



Molecule to Market: Safety Assessment

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Safety Assessment - RTP



Safety Assessment - Mission Statement



GlaxoSmithKline

*Enabling safe clinical trials and
successful, efficient compound
registration via non-clinical hazard
identification and risk assessment.*



CLINICAL TRIALS

- **Phase I studies** – Human Pharmacology studies
 - single dose studies, dose escalation and short term repeated dose studies to evaluate pharmacokinetic parameters and tolerance
 - usually in healthy volunteers but may include ‘disease models’ or patients
- **Phase II studies** – Therapeutic Exploratory studies
 - exploratory efficacy and safety studies in patients
- **Phase III studies** – Therapeutic Confirmatory studies
 - efficacy and safety in patient populations

Toxicology

Definitions:

- **Toxicology:** The study of the adverse effects of chemicals on living things (organelles, cells, tissues, organs, organisms, populations, ecosystems)
- **Regulatory Toxicology:** Identify the adverse effects associated with the administration of a test material.
Contribute to the decision making process leading to the further development or abandonment of a test material

Toxicology

Why?

- To determine if the compound is safe
- To protect volunteers
- To protect patients
- To protect workers
- To fulfil regulatory requirements

ICH Safety Topics - Checklist

S1	Carcinogenicity studies
S2	Genotoxicity studies
S3	Kinetics
S4	Toxicity testing
S5	Reproductive toxicology
S6	Biotechnological products
S7	(Safety) Pharmacology studies
M	Multidisciplinary
E	Efficacy
Q	Quality

What is required for each stage of development ?

**CANDIDATE
SELECTION**

**BEFORE FIRST
DOSE TO
MAN**

**TRIALS IN
PATIENTS**

**FOR MARKETING
APPROVAL**

SCREENING

FORMAL SAFETY ASSESSMENT

**GENETIC TOX.
SAFETY PHARM.
RODENT screen**
Other 'Specials'
Cytotoxicity
S.A.R alerts
Photo & UV

**GENETIC TOX
SAFETY PHARM
ACUTE TOXICITY
14-28 DAY STUDIES
(rodent + non-rodent)**

**Add 3-12 MONTH
STUDIES
(rodent + non-rodent)
REPRODUCTION &
DEVELOPMENT
H&S, ENVIRONMENT**

**CARCINOGENICITY.
DEVELOPMENT.
EXTRA STUDIES?**
Juvenile Toxicology
Local tolerance
Immunotoxicity
Phototoxicity

TOXICITY STUDIES

Clinical Progression

- Safety pharmacology
- Genetic toxicity
- Single/repeat dose toxicity
- Local tolerance
- Fertility & early embryonic development
- Embryo-fetal development

Marketing

- Pre- & post-natal development
- Carcinogenicity

CHARACTERIZATION OF TOXIC EFFECTS

- Undesirable pharmacodynamic effects on specific physiological systems
- Gene mutation and chromosome damage
- Target organ toxicity/local tolerance
- Reproductive capacity

- Carcinogenicity

- Special studies

Safety pharmacology

Genetic toxicity

Single/repeat dose toxicity

Fertility & early embryonic development
Embryo-fetal development, Pre- & postnatal development

Carcinogenicity

Local tolerance, immunotox., phototox, environmental, health & safety

Pre-Clinical Activities and Deliverables Launch to End of Life Cycle

Launch

End of Life Cycle

ALL DEPARTMENTS

DMPK

PHARMACY

CHEMISTRY

SAFETY ASSESSMENT

Line Extensions
Intellectual Property defence
Regulatory Updates
New submissions
Emerging issues
OTC products

GENETIC TOXICITY (ICH S2B)

- Prior to Phase I, in vitro tests for evaluation of mutations and chromosomal damage are generally needed
 - Gene mutation in bacteria (eg: AMES)
 - Chromosome damage; in vitro mammalian cell assay (mouse lymphoma)
- The standard 3 test battery for genotoxicity should be completed prior to the initiation of Phase II studies
 - as above plus in vivo chromosome damage study (Micronucleus test - rodent haematopoietic cells)
- If an equivocal or positive finding occurs, additional testing should be performed

Genetic Toxicology

What are genetic toxicity studies?

- In vitro and in vivo tests designed to detect compounds which induce genetic damage directly or indirectly by various mechanisms
- Prediction of genetic damage to humans by:
 - gene mutation studies (bacteria, mammalian cells)
 - chromosome damage (in vitro and in vivo)
- Simple, short-term assays
- Required by Regulatory Authorities for clinical trial and marketing applications

Genotoxicity Assays

- **In Vitro**
 - **Bacterial mutation assay:** detects single base substitutions and very small insertion/deletion events (positive – direct acting mutagen)
 - Concentrations of up to maximum solubility or cytotoxicity or 5000 mg/plate
 - **Mouse lymphoma assay:** detects non-lethal gene mutations and clastogenicity (chromosome deletions, insertions, rearrangements, and number)
 - Concentrations of up to maximum solubility or cytotoxicity or 500 mg/plate
- **In Vivo (rodent)**
 - **Micronucleus assay:** detects the ability to break chromosomes
 - Same doses as 2 or 4 week toxicology studies or up to 2000 mg/kg/day
 - Daily dosing for 2 days

SAFETY PHARMACOLOGY (ICH S7A) 2001

Safety Pharmacology Studies for human Pharmaceuticals

- Safety pharmacology studies are designed to assess undesirable pharmacodynamic effects of a drug on specific physiological systems
- Standard approach - cardiovascular, central nervous and respiratory systems. Other organ systems picked up in toxicity studies
- Usually single dose, High dose in toxic range – same as 2/4 week studies

SAFETY PHARMACOLOGY (S7B) 2005

The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) By Human Pharmaceuticals

- QT interval of the ECG is a measure of the duration of ventricular depolarization and repolarization – prolongation predictive for risk of ventricular tachycardia
- In vitro I_{Kr} and In vivo QT assay should be conducted

SAFETY PHARMACOLOGY (ICH S7A/B)

GSK Prior to Phase 1

- Rat Irwin: Behavioral / observational study that predicts CNS toxicity
- Rat Respiratory: Predicts drug-related changes on respiratory function / body temperature
- Non-Rodent Cardiovascular: Predicts drug related changes in cardiovascular function or electrocardiographic intervals
- hERG: In vitro system predictive for cardiac electrophysiology
 - The most common mechanism of QT interval prolongation by pharmaceuticals is inhibition of the delayed rectifier potassium channel that is responsible for repolarization phase from the from the efflux of K (IKr).
 - Compounds that have been shown to inhibit hERG current have been shown to prolong the cardiac action potential and hence QT interval in man

Overt Central and Peripheral Effects in Rodents

- Study Design:
 - The objective of this study is to assess potential neurobehavioural effects
 - Same doses as 2 or 4 week toxicology study
 - 1 day dosing period with monitoring hourly for 24 hours post dose
 - Usually males rats only
 - Standard Observation Battery Assessment (clinical observations from testing)
 - Awareness (to touch)
 - Mood (aggressiveness)
 - Motor activity
 - Excitation/Muscle Tone/Reflex
 - Cardiovascular, Respiration, Autonomic Systems
 - Body temperature

Effect on Respiratory Function

- Study Design:
 - The objective of this study is to assess effects on ventilatory function
 - Same doses as 2 or 4 week toxicology study
 - Usually males rodents only
 - 4X4 latin square cross-over study design
 - 1-day dosing period
 - Telemetered animals
 - Monitoring hourly for 24 hours post dose
 - Assessments
 - Ventilatory function (respiration rate)
 - Lung Function (pulmonary resistance)
 - Body temperature

Non-rodent CV Study Design

- Study Design
 - The objective of the study is to assess effects on cardiovascular function
 - Same doses as 2 or 4 week toxicology study
 - Usually males only
 - 4X4 latin square cross-over study design
 - 1-day dosing period
 - Telemetered animals
 - Measurements hourly up to 24 hours post dose
 - ECGs
 - QT, PR, QRS, QTc
 - Arterial Blood Pressure
 - Heart Rate
 - Pulse Pressure
 - Body Temperatures

hERG Inhibition

- Assay assesses direct effects on cardiac potassium channels
 - HEK-293 cells expressing the hERG (IKr)
 - Concentrations of up to maximum solubility or cytotoxicity
- Calculation:
 - Fold coverage free concentration to hERG IC50 (or lower)
 - Comparing in vivo effects (CV) study to in vitro inhibition (IC30 or lower) may indicate from direct effects on cardiac potassium channels (QTc prolongation (Torsades de Pointes))

REPEAT DOSE TOXICITY STUDIES (ICH S4)

- TWO SPECIES typically Rat and Dog
 - Dog is default non-rodent species at GSK
 - Monkey used on case by case basis
- ~10-12 rodents, ~4 non-rodents /sex/ group
- Control group and often 3 drug-treated groups
 - Low dose: no toxic effect, small multiple of the therapeutic dose
 - Mid-dose: somewhere in between low and high
 - High dose: limited by toxicity, exposure, pharmacology or pharmacy
- Typically by clinical route
- 14 days to 12 months of treatment
- May include 'RECOVERY' post-treatment

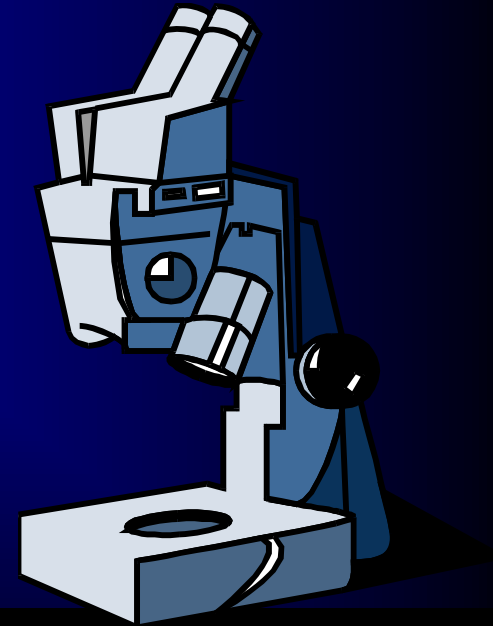
REPEAT DOSE TOXICITY STUDIES

- Objective is to assess target organ toxicity and safety associated with repeat dosing
- Demonstrate dose-response
- Predict risk associated with administration to humans
- Chronic toxicity: Detection of slower developing toxicity (eg CNS)
- Establish a “No observed effect level” (NOEL) or “No observed adverse effect level” (NOAEL)

REPEAT DOSE TOXICITY STUDIES

TARGET ORGAN TOXICITY

- **Clinical observations:**
 - condition, behavior, food intake, body weight
- **Functional measurements:**
 - ECG, Ophthalmoscopy, Otology
- **Clinical pathology:**
hematology, clinical biochemistry, urinalysis
- **Organ weights**
- **Macropathology**
- **Micropathology 50+ tissues**



Tissue List for Microscopic Evaluation

	Tissues Examined	
Abnormalities	Kidneys	Skeletal muscle (hindlimb)
Adrenal glands	Larynx ¹	Skin
Animal identification site ¹	Liver (two lobes)	Spinal cord (cervical ¹ and lumbar)
Aorta (thoracic)	Lung	Spleen
Bone marrow smear ² (sternum)	Lymph node - mandibular	Sternum with bone marrow
Brain	Lymph node - mesenteric	Stomach
Cecum	Lymph node - popliteal ¹	Testes
Cervix	Mammary gland	Thymus gland or thymic area
Colon	Nasal cavity ¹	Thyroid glands
Duodenum	Nasopharynx ¹	Tongue
Epididymides	Optic nerves ³	Tonsils ¹
Esophagus (distal)	Ovaries	Trachea
Eyes ³	Pancreas	Urinary bladder
Femur (femoral head)	Parathyroid glands	Uterus
Gallbladder	Pituitary gland	Vagina
Heart	Prostate gland	
Ileum	Rectum ¹	
Jejunum	Salivary glands (mandibular and parotid)	

1. Collected but not examined

2. Prepared at scheduled necropsy but not examined unless required to interpret peripheral blood changes as per study director or pathologist

3. Only one examined

REPEAT DOSE TOXICOLOGY DURATIONS - ICH M3

Phase 1 and 2 - EUROPE
Phase 1, 2 and 3 USA and Japan

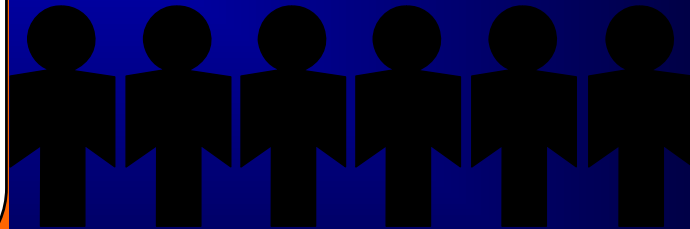
Duration of Clinical Trials	Minimum Duration of Repeated Dose Toxicity Studies	
	Rodents	Non-rodents
Single Dose	2-4 weeks ⁽¹⁾	2 weeks
Up to 2 Weeks	2-4 weeks ⁽²⁾	2 weeks
Up to 1 Month	1 month	1 month
Up to 3 Months	3 months	3 months
Up to 6 Months	6 months	6 months
> 6 Months	6 months	9/12 months ⁽³⁾

(1) In the US, single dose toxicity studies with extended examinations can support single-dose human trials

(2) In EU and US, 2 week studies are the minimum duration (Japan may prefer 4 week rodent)

(3) For trials >3 Months provide 6-month non-rodent to initiate, or provide 9 or 12 month non-rodent before duration of existing toxicology studies is exceeded.

REPRODUCTIVE & DEVELOPMENTAL TOXICOLOGY (ICH S5, S5B(M))



REPRODUCTIVE & DEVELOPMENTAL TOXICOLOGY

KEY STAGES FOR EXAMINATION

RAT

Three routine screening protocols

RABBIT

1

- Pre-mating to conception
- Conception to implantation

2

- Embryo-Fetal development – mid pregnancy

2

3

- Fetal development - late pregnancy
- Birth to weaning
- Weaning to sexual maturity

REPRODUCTION TOXICITY STUDIES

TIMING OF STUDIES RELATIVE TO CLINICAL TRIALS (WCBP = Women of childbearing potential)

PHASE I

Males

- Evaluation of reproductive organs in repeat dose toxicity studies

Females (WCBP)

- Japan, fertility and embryo/fetal studies
- EU, embryo/fetal studies
- US, Case by Case

PHASE II

Males

- As phase I

Females (WCBP)

- As phase I

PHASE III

Males

- Fertility study

Females (WCBP)

- Japan, as phase I
- EU, fertility study
- US, fertility and embryo/fetal studies

MARKETING

- Pre- & post-natal study

CARCINOGENICITY (ICH S1)

- Rat and mouse 2 year studies
- Completed carcinogenicity studies are NOT usually needed in advance of the conduct of clinical trials unless there is cause for concern.

For example....

- Chemical class effects, prior knowledge
 - PPARs prior to clinical studies of > 6 months duration
- Preneoplastic changes in repeated dose toxicity studies
- Long-term tissue retention of parent or metabolites
- Genotoxicity (for chronic administration)
- For pharmaceuticals developed to treat certain serious diseases (oncology, HIV), carcinogenicity testing, if needed, may be concluded post-approval

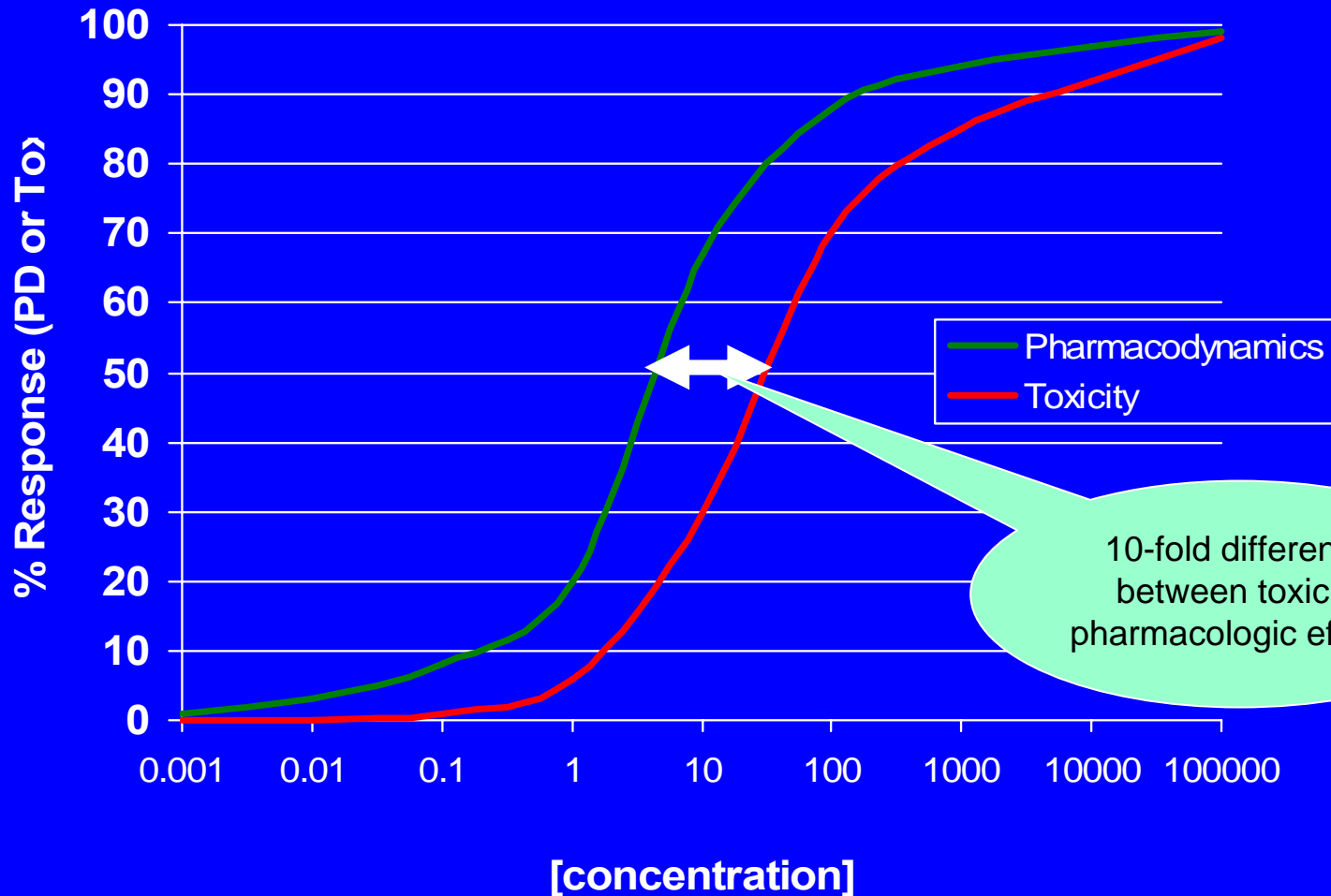
ADDITIONAL STUDIES / SPECIAL TOXICITY STUDIES

- Additional studies may be required when there are:
 - high levels of metabolites (either active or prolonged exposure)
 - changes to the impurity profile of the active (CPMP/ICH/142/95)
 - degradants, device/pack extractives (CPMP/ICH/282/95)
 - changes to the formulation, route or method of administration

ADDITIONAL STUDIES / SPECIAL TOXICITY STUDIES

- **Additional studies may be:**
 - **local tolerance and special toxicity studies to support a route change (e.g. dermal irritancy or sensitisation for topical formulations)**
 - **Juvenile Toxicity Studies**
Usually repeated daily dosing for \approx 1 month in the rat (\approx 4 days old) or dog (\approx 6 weeks old)
 - **Combination toxicity studies**
 - **Combination products, adjunctive therapy, concomitant administration and drug/drug interaction studies**
 - **Bridging studies designed to detect potential adverse interactions between the individual components of a combination product for which there are existing nonclinical and clinical data**
 - **GSK: 14 Day – 3 Month rat toxicity study**

Separation of Pharmacodynamic and Toxicologic effects



The toxicologic effects seen may be mechanistically unrelated to pharmacology, but separation remains important!

Exposure Multiples – Ratios of Nonclinical vs Human Exposure

What safety margin is required ?

No set multiple - dependent on:

- **Adverse finding that sets preclinical NOAEL**
- **Dose dependency of toxic effect / relationship to exposure**
- **Toxicokinetics / Pharmacokinetics - Accumulation? Metabolites?**
- **Reversibility of adverse nonclinical finding**
- **Clinical relevance of toxic effect to humans**
- **Clinical markers of toxicity for monitoring**
- **Duration of clinical study**
- **Therapeutic indication**
- **What is known clinically about similar compounds in the same chemical / pharmacological class**

Regulatory Library

- <http://www.biologicsconsulting.com/regsdocs.htm>

Training Sites -

Pharmaceutical Education and Research Institute

www.peri.org or www.rs.peri.org

- **Good Laboratory Practices**
- **A Primer in Non-Clinical Safety Assessment of New Pharmaceuticals**
- **Basic Training Course in Clinical Monitoring**
- **Basic Training Course on Drug Development**

Training Sites

- **SOT www.sot.org**
 - **Continuing Education Courses**
- **GLP www.glp guru.com/documents.shtml**
 - **GLP downloads**

Employment Websites

- GlaxoSmithKline - www.gsk.com
- SOT - www.sot.org
- CIIT - www.ciit.org
- NIEHS- www.niehs.nih.gov
- USEPA - www.usajobs.opm.gov
- ACT - www.actox.org

Interview Websites

- purdue.placementmanual.com/interviewing/index.html
- www.ddiworld.com
- STAR
 - Situation/Task
 - Action
 - Result