Historical Perspective

“The adverse drug reactions which the standard toxicological test procedures do not aspire to recognize include most of the functional side-effects.

Clinical experience indicates, however, that functional side-effects are much more frequent than the toxic reactions due to morphological and biochemical lesions…”

Gerhard Zbinden, 1979
Main reasons for compound attrition during non-clinical & clinical development

- ABPI review of reasons for attrition (FGLPD to phase III) was carried out by Safety Biomarker Working Group.
- In total 225 drugs were analysed.
- The summary does not classify the therapeutic area, solely the organ where the pathology of the adverse drug reaction is expressed.

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>64</td>
<td>28</td>
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<tr>
<td>Liver</td>
<td>42</td>
<td>19</td>
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<td>Musculoskeletal</td>
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<tr>
<td>Immune system</td>
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<tr>
<td>Kidney</td>
<td>12</td>
<td>5</td>
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<tr>
<td>CNS</td>
<td>10</td>
<td>5</td>
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<tr>
<td>Testes</td>
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<tr>
<td>Gastro-intestinal</td>
<td>8</td>
<td>4</td>
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<tr>
<td>Eye</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Genetic toxicology</td>
<td>7</td>
<td></td>
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<tr>
<td>Reproductive toxicity</td>
<td>7</td>
<td></td>
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</table>
## Causes of AEs/attrition

<table>
<thead>
<tr>
<th>Phase</th>
<th>Nonclinical</th>
<th>Phase I</th>
<th>Phase 1-III</th>
<th>Phase III/Marketing</th>
<th>Marketing</th>
<th>Marketing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Information</strong></td>
<td>Causes of attrition</td>
<td>Serious ADRs</td>
<td>Causes of attrition</td>
<td>ADRs on label</td>
<td>Serious ADRs</td>
<td>Withdrawal from sale</td>
</tr>
<tr>
<td><strong>Sample size</strong></td>
<td>88 CDs stopped</td>
<td>1,015 subjects</td>
<td>82 CDs stopped</td>
<td>1,138 drugs</td>
<td>21,298 patients</td>
<td>47 drugs</td>
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<tr>
<td><strong>Cardiovascular</strong></td>
<td>27%</td>
<td>9%</td>
<td>21%</td>
<td>36%</td>
<td>15%</td>
<td>45%</td>
</tr>
<tr>
<td><strong>Nervous system</strong></td>
<td>14%</td>
<td>28%</td>
<td>21%</td>
<td>65%</td>
<td>39%</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Respiratory</strong></td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>32%</td>
<td>8%</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Gastrointestinal</strong></td>
<td>3%</td>
<td>23%</td>
<td>5%</td>
<td>67%</td>
<td>14%</td>
<td>2%</td>
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<tr>
<td><strong>Renal</strong></td>
<td>2%</td>
<td>0%</td>
<td>9%</td>
<td>19%</td>
<td>2%</td>
<td>0%</td>
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<tr>
<td><strong>Hepatotoxicity</strong></td>
<td>8%</td>
<td>7%</td>
<td>21%</td>
<td>13%</td>
<td>0%</td>
<td>32%</td>
</tr>
<tr>
<td><strong>Reprotox</strong></td>
<td>13%</td>
<td>0%</td>
<td>1%</td>
<td>11%</td>
<td>0%</td>
<td>2%</td>
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<tr>
<td><strong>Genetic tox</strong></td>
<td>5%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Carcinogenicity</strong></td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Haematology/BM</strong></td>
<td>7%</td>
<td>2%</td>
<td>4%</td>
<td>16%</td>
<td>10%</td>
<td>9%</td>
</tr>
<tr>
<td><strong>Musculoskeletal</strong></td>
<td>4%</td>
<td>0%</td>
<td>1%</td>
<td>?</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Immunotox; photosensitivity</strong></td>
<td>7%</td>
<td>16%</td>
<td>11%</td>
<td>5%(+)</td>
<td>34%</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>0%</td>
<td>0%</td>
<td>4%</td>
<td>?</td>
<td>2%</td>
<td>2%</td>
</tr>
</tbody>
</table>

Redfern, et al, 2010
Agenda

Introduction (5 minutes)
Background: (10 minutes)
Safety pharmacology evaluation: (ICH S7A and S7B; 60 minutes)
  - The core battery (CV, CNS, and Respiratory)
  - Study design
  - Follow-up studies
  - Supplemental studies
  - Combination studies
  - The QT issue
  - Special situations (novel excipients, human-specific metabolites)
  - Special considerations including biopharmaceuticals
  - Strategic considerations
  - Exploratory clinical trials
Interpretation of safety pharmacology studies
Lecture Goals & Metrics

Participants should be able to:

- Explain why safety pharmacology evaluation is performed and what SP is intended to provide that is in addition and complementary to general toxicology evaluations
- Identify the human populations that current SP evaluations are most likely to protect
- Identify the ICH guidance that impact the conduct of SP studies for particular human pharmaceuticals and situations
- Identify the SP Core Battery organ systems and the types of models most often used
- Discuss the most important components of safety pharmacology study designs
- Discuss the critical elements of analysis, interpretation, and risk assessment derived from of safety data
- Discuss differences in SP evaluations for small and large molecules
Origins of Safety Pharmacology

General Pharmacology Studies

• The US, Europe and Japan considered general pharmacology studies to be an important component of drug safety assessment, and accepted the data for assessment of marketing applications.

A seminal paper published in 1994 delineated general pharmacology studies into 2 categories:
• Pharmacological profiling studies (e.g. secondary pharmacology)
• Safety profiling studies (e.g. safety pharmacology)

The authors posited that while pharmacological profiling was limited only by imagination (and budget), safety profiling could logically be confined to those organs where acute deficits in function could constitute a clinical emergency. The authors identified the priority for clinicians to know in advance of any potential threat to a critical organ system, and any rescue or antidotes identified.

Guidance on Safety Pharmacology


Section 4.1: ‘The aim of the safety pharmacology studies should be to reveal any functional effects on the major physiological systems (e.g., cardiovascular, respiratory, renal and central nervous system).’

ICH M3(M): Nonclinical Safety Studies for Conduct of Human Clinical Trials for Pharmaceuticals, 2000

Section 2: ‘Safety pharmacology includes the assessment of effects on vital functions, such as cardiovascular, central nervous, and respiratory systems and these should be evaluated prior to human exposure. These evaluations may be conducted as additions to toxicology studies or as separate studies.’
ICH S7A: Safety Pharmacology Studies for Human Pharmaceuticals, 2000

Section 2.3.2: ‘In conducting in vivo studies on vital functions, it is preferable to use unanesthetized animals. Data from unrestrained animals that may be chronically instrumented for telemetry … are preferable to data from restrained or unconditioned animals.’

ICH S7B: The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT prolongation) by Human Pharmaceuticals, 2005

Section 2.3.2: ‘An in vivo QT assay measures indices of ventricular repolarization such as QT interval. This assay can be defined to meet the objective of both ICH S7A and S7B. This will reduce the use of animals and other resources.’
ICH M3(R2): Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorizations for Pharmaceuticals, June, 2009

Section 2: Safety pharmacology and pharmacodynamic (PD) studies are defined in ICH S7A.

The core battery of safety pharmacology studies includes assessment of effects on cardiovascular, central nervous, and respiratory systems, and should generally be conducted before human exposure, in accordance with ICH S7A and S7B.

Primary PD studies (in vivo & in vitro) are intended to investigate the mode of action and/or effects of a substance in relation to its desired therapeutic target… These studies can contribute to dose selection for both nonclinical and clinical studies.
General Concepts

3 categories of pharmacology studies (Section 1.5):

• 1\textsuperscript{o} and 2\textsuperscript{o} pharmacodynamic (PD) studies
• Safety pharmacology studies

Vital organ systems, most important to assess, are those whose functions are acutely critical for life

• Safety Pharmacology Core Battery: Cardiovascular, respiratory, and central nervous systems … should be studied (Section 2.7)
• Other organ systems (e.g., renal, GI) whose functions can be transiently disrupted without causing irreversible harm are of less immediate investigative concern and studied for cause (Section 2.2)

In conducting \textit{in vivo} studies, it is preferable to use unanesthetized animals.

• Data from unrestrained animals that may be chronically instrumented for telemetry, or other suitable instrumentation… or animals conditioned to the laboratory environment are preferable to data from restrained or unconditioned animals. (Section 2.3.2)
Study Objectives

1. Identify undesirable PD properties ...that may have relevance to human safety

2. Evaluate adverse PD and/or patho-physiological effects ...observed in toxicology and/or clinical studies

3. Investigate the mechanism of the adverse PD effects observed and/or suspected

The investigational plan to meet these objectives should be clearly identified and delineated.
Test Systems
(Section 2.3)

Selection of relevant animal models or other test systems so that scientifically valid information can be derived.

*In vivo, ex-vivo, and in vitro* preparations can be used and include:

- Isolated organs and tissues
- Cell cultures
- Cell fragments
- Subcellular organelles
- Receptors
- Ion channels
- Transporters
- Enzymes
- Etc.
Test Systems

Test Article
Test Systems

Test Article

Bioassay

Hypothesis-based
Test Systems

Hypothesis testing

- Directed at a prospectively identified molecular target (or targets) or pathways
- Anticipated pharmacologic, pharmacokinetic, pharmacodynamic, and/or molecular responses (specific experimental endpoints)
- Possibility to use positive control agents
- Reductionist: Applicable only to previously identified mechanisms
- Applications in mechanism of action, Proof of Principle, etc.
- ED\(_{50}\), ID\(_{50}\), threshold response, maximum response
- Ion channels (e.g., hERG), Follow-up and Supplemental studies
- Cross-species translation via mechanism and pathway analyses
  - Safety margins based upon test article affinity/potency, as well as exposure multiples
Test Systems

Bioassay testing:

- Characterization of test article responses
- No specific prospective experimental hypothesis
- No anticipated responses (routine experimental endpoints; Clin Obs, Clin Path, etc.)
- Integrative: Responses are a summation of individual actions/effects
- Positive control agents less informative
- Applications in screening: therapeutic identification, hazard identification
- MTD/MFD, LOEL/LOAEL, NOEL/NOAEL, LD$_{50}$
- SP Core Battery studies (except hERG)
- Cross-species translation is qualitative
  - Most sensitive species
  - Severity, monitorability, and reversibility of effect
  - Suitable biomarkers available?
  - Safety margins based upon human equivalent dose (HED) conversion, and exposure margins
Critical Determinants

- Genetics
  - Expression of critical genes (specific mechanisms of action)
  - Genetic stability over time (e.g., management of genetic drift)

- Environmental factors
  - Diet (food and water)
    - Contaminants
    - Nutrient sources (animal- vs. vegetable-sourced proteins, fats)
  - Environmental conditions (lighting, temperature, humidity)
  - Animal welfare parameters (caging, socialization, enrichment, etc.)

- Human factors
  - Training/experience with specific in-life procedures
  - Test article/control group cross-contamination
Hypothesis-based

Model selection
  • Mechanism-driven (access, sensitivity, pathway linkages, etc.)

Study design
  • Test article and controls (vehicle, sham, etc.)
  • Positive and negative controls
  • Group size (by prospective statistical power analysis)
  • Surgical interventions (e.g., catheters, telemetry)
  • Randomized vs. blocked study designs
  • Test article exposure

Dose selection
  • Establish dose-response

Route and duration of administration

Prospective in-life procedures and endpoints
  • Instrumentation for endpoint detection

Animal welfare considerations (3Rs)
Bioassay

Model selection
• ‘Fit for purpose’ (often driven by regulatory guidance)

Study design
• Test article & control groups
• Group size (often driven by regulatory guidance/expectation)
• Age/weight ranges, sex (by regulatory guidance/expectation)
• Satellite groups (TK, telemetry, etc.)

Dose selection
• Maximum tolerated exposure vs. exposure multiples
• NOEL/NOAEL (for calculation of first dose in humans)

Route and duration of administration (by regulatory guidance)

Proscriptive in-life procedures and endpoints

Animal welfare considerations (3Rs)
Experimental Design
(Section 2.3.3)

- Sample size and use of controls
  - Group sizes sufficient to allow meaningful scientific interpretation of the data
  - Adequate to demonstrate or rule out the presence of a biologically significant effect

- Route of administration
  - Intended clinical route
  - Exposure to parent and major metabolites should be similar to or greater than that achieved in humans when such information is available
Experimental Design
(Sections 2.4, 2.5)

Dose levels or concentrations of test substance

• Dose- or concentration-response relationships of the adverse effect
• Time course (onset, duration) of the adverse effect, when feasible
• Doses/exposures should include and exceed the primary PD or therapeutic range
• High dose/exposure should be one that produces moderate adverse effects in the present study or others of similar route and duration, or is limited by physio-chemical properties
• Testing of a single group at the limiting dose may be sufficient in the absence of adverse effects.

Safety pharmacology studies are generally single dose administration.
Experimental Design
(Section 2.6)

Metabolites, Isomers, and Finished Products

• Parent compounds and major metabolites that achieve/anticipate significant human exposure should be evaluated.
  • Disproportionate or unique human metabolites

• Testing of individual isomers should be considered with the product contains an isomeric mixture

• Testing of finished product formulations should only be conducted for formulations with substantially altered PK or PD of the active substance in comparison to formulations previously tested.
Core Battery
(Section 2.7)

Central Nervous System
  • Endpoints include motor activity, behavioral changes, coordination, sensory/motor reflex responses, body temperature
  • Suggested systems: Functional observation battery (FOB), modified Irwin’s Screen

Cardiovascular System
  • Endpoints include blood pressure, heart rate, ECG
  • Suggested systems: *In vivo* and *ex vivo/in vitro* evaluations, including repolarization and conductance abnormalities

Respiratory System
  • Endpoints include respiratory rate, tidal volume and hemoglobin O$_2$ saturation
  • Suggested systems: Appropriate methodologies (plethysmography)
    • N.B.: Clinical observations of animals are generally not adequate to assess respiratory function
Core Battery: Central Nervous System Function Study Sequence - Modified Irwin’s Screen

### HOME CAGE OBSERVATIONS
Posture and unusual behaviors (possible convulsion; shivering; vocalisation; stimulation; stereotypy) [~1-2 min]

### OPEN FIELD ACTIVITY
Spontaneous motor activity; supported rears; unsupported rears; gait abnormalities; stereotypic behaviour; irritability; body tone; salivation; lacrimation; piloerection; catalepsy; micturition; defecation [~5 min]

### AUTONOMIC ACTIVITY ASSESSMENT (SENSORIMOTOR REFLEX)
Touch response (passivity); startle response; righting reflex; palpebral reflex; tail pinch; thermal nociception; grasping reflex; ataxia; rectal temperature; pupil response [~10 min]
Modified Irwin’s Screen Parameters

**AUTONOMIC**
- salivation
- lacrimation
- piloerection
- excessive urination
- diarrhoea/loose faeces
- rectal temperature

**NEUROMUSCULAR**
- posture
- gait
- body tone
- grasping reflex
- tremor/convulsions
- shivering

**SENSORIMOTOR**
- grasping reflex
- pupil response
- touch response
- palpebral reflex
- tail pinch response
- startle reflex (air puff)
- righting reflex

**BEHAVIOURAL**
- stimulation
- vocalisation
- irritability
- stereotypy
- unusual behaviour
- supported rears
- unsupported rears

*Also: mortality at 24 hr*
Core Battery: Cardiovascular Function

• Assess appropriately:
  – blood pressure
    systolic, diastolic, mean
  – heart rate
  – Lead II ECG

• Usually from freely moving, conscious telemetered dogs or monkeys
  • Anesthetized study if toxicity or lack of exposure prevent above

• Measurement of QT interval used to comply with ICH S7B

• Consider also:
  – in vivo, in vitro and/or ex vivo evaluations, including methods for repolarization and conductance abnormalities
Core Battery: Respiratory Function

- Freely moving, conscious rats
  - Clinical observations of animals generally not sufficient
- Measurement of ventilatory parameters using indirect or direct methods
  - Respiratory rate
  - Tidal volume
  - Minute volume
  - Resistance and/or compliance (direct method only)
- Typically performed using whole body plethysmography
- Multidose dose study with parameters being monitored for several hours
  - Dose should reach 100 times predicted therapeutic plasma concentration (if possible)
Rodent Whole Body Plethysmograph
Follow-up Studies
(Section 2.8.1)

Central Nervous System
• Behavioral pharmacology, learning and memory, ligand-specific binding, neurochemistry, visual and auditory, and/or electrophysiological examinations, etc.

Cardiovascular System
• Cardiac output, ventricular conductivity, vascular resistance, effects on endogenous and exogenous substances on cardiovascular responses, etc.

Respiratory System
• Airway resistance, lung compliance, pulmonary hemodynamics, blood gases, blood pH, etc.
Supplemental Studies
(Section 2.8.2)

Renal/Urinary System
- Urine volume, specific gravity, osmolality, pH, fluid/electrolyte balance, proteins, cytology, and blood chemistry (e.g., creatinine, urea, plasma proteins)

Autonomic Nervous System
- Receptor binding, functional responses to agonists and antagonists *in vivo* and *in vitro*, direct stimulation of autonomic nerves and measurement of cardiovascular responses, baroreflex testing, heart rate variability

Gastrointestinal System
- Gastric secretion, gastrointestinal injury potential, bile secretion, transit time *in vivo*, ileal contraction *in vitro*, gastric pH and emptying, intestinal pooling

Other Organ Systems (only when there is reason for concern)
- e.g., Abuse/dependency potential
Combination studies

- Integrative pharmacology approaches determine the inter-relationships of drug-mediated effects on different organs
  - e.g., combination of CV telemetry and respiratory plethysmography (shown)

- Allows for:
  - Time-dependent effects
  - Insight into possible mechanism
  - 3R’s benefits
  - Cost and resource benefits

- Other examples:
  - FOB/Irwin’s with tox studies
  - FOB/Irwin’s with EEG
  - CV, EEG and renal with blood sampling
Automated Blood Sampling + Telemetry (ABST)

BASi ABS System

DSI Radio Telemetry

ABST Parameters Measured Simultaneously

- Cardiovascular
  - Heart Rate
  - Mean Arterial BP
  - Systolic BP
  - Diastolic BP
  - Pulse pressure

- CNS
  - Electroencephalogram (EEG) with spectra
  - Behaviors (limited)
  - Activity counts

- Body temp

- Renal
  - Urinary electrolytes
  - Biomarker of injury
  - Glomerular filtration rate and renal plasma flow

- Plasma exposures (DBS or microsamples)

- Blood constituents
Case study #1: Combination parameters with toxicokinetics

- Spike & wave
- Interictal activity
- Continuous seizure

**Graphical Representation:***

- X-axis: Time post 1st oral dose (minutes)
- Y-axis 1: beats/min
- Y-axis 2: mm Hg or [CMPD X (μM)]
- Y-axis 3: % Spectral power

**Data Points:***

- 50 mg/kg po
- 150 mg/kg po

**Graph Details:***

- Spectral analysis chart showing changes in spectral power over time.
Case Example #2: Value of Longitudinal Observation

Methods:

- 8 conscious Han Wistar rats (~300 gm) were pre-implanted with radiotelemetry transmitters and externalized cannula.
- After 1-day acclimation and baseline recording in ABST, rats were treated with a single dose of Cisplatin (15 mg/kg, i.p.) and the same parameters recorded for 3 days.
- Parameters included:
  - Blood samples (PK; automated sampling)
  - Cortical electroencephalography (EEG)
  - Blood pressures (systolic, diastolic, mean) and heart rate (HR)
  - Body temperature
  - Renal hemodynamics (GFR and RPF) and excretory functions
  - Biomarkers of drug-induced kidney injury (DIKI)
  - Histopathology
Case Example #2 (cont.):
Value of Longitudinal Observation

Results:
- Pharmacokinetics (PK):

<table>
<thead>
<tr>
<th>$T_{\text{max}}$ (hr)</th>
<th>$t_{1/2}$ (hr)</th>
<th>$C_{\text{max}}$ (µg/mL)</th>
<th>AUC (µg*h/mL)</th>
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</thead>
<tbody>
<tr>
<td>0.25</td>
<td>33.7</td>
<td>10.1</td>
<td>66.6</td>
</tr>
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</table>
Case Example #2 (cont.): Value of Longitudinal Observation

- Pharmacodynamics (PD):

  Compared with control, Cisplatin significantly (*p<0.05; t-test) decreased HR, Temp, GFR, RPF, and elevated DIKI biomarkers which correlated with histopathological renal tubular injury on day 3.

<table>
<thead>
<tr>
<th>PD parameters</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV</td>
<td>(% change over control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>1%</td>
<td>–2%</td>
<td>4%</td>
</tr>
<tr>
<td>HR</td>
<td>–9%</td>
<td>–28%</td>
<td>–30%*</td>
</tr>
<tr>
<td>EEG spectral analysis</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Body Temp</td>
<td>–2%</td>
<td>–6%</td>
<td>–8%*</td>
</tr>
<tr>
<td>Renal GFR (mL/min)</td>
<td>–28%</td>
<td>–62%*</td>
<td>–73%*</td>
</tr>
<tr>
<td>ERPF (mL/min)</td>
<td>9%</td>
<td>–8%</td>
<td>–24%*</td>
</tr>
<tr>
<td>DIKI biomarkers (fold increase over control)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-GST</td>
<td>4*</td>
<td>40*</td>
<td>222*</td>
</tr>
<tr>
<td>GSTYb1</td>
<td>3*</td>
<td>24*</td>
<td>24*</td>
</tr>
<tr>
<td>clusterin</td>
<td>2</td>
<td>8*</td>
<td>16*</td>
</tr>
<tr>
<td>albumin</td>
<td>2*</td>
<td>171*</td>
<td>57*</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>1</td>
<td>2</td>
<td>4*</td>
</tr>
</tbody>
</table>
Case Example #2 (cont.):
Value of Longitudinal Observation

• Renal histopathology:
Acute renal tubular necrosis were observed on day 3 after a single administration of Cisplatin in rats.

Histopathology of rat renal cortex on study day 3. (A) saline control; normal. (B) Cisplatin (15 mg/kg, i.p.) induces necrosis of tubular epithelium (black arrows) and minimal luminal dilatation (asterisks). H&E, 200X
Relation to Clinical Development
(Section 2.10)

Prior to First Administration to Humans (FTIH)

- Core battery studies
- Any follow-up or supplemental studies identified, based upon cause for concern
- Supports IND

Studies During Clinical Development

- SP studies to clarify observed or suspected adverse effects in animals or humans during clinical development
- e.g., abuse potential studies in animals for CNS active compounds

Studies Before Approval

- Follow-up and supplemental studies listed in Section 2.8, unless not warranted, in which case this should be justified.
Applicability of GLP  
(Section 2.11)

Core Battery Studies
• Ordinarily conducted in compliance with GLP

Follow-up and Supplemental Studies
• Conducted in compliance with GLP, to the greatest extent feasible

Primary and Secondary Pharmacology Studies
• GLP compliance not required unless the data is used as a pivotal contribution to the safety evaluation for potential adverse effects in humans, in which case GLP compliance is expected

When SP studies or portions of studies are not conducted in compliance with GLP:
• Study reconstruction should be ensured through adequate documentation of study conduct and archiving of data
• Non-compliance with GLP should be justified, and potential impact on evaluation of SP endpoints explained
SP Studies May Not Be Necessary
(Section 2.9)

In cases of:

- Locally applied agents (dermal or ocular) where the pharmacology is well characterized and systemic exposure or distribution to other organs is demonstrated to be low

- Cytotoxic agents for treatment of end-stage cancer; however, for agents with novel mechanisms of action SP studies may be of value

- Biotechnology-derived products

- New formulations having similar PK and PD properties
ICH S7B: ‘The QT issue’

• Consequences for drug prolonging QT Interval
  – Withdrawn from market
    – Prenylamine
    – Terodiline
    – Astemizole
    – Grepafloxacin
    – Terfenadine
    – Cisapride
    – Droperidol
    – Sertindole
  – Prescribing restrictions
    – Pimozide
    – Thioridazine
    – Halofantrine
  – Approval delayed
    – Ziprasidone
  – Labelling implications
    – Far too many examples!…incl. Zomig, Nolvadex, Seroquel
ICH S7B: Objectives
(Sections 1.1, 2.1)

Describes a non-clinical testing strategy for assessing the potential of a test substance to delay ventricular repolarization

- Non-clinical assays
  - Identify the potential of a test substance and its metabolites to delay ventricular repolarization

- Integrated risk assessment
  - Relate the extent of delayed ventricular repolarization to concentrations of test substance and its metabolites
What does the QT interval represent?

- **Biological background**
  - The electrocardiogram (ECG) reflects the activity of every cardiac cell when recorded at the body surface.

The QT interval represents the period from the depolarization of atrial cells (from P wave to QRS complex) to the repolarization of ventricular cells (from QRS complex to T wave). The diagram illustrates the depolarization and repolarization processes in the heart, with arrows indicating the direction of electrical activity.

- **Depolarization of atrial cells**
- **Depolarization of ventricular cells**
- **Repolarization of ventricular cells**

The amplitude of the ECG waves is indicated as approximately 1 mV.
Importance of the hERG-encoded channel

- Biological background
  - QT interval is a measure of overall ventricular cell action potential duration
  - K⁺ ion efflux is critical for repolarisation of the cardiac action potential
  - The K⁺ current known as $I_{kr}$ (hERG) plays a key role
  - An intense burst of hERG channel activity carrying $I_{kr}$ ensures rapid repolarisation
QT and Torsades de Pointes

- Biological background
  - Selective block of hERG-encoded channel
  - Increases action potential duration and QT interval
  - This can lead to a fatal cardiac arrhythmia – Torsades de Pointes
  - Delayed repolarization leading to early after depolarisations (EADs) is a key initiating event

hERG blocker

EADs

Torsades de Pointes
Background biology

• Cardiac action potential is generated by current flow through several ion channel types

The channel carrying $I_{Kr}$ current (hERG-encoded channel) is central to the QT issue

However, action potential prolongation may result from several pharmacological effects (e.g. block of $I_{Ks}$ or $I_{Kr}$, enhancement of $I_{Na}$ or $I_{Ca}$)
**hERG is pharmacologically promiscuous**

- **Biological background**
  - Aromatic side chains line the channel – favors binding of lipophilic bases
  - Other channels have aliphatic side chains

<table>
<thead>
<tr>
<th>Channel</th>
<th>S6 Domain Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv1.1</td>
<td>AGVLTI ALPV PVIV</td>
</tr>
<tr>
<td>Kv1.5</td>
<td>AGVLTI ALPV PVIV</td>
</tr>
<tr>
<td>Kv2.1</td>
<td>AGVLI I ALPV PVII</td>
</tr>
<tr>
<td>Kv3.1</td>
<td>AGVLTI AMPV PVIV</td>
</tr>
<tr>
<td>Kv4.1</td>
<td>SGVLTI ALPV PVIV</td>
</tr>
<tr>
<td>Kv4.3</td>
<td>SGVLTI ALPV PVIV</td>
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<tr>
<td>KCNQ1</td>
<td>FAISF ALPA GILG</td>
</tr>
<tr>
<td>hERG</td>
<td>IGSLMY ASIF GNVS</td>
</tr>
</tbody>
</table>

- Probable greater cavity volume in the closed state contributes to pharmacological promiscuity
QT Prolongation/Arrhythmia ADRs

Drugs

IKr

Other Ion Channels

Other Factors

Concomitant Risk Factors

↑APD

↑QT

TdP

TdP Death
ICH S7B: hERG and QT Studies

• “In vitro IKr assay” data generated using automated patch clamp electrophysiology (e.g., IonWorks planer patch clamp technology) or GLP-complaint conventional patch clamp electrophysiology
  - Test to complete inhibition or limit of solubility
  - Consider other cardiac relevant ion channels

• “In vivo QT assay” data come from QT interval measurements for ICH S7A CV study
  - Lead II ECG in conscious telemetered dog or monkey
Testing Strategy

Non-clinical Testing Strategy

- In Vitro \( I_{Kr} \) Assay
- In Vivo QT Assay

Chemical/Pharmacological Class

Follow-up Studies

Integrated Risk Assessment

Evidence of Risk

Relevant Non-clinical and Clinical Information
Integrated Risk Assessment

Effect of M123456 in QT-related assays

Margins from predicted free $C_{\text{max}}$ in man at efficacious dose to:
- hERG IC$_{50}$
- 10% increase APD
- 10% increase QTcV
New Approaches…

MICE Models: Superior to the HERG Model in Predicting Torsade de Pointes


1Chantest Corporation, 14656 Neo Parkway, Cleveland, OH 44128, 2Leadscope, Inc., 1393 Dublin Rd, Columbus, Ohio 43215, 3The Ohio State University, 440 N Cockins Hall, 1958 Neil Ave., Columbus, OH 43210.

Drug-induced block of the cardiac hERG (human Ether-à-go-go-Related Gene) potassium channel delays cardiac repolarization and increases the risk of Torsade de Pointes (TdP), a potentially lethal arrhythmia. A positive hERG assay has been embraced by regulators as a non-clinical predictor of TdP despite a discordance of about 30%. To test whether assaying concomitant block of multiple ion channels (Multiple Ion Channel Effects or MICE) improves predictivity we measured the concentration-responses of hERG, Nav1.5 and Cav1.2 currents for 32 torsadogenic and 23 non-torsadogenic drugs from multiple classes. We used automated gigaseal patch clamp instruments to provide higher throughput along with accuracy and reproducibility. Logistic regression models using the MICE assay showed a significant reduction in false positives (Type 1 errors) and false negatives (Type 2 errors) when compared to the hERG assay. The best MICE model only required a comparison of the blocking potencies between hERG and Cav1.2.
New Initiatives…

- Comprehensive In Vitro Proarrhythmia Assay (CIPA)
- Objective: “…to facilitate the adoption of a new paradigm for assessment of clinical potential of TdP that is not measured exclusively by potency of hERG block and not at all by QT prolongation”
- The new model to consist of mechanistically based in vitro assays coupled to in silico reconstructions of cellular cardiac electrophysiologic activity
- Verification of predicted and observed responses in human-derived cardiac myocytes
- Once operational, CIPA will require modification or replacement of ICH S7A/B guidelines and elimination of E14 guidelines
  - Although progress can be made in the short term under the existing regulatory construct
Evolution of methodologies to detect QT risk preclinically
Relation to Clinical Development
(Section 2.4)

First Administration to Humans (FTIH)

- Nonclinical studies assessing risk of delayed ventricular repolarization and QT interval prolongation

During Clinical Development

- Nonclinical results, as part of an integrated risk assessment, can support the planning and interpretation of subsequent clinical studies (e.g., Clinical Robust QT Prolongation Study; ICH E14)
Applicability of GLP
(Section 1.4)

_in vitro_ \(I_{Kr}\) and _in vivo_ QT assays (described in sections 2.3.1 and 2.3.2), when performed for regulatory submission:

- Conducted in compliance with GLP

Follow-up studies (section 2.3.5)

- Conducted in compliance with GLP, to the greatest extent feasible

When SP studies or portions of studies are not conducted in compliance with GLP:

- Study reconstruction should be ensured through adequate documentation of study conduct and archiving of data
- Non-compliance with GLP should be justified, and potential impact on evaluation of SP endpoints explained
Special Considerations

Novel excipients (FDA Guidance)

Disproportionate or unique human metabolites (FDA MIST guidance)

Biopharmaceuticals

Exploratory clinical trials (FDA Guidance on Exploratory INDs)

Juvenile animals
Biopharmaceuticals

Highly-specific receptor targeting (e.g humanized mAbs)

• Often sufficient (e.g. feasible) to evaluate SP endpoints as part of toxicology and/or PD studies
  • Non-rodent species only (usually a non-human primate)
  • Limited to cardiovascular and respiratory endpoints

Novel therapeutic classes and/or products that do not achieve highly specific targeting – a more extensive SP evaluation should be considered.

Surrogate molecules

• Preclinical species homolog of a human-specific biopharmaceutical

Transgenic approaches

• Knock-out and knock-in models
• Surrogate constructs
### Exploratory CTA Requirements

**ICH M3(R2) 2009**

<table>
<thead>
<tr>
<th>eCTA</th>
<th>Preclinical Species</th>
<th>High Dose Selection</th>
<th>GeneTox &amp; Safety Pharm</th>
<th>Clinical Start Dose</th>
<th>Clinical Stop Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-Dose 1</td>
<td>SD rodent*, w/ extended observations</td>
<td>~10 mg/kg (1000x, mg/kg or mg/m²)</td>
<td>Not Applicable (but include any SAR w/CTA)</td>
<td>≤100 µg</td>
<td>≤100 µg</td>
</tr>
<tr>
<td>Micro-Dose 2</td>
<td>7-day rodent</td>
<td>~10 mg/kg (1000x, mg/kg or mg/m²)</td>
<td>Not Applicable (but include any SAR w/CTA)</td>
<td>≤100 µg x5</td>
<td>≤100 µg x5</td>
</tr>
<tr>
<td>Single-Dose</td>
<td>Pharm model, SD rodent* &amp; nonrodent*</td>
<td>MTD, MFD, or limit dose (1g/kg)</td>
<td>Ames assay; SP core battery</td>
<td>1/50th NOAEL (mg/m² basis)</td>
<td>½ NOAEL (mg/m² basis)</td>
</tr>
<tr>
<td>Repeat-Dose 1</td>
<td>Pharm model, 14-day rodent &amp; nonrodent</td>
<td>10x (anticipated human exposure)</td>
<td>Ames, clastogen assays SP core battery</td>
<td>1/50th NOAEL (mg/m² basis) or 1/50th AUC at NOAEL</td>
<td>1/10th, ½, or AUC at NOAEL</td>
</tr>
<tr>
<td>Repeat-Dose 2</td>
<td>Pharm model, 14-day rodent &amp; confirmatory nonrodent</td>
<td>MTD, MFD, or limit dose (1 g/kg)</td>
<td>Ames, clastogen assays SP core battery</td>
<td>1/50th NOAEL (mg/m² basis)</td>
<td>½, or AUC at NOAEL</td>
</tr>
</tbody>
</table>
Exploratory Clinical Trials

**Tactical Application**
- Candidate Drug (CD, or later)
- De-risk a compound or compound selection
- GMP chemistry
- Investigational drug is possible launch candidate
- Clinical endpoints:
  - PK, ADME
  - PD
  - Efficacy biomarker
  - Select Phase I CD
  - Confirm back-up CD

**Strategic Application**
- Lead compounds
- De-risk basic biology (e.g., therapy target)
- Pre-GMP chemistry
- Investigational drug (probably) not a launch candidate
- Clinical endpoints
  - Proof of Principle (PD, biomarker)
  - Select chemistry platform (PK, ADME)
Integration of SP Endpoints in Traditional Toxicology Studies

Integration of SP endpoints in general toxicity (GT) studies can reduce the total number of individual studies and total numbers of animals used, and aid in integrative interpretations.

- SP modules (or satellite groups) in GT studies
  - CNS (FOB, modified Irwin screen)
  - Respiratory (plesythmography)
  - Cardiovascular
    - ECG and heart rate
    - Blood pressure (?)
  - Toxicokinetics

Technical considerations:

- SP requirements must not confound interpretation of GT endpoints, and *visa versa*:
  - Introduction of pathological artifacts (e.g., surgery for telemetry implants)
  - Introduction of stress artifacts (e.g., extra handling, novel environments)
  - MTD (GT) vs. MTD (SP)
  - NOEL vs. low multiple therapeutic exposure
SP Data Integration & Risk Assessment

Chemical / Pharmacological Classes

Core Battery Assays

Follow-up Assays

Supplemental Assays

General Tox Studies

Integrated risk assessment

Follow-up studies If necessary

Signal of Risk

None  Weak  Strong
Safety Margin Considerations

Standard approach compare IC_{50} values

IC_{20} values may be more appropriate but not always accurate

RESPONSE

50%

log(EXPOSURE)

AGONIST low occupancy
ANTAGONIST high occupancy
Adverse event

DESIR ED EFFECT

ADVERSE EVENT LIABILITY
Transition to Human Trials

For small molecules
- NOAEL dose in most sensitive species/model
- Calculate human equivalent dose using body surface area scaling (HED)
- Start does is 1/50th HED (for human volunteers)

Alternatively, for some new drugs (e.g., some biopharmaceuticals) the minimum active biological effect level (MABEL) in preclinical species may be more appropriate than the NOAEL for estimating a safe first human dose.

Acknowledgements

• Lew Kinter, Ph.D.
• Tim Hammond, Ph.D.
• Jean-Pierre Valentin, Ph.D.
• Silvana Lindgren, Ph.D.
Advice in Searching for Risk

(from G. Zbinden)

1. Do not do something just because you can.
2. Do not do something just because it has always been done.
3. Do not do something just because others do it.
4. Do not do something because (you believe) it is expected.
5. Do not do something the results of which cannot be interpreted.

Do something because there is a reasonable expectation it will provide knowledge necessary for an accurate decision.

Selected Reading


Selected Reading (cont.)


Please click on the link below to enter your comments on this talk

https://www.surveymonkey.com/s/VNRH97V