Lead Optimization Strategies and Integrated Assessment of *In Vitro* and *In Vivo* Toxicology Studies for the Rapid Identification of Clinical Candidates

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Conflict of Interest Statement

Neither myself nor any of my coauthors, including members of our immediate families, have any financial interest or affiliation of the type described above with a commercial organization that has a direct or indirect interest in the subject matter of my presentation.
Presentation Outline

- Goals of lead optimization
- The LO flow scheme: putting it all together
- Case Study 1: common issue
- Case Study 2: unanticipated issue
- How to expedite candidate selection
Proactive staged approach to understand target and drug liabilities in order to inform project progression and clinical candidate selection, and reduce late stage attrition
Primary toxicity was associated with the following target organs:
- Cardiovascular (18%), Liver (16%), GI (12%), and CNS (13%)

Cardiovascular was most prominent reason for termination
- Electrophysiology/hemodynamic and histological findings
Safety Goals for Lead Optimization

- Establish safety profile of clinical candidate to support candidate selection and progression into the clinic
  - Identify maximum tolerated dose
  - Identify target organs of toxicity (and MOA if possible)
  - Preliminary estimate of safety margins
  - Inform design of FIH-enabling GLP tox studies
What do I Need from Other Functions?

• Pharmacology:
  • Estimate of efficacious plasma concentrations (i.e. PD biomarker)
  • Drives dose selection and initial estimates of safety margin

• Pharmacokinetics:
  • PK profile in nonclinical species (e.g. AUC, Cmax, half-life, etc)
    • Informs dose selection for toxicology studies
  • Metabolite profile
    • Risk of reactive metabolites (e.g. hepatotoxicity risk)
What is a Lead Optimization Flow Scheme

• An outline for how molecules will be evaluated for PD, PK and safety
• Establishes the order of testing, and typically the desired criteria to advance molecules to the next stage of testing
  • Goal is to fail fast
• Helps project teams understand
  • Resources for assays and studies needed
  • Timing considerations
  • Compound needs (how much, and when)
Conceptual LO Flow Scheme

Small (mg) batches
- In vitro target potency
- Rodent PK/PD

Tier 1 (HTS)
- in vitro ADME & Selectivity

Tier 2 in vitro
- ADME & Selectivity

8-12 g
- Short term (4d) rodent tox
- Non-rodent (dog) PK

Non-rodent (dog) PK

Cardiovascular Assessment

Acceptable PK, PD?

100-300 g
- Longer-term (14d) rodent tox
- Non-rodent dose escalation

Non-rodent (dog) PK

Longer-term (14d) non-rodent tox

Predicted safe & efficacious in humans?
“Real Life” Example of LO Flow Scheme

Target X biochemical and cell assay

Rat PD (single dose)
Protein binding

Rat PK (IV/PO)

Microsome stability Permeability/Efflux/ P450 Inhibition

hERG Ki Bsep IC50

4d rat tox

In vitro genotoxicity

Rat PD (repeat dose IC50)

Extended selectivity screens

14d repeat dose rat tox

Non-rodent dose escalation

Decision Point

Tier 2 Hepatocyte Induction/ Clearance/ Metabolite ID

Non-rodent PD/ Human dose projection/Species selection

Ex vivo heart/ rat telemetry assessment

Decision Point

Decision Point

Extended selectivity screens

Candidate Selection Decision

Non-rodent 14d repeat dose tox
In Vivo Study Design Considerations

• Rat and dog are typical small molecule test species
  • Practical, large historical database, readily available
• 4 to 14 days in duration
  • Assumes 28-day GLP study needed to support Phase 1
• Dosing designed to establish maximum tolerated dose
  • Up to limit dose (1000 mg/kg) or saturation of exposure
Additional Safety Considerations During LO

- On-target liabilities
  - Non-standard endpoints to inform target liabilities
- Phototoxicity
  - Absorbance, and potential in vitro evaluations (see ICH S10)
- Teratogenicity risk
  - Early assessment if outcome is critical for clinical indication
- Other safety endpoints important for clinical differentiation
  - E.g. better selectivity versus competition
Case Study 1: CV and Mutagenicity Liabilities

• Issue: chemical series has moderate affinity for hERG, and many tested compounds are mutagenic in Ames

• Project Goal:
  • Ensure an adequate safety margin to QT prolongation
  • Eliminate the mutagenicity

• Approach:
  • Front load the in vitro hERG assay and micro Ames assay prior to investment in significant compound scale-up or in vivo tests
  • Confirm safety margin to QT prolongation in whole heart/whole animal CV model according to in vitro risk
Case Study 1: CV and Mutagenicity Liabilities

- **In vitro target potency**
- **Tier 1 (HTS) in vitro ADME & Selectivity**
  - hERG
- **Rodent PK/PD**
- **Tier 2 in vitro ADME & Selectivity**
  - MicroAmes

**Cardiovascular Assessment**
- **Short term (4d) rodent tox**
- **Non-rodent (dog) PK**
- **Longer-term (14d) rodent tox**
- **Non-rodent dose escalation**

**Acceptable PK, PD?**
- **Acceptable Safety, PK?**
  - Predicted safe & efficacious in humans?
Case Study 2: Unanticipated Liver Toxicity

• Issue: unanticipated liver toxicity observed in 4-day rat tox studies

• Project Goal:
  • Screen compounds to identify candidate with no liver tox (or better safety margin)

• Approach:
  • Evaluate mechanisms of liver toxicity to identify in vitro model to counter screen and prioritize compounds for in vivo testing
  • Test compounds in 4-day rat tox studies to confirm
Case Study 2: Unanticipated Liver Toxicity

In vitro target potency

Tier 1 (HTS) in vitro ADME & Selectivity

Rodent PK/PD

Tier 2 in vitro ADME & Selectivity

In vitro ADME & Selectivity

Short term (4d) rodent tox

Non-rodent (dog) PK

Longer-term (14d) rodent tox

Non-rodent dose escalation

4d rat tox

Cardiovascular Assessment

Delay?

Acceptable Safety, PK?

Predicted safe & efficacious in humans?

Acceptable PK, PD?
Decision Making: Is My Drug Safe Enough?

• Severity of toxicity (eg. nausea vs. fatal arrhythmia)?
• Adequate margin of safety?
• Will the toxicity get worse over time?
• Is the toxicity monitorable in the clinic, and reversible?
• Consider the intended patient population and medical need
  • Chronic vs acute treatment?
  • Life-threatening disease?
  • Other treatment options?
• Consult with your clinical stakeholders
Conclusions

• Compound attrition is likely, so plan to fail fast
• LO is a dynamic and flexible process
• LO is a highly integrated process with pharmacology, pharmacokinetics and safety partners
• Identifying the clinical candidate is the starting point for drug development, so think ahead
References


