – Targets at the Cellular Level
Cytotoxicity – Targets of Toxicity within the Cell
Toxicity at the Mitochondria Level
Mitochondrial Metabolism

- Complex I and Complex III are the major sources of ROS
- ROS generation ↑ with inhibitors or uncouplers
Toxicity at the Gene Level

Mutagenicity
- CCCG
- GGGC
- cCcg
- GG GC

Clastogenicity
- cCCG
- cCCG

Aneuploidy
- Loss of chromosomes

Point mutation
- Frame shift
- Deletion

Chromosome breaks

Loss of chromosomes
Genome-based Safety Testing

Non-clinical Drug Safety Evaluation
Drug Discovery → Development

Master Schedule of Toxicity Studies

Non-clinical Drug Safety Evaluation

<table>
<thead>
<tr>
<th>Function</th>
<th>Lead finding/optimization</th>
<th>Clinical Candidate Selection</th>
<th>EIP</th>
<th>Phase I to III</th>
<th>4 years</th>
<th>Documentation</th>
<th>NDA</th>
<th>post NDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotoxicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reprotoxicology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermatotoxicology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General Toxicology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lab. Services-Pathology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Genotoxicity: 2-3 in vitro tests, 1-2 in vivo tests
- Reprotoxicology: Pilot Seg II, in vitro screen, in vivo screen, male fertility
- Dermatotoxicology: in vitro screen, in vivo screen
- General Toxicology: in vitro screen, pilotox, 13 wk R+D, 6 month R
- Lab. Services-Pathology: DRF, 4 wk R+D, 13 wk DRF in mouse

R=Rat, D=Non-Rodent (Dog or Monkey), M=Mouse, DRF = Dose range finding, wk = week

Studies which are regularly contracted out: ____________
Studies normally performed in-house: ____________
Non-clinical Drug Safety Evaluation

Phase 0 /I: “Entry-into-human enabling”

Safety Studies

- General Toxicology
  - 2- / 4-week toxicity study in rodent and non-rodent animal species, including toxicokinetics and recovery; GLP-compliant
  - Acute toxicology study in rodents
  - Local tolerance studies

- Genotoxicity (GLP-compliant)
  - Ames test
  - Mouse lymphoma test / Human Chromosome Aberration
  - (in vivo Micronucleus test in rats/ mice)

- Reproduction Toxicology
  - Embryo-fetal toxicity (Pilot Segment II) in rats
  - Maximally Tolerated Dose (MTD) study in rabbits

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

Non-clinical Drug Safety Evaluation

- General Toxicology
  - 13-week toxicity study in rodent and non-rodent animal species, including toxicokinetics and recovery; GLP-compliant
  - 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 (Segment II) in rats
  - Embryo-fetal toxicity (Pilot Segment II) in rabbits

- Special studies
  - Local Tolerance / Sensitization / Phototoxicity
Phase III: Entry into ‘life-cycle management’

Safety Studies

Non-clinical Drug Safety Evaluation

- Chronic toxicity studies (completion)
  - 6-month in rodent
  - 9-month toxicity study in non-rodent animal species

- Reproduction toxicity studies
  - Fertility (Segment I)
  - Embryo-fetal toxicity (Segment II) in rabbits
  - Perinatal Development (Segment III)

- Carcinogenicity studies
  - In 2 rodent species or
  - In 1 rodent species and “alternative” test

- Environmental risk assessment
Non-clinical Drug Safety Evaluation

Early Safety Evaluation

In silico:
Activity / Toxicity related to chemical structure & physico-chemical properties

In vitro:
Subcellular systems, cell lines & primary cells

In vivo:
“Short-term” animal studies
“Conventional” endpoints
Inclusion of new technologies
Approaches of *in silico* Prediction

**Expert tools for (Quantitative) Structure Activity Relationships - (Q)SARs**
- Starting from the chemical structure of the toxicant and mainly prediction of the reaction with the target

**Development of Local SARs**
- Modeling of target-specific toxic effects using results from *in vitro* experiments (=tailored systems)
- Three-step approach
  » Conduct representative in vitro (and in vivo) experiments
  » Develop model for targeted toxicity
  » Validate with reliable dataset, e.g. for
    - phospholipidosis
    - Phototoxicity
    - ...
Non-clinical Drug Safety Evaluation

**Expert Tools for (Q)SARs**

- **DEREK**
  - Genotoxicity, Skin sensitization, Irritation, Phototoxicity

- **VITIC database (LHASA; ILSI/HESI, 2004)**
  - Genotoxicity, Carcinogenicity, hERG, Hepatotoxicity, Skin sensitization

- **Multi-CASE**
  - Carcinogenicity, Teratogenicity, Hepatotoxicity in humans
Non-clinical Drug Safety Evaluation

Early Safety Testing Tools: *In Vitro*

- **Ames microsuspension (Genotoxicity)**
  - *Salmonella typhimurium*
- **Micronucleus test (Clastogenicity)**
  - Lymphoma cell lines or human lymphocytes
- **Phototoxicity**
  - 3T3 murine fibroblast cell line
- **Phospholipidosis**
  - Bovine corneal fibroblast, primary cells
- **Embryonic Stem Cell Test (Embryotoxicity)**
  - Mouse embryonic stem cell line
- **hERG inhibition (Cardiotoxicity)**
  - CHO-transfected cells
- **Toxicogenomics (Hepatotoxicity)**
  - Several hepatic cell lines
  - Primary hepatocytes
- **Primary cell cultures for organ toxicity**
  - Hepatocytes, Kidney cells, Cardiomyocytes, etc...
Non-clinical Drug Safety Evaluation

**Early Safety Testing Tools: In Vitro**

- **P450 interaction**
  - CYP inhibition in recombinant enzymes and human microsomes
- **Time dependent P450 interaction**
  - In recombinant enzymes and human microsomes
- **P450 induction**
  - In human hepatocytes or in recombinant systems
- **Reactive metabolites**
  - Trapping (e.g. GSH) or covalent binding with radiolabelled material
- **Microsomal (hepatocyte) stability**
  - Hepatocyte microsomes from different species
- **Stability in plasma (first assessment)**
  - In human and animal plasma
- **Permeability**
  - E.g. CaCo-2-cell monolayer, PAMPA
- **Protein binding (plasma shift)**
  - In Plasma, serum albumin or AGP
- **Transporters**
  - In hepatocytes, transfected cells or vesicles measuring transport or inhibition of transport
Early Safety Testing Tools

In Vivo

Conventional endpoints with tailor-made study design
- Pharmacology studies
- Disease animal models
- „MiniTox“ studies

Inclusion of new Technologies
- Toxicogenomics: Gene expression profiling, genome-wide screening of expressed mRNA in a given tissue or cell culture
- Proteomics: Evaluation of all proteins in a biological sample (e.g. tissue, urine)
- Metabonomics: Metabolic profiling in body fluids (e.g. urine, plasma)
- Additional biomarkers; Imaging; …
Examples of Biopharmaceuticals

**Therapeutic Proteins**
- EPO
- CERA
- Pegasys®

- Erythropoiesis stimulating agents
- Granulocyte colony stimulating factor G-CSF
- Interferones against multiple sclerosis
- Interferones against HCV - infection
- Human growth hormone

**Monoclonal Antibodies (Mabs)**
- Mabs HER2 / EGF-R / CD 20 / VEGF - cancer
- Mabs binding TNF alpha – rheumatoid arthritis / autoimmune diseases
- Mab alpha4-Integrin (Tysabri™) – multiple sclerosis
Mode of Action of Mabs in Oncology - Examples

- Certain tumors overexpress receptors mediating cell proliferation and survival
- Herceptin (HER2 blockade), Erbitux (EGF-R blockade)
Inhibition of receptor signalling is not the only mode of action of Mabs

- Antibody inhibits receptor signalling
- NK-cells release cytokines, target cells can be killed: Antibody Dependent Cell-mediated Cytotoxicity (ADCC)

Cells of the immune system, e.g., natural killer cells (NK-cells) bind to antibody via their Fc gamma receptors
Safety Testing of Biopharmaceuticals

Non-clinical Drug Safety Evaluation

VHI
- Tissue Cross Reactions
- Early Safety

POC
- Life Cycle

CLS
- Regulatory Toxicology
  - (Short-term Toxicology, Safety Pharmacology)
- Immunosafety
- Antibody detection

EIGLP

EIH
- Regulatory Toxicology
  - (Sub)chronic toxicology, Reproduction toxicity
- Immunosafety
- Antibody detection

Phase I-III
Biopharmaceuticals: High Tissue Specificity

What is the relevant animal species?

Due to the high specificity of biopharmaceuticals, activity and toxicity can often only to be tested in (non-human) primates.
Immunosafety Assessment with Biologics

Non-clinical Drug Safety Evaluation

Immunogenicity Risk Minimization:
(Human cell-based)
Ranking of Lead Candidates

Lead Identification

Lead Optimization

Enabling EIH

Phase I

Phase II-IV

Cytokine Release Risk:
(First Infusion-related)
Human Whole Blood Assay

Immunogenicity Risk Minimization:
(Human cell-based & in silico)
Epitope Identification & Removal
(De-immunization)

Anti-Drug Antibody Screening

Immunosuppression:
T-Cell-Dependent Ab Response (TDAR)
Host Resistance Testing
Immunophenotyping
Safety Marker
"Human Whole Blood Cytokine Test"

**Biopharmaceuticals**

Antibody links to blood cells

Cytokine Release:
- TNF–α
- IL-6
- IFN-γ

Activated neutrophiles

Leakage of small blood vessels

**Determination in vitro**

Non-clinical Drug Safety Evaluation
Converging:
- Effects produced in laboratory animals when appropriately qualified are relevant to humans
- Exposure of experimental animals to high doses to discover possible hazards to humans

Diverging:
- Species differences
  » Physiology
  » Metabolism
  » Organotrophy (e.g. Gl-tract in dog !!!)
- Healthy animal versus human patient
Prediction / Non-Prediction of Human Toxicities (HT) by Animal Species

Non-clinical Drug Safety Evaluation

ILSI concordance analysis
Can Tumors in Rodents Predict Tumor Patterns in Humans?

There is poor correlation of tumor incidences in rats and humans and predictability of human tumors is not enhanced by rat data.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Human</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon / Rectum</td>
<td>+++</td>
<td>(+)</td>
</tr>
<tr>
<td>Breast</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Prostate</td>
<td>+++</td>
<td>(+)</td>
</tr>
<tr>
<td>Liver</td>
<td>(+)</td>
<td>+++</td>
</tr>
<tr>
<td>Thyroid</td>
<td>(+)</td>
<td>+++</td>
</tr>
<tr>
<td>Pituitary</td>
<td>(+)</td>
<td>+++</td>
</tr>
</tbody>
</table>
Safety Assessment in Transition

Non-clinical Drug Safety Evaluation

- **Past**
  - Descriptive activities complemented by
  - Analytical and “measuring science”

- **Current challenges**
  - *in vitro* testing (e.g. micro-methods)
  - Alternatives to animal testing
  - Evaluation of Toxicogenomics, Proteomics, Metabonomics
  - Biomarker development
  - Genetically modified cells and animals; stem cell research
  - Regulatory needs /complexity / rigidity

- **Future: Personalized, predictive and preventive (3P’s)**
  - “Predictive” Toxicology: Toxico- / Proteo- / Metabonomics
  - Integrated approaches: *Systems Toxicology; Virtual Metabolic Network; Genome-based Safety Testing Strategy*
  - Shift from non-clinical to clinical studies (e.g. micro-dosing)
Paracelsus: Medicines and Poisons are Differentiated only by the Dose

What is the point of always dosing to the MTD – what does it tell us if pathways, systems are saturated?

It is the dose response and relationship of effective dose to toxicity threshold that matters!

New technologies not about simply resetting the NOEL. They are about …

– understanding the mechanism
– its relevance to populations and therefore
– better assessing the risk
Liver and Kidney Biomarkers - Already Accepted or in Evaluation

The set of kidney biomarkers accepted by FDA and EMEA
- KIM-1, Albumin, Clusterin and Trefoil Factor-3
  - acute kidney tubular alterations in Good Laboratory Practice (GLP) rat studies used to support clinical trials
- Total Protein, β2 Microglobulin and Cystatin C
  - acute drug-induced glomerular alterations/damage and/or impairment of kidney tubular reabsorption in GLP rat studies used to support clinical trials

Liver biomarkers under validation by C-Path
- PON-1, MDH, PNP and GLDH
  - (identified for initial cross-qualification)
- Under discussion
  - alpha-GST (multiplex assay expected)
  - Proposed: ALT isoforms 1 (liver) and ALT 2 (mitochondria)
The Troponins as Biomarkers

Involved in regulation of muscle contraction

Different isotypes

- Troponin C: binds Ca++ (identical in heart and skeletal muscle)
- Troponin I: inhibitor of actin-myosin interaction (cardiac and skeletal muscle isoforms) – Sensitive test available
- Troponin T: links troponin complex (C, I & T) to tropomyosin (cardiac and skeletal muscle isoforms)
The Toxicologist: Mission Impossible?

Non-clinical Drug Safety Evaluation

- Safety Pharmacology
- Mutagenicity
- Mechanistic Toxicology
- Pharmacology
- Project Team
- ‘omics
- Pharmacokinetics
- Metabolism
- in silico tools

COORDINATOR
Senior Expert in Nonclinical Safety

Development Candidate(s)
George Seurat (1859 – 1891)
Detail from “La Parade”, 1889