Early Target De-Risking Strategies for Oncology Small Molecule Drugs

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Global Safety Pharmacology
Outline

• Oncology Small Molecules and safety
• Differences for Oncology Drugs pre and post ICHS9
• Attrition Rates
• Cardiovascular Safety Testing: early de-risking strategies
• Antibody-drug conjugates (ADCs) special considerations
• Cardiovascular de-risking: Examples for small and large animals
• Summary
Background

- Why do we need a cardiovascular strategy for oncology?

  - **ICHs9 updated guidance for oncology**
    - Stand alone GLP Safety Pharmacology studies not required if FIH is in patients with late-stage cancer
    - General Tox endpoints are acceptable but may not provide strong confidence of lack of CV or CNS activity

- What is the industry Goal? - First in class or best in class
  - Oncology patients are living longer due to advancement of multiple therapies
  - Safety needs to be a factor to help differentiate drugs
  - Evaluating early is key to safer drugs and decrease attrition

- Combination therapy in Oncology
  - Better safety profile potentially the easier it will be to combine with other products which is standard for treatment of cancer
  - New Technologies / modalities i.e. ADCs = new safety concerns
Comparison of Small Molecule FIP-Enabling Packages

Pre-ICH S9

• Non-GLP Studies (Discovery)
  – Dofetilide/hERG
  – CEREP

• GLP Package
  – Ames (in vitro)
  – Chromosomal Aberrations (in vitro)
  – CV Safety Pharmacology
  – CNS Safety Pharmacology
  – Repeat Dose Rodent + MN + Recovery
  – Repeat Dose Non-Rodent + ECG + Recovery

Post ICH S9

• Non-GLP Studies (Discovery)
  – Dofetilide/hERG
  – CEREP
  – Screening Ames (in vitro)
  – Screening in vitro MN (opt.)
  – Screening rodent CV Safety Pharm
  – Early toxicity study (14D) + CNS FOB + echo Rat
  – Early toxicity study dog/NHP (14D) + JET

• GLP Package
  – Repeat Dose Rodent + MN + Recovery
  – Repeat Dose Non-Rodent + ECG + Recovery

MN = micronucleus; ETS = exploratory toxicity study
Attrition Rates: Historical data highlight the importance of early detection of CV & CNS issues.

Impact of adverse effects of drugs by organ function throughout the pharmaceutical life cycle.

<table>
<thead>
<tr>
<th>Phase</th>
<th>‘Nonclinical’</th>
<th>Phase I</th>
<th>Phase I-III</th>
<th>Phase III/Marketing</th>
<th>Post-Marketing</th>
<th>Post-Marketing</th>
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<td><strong>Information:</strong></td>
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<tr>
<td></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>
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<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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<td><strong>Cardiovascular:</strong></td>
<td>27%</td>
<td>9%</td>
<td>21%</td>
<td>36%</td>
<td>15%</td>
<td>45%</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>
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<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>NERVOUS SYSTEM:</strong></td>
<td>14%</td>
<td>28%</td>
<td>21%</td>
<td>67%</td>
<td>39%</td>
<td>2%</td>
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</table>

**2010 Update:**

- Trial halted/delayed/development stopped 2010
- 18 CDs
- Cardiovascular: 22%
- Nervous System: 22%
- No change in 10 years!

- Withdrawal from sale 2010
- 8 products
- Cardiovascular: 75%
- Hepatotoxicity: 25%
- Nervous System: 25%
- Increased contribution from Nervous System 2010
Cardiovascular Parameters
- Which Are Important To Evaluate?

• **In Vitro / Ex Vivo**
  – CEREP
  – hERG / ion Channels
  – Isolated heart / tissue baths (** as needed)**

• **Hemodynamic**
  – Blood pressure and heart rate (telemetry/JET)
  – Isolated heart or atria, vascular preps

• **Cardiac contractility**
  – Direct effects on isolated heart model
  – Catheterized in vivo studies
  – Non-invasive in vivo studies using echocardiography

• **Cardiac structure and function**
  – Echocardiography

• **ECG**
  – PR, QRS, QT
  – Isolated heart model and telemetry/JET studies
Profiling Of Compounds For Cardiovascular Risk
- Information Required Prior To CV Testing

- **Target Knowledge Review**
  - Is there information that suggests CV risk associated with target?
    - Target Literature reviews
    - Knock out model information

- **Cerep Panel/ Kinase Panel**
  - Does the compound have 2° pharmacology that is associated with CV risk?
  - > 60 targets profiled in Cerep, including receptors, channels, transporters

- **Dofetilide Binding Data (hERG) & ion Channels**
  - Is there a potential for QT risk?
  - Is there a potential for blockade of other ion channels? (sodium, calcium, etc)

- **PK information**
  - Is the PK appropriate for in vivo testing?
  - Consider species specific ADME properties / exposures

- **Antibody –drug conjugate**
  - Does naked mAb have target CV expression / risk
  - Consider off-target toxin effects on cardiac and vascular tissues
  - How much is know about your linker and payload separately and combined, stability?
Oncology Cardiovascular De-risking Strategy
- Generic Profiling Pathway

Target Knowledge Review

In Vitro Secondary Pharmacology
CEREPR, Kinase Panel, Ion Channel (hERG, Calcium, Sodium), PK

- < 100 Fold versus hERG
- Na⁺/Ca²⁺ Channel Activity

- < 100 Fold versus hERG
- No overt 2° pharmacology

- > 100 Fold versus hERG
- +/- 2° pharmacology

Isolated Heart
(Contractility, ECG, LVP)
Aortic Ring Bath

- No Effect
- Change In Contractility/ vasoactivity
- Change In ECG

Rat CV Study
BP/HR/Echo

- Rat 14 Day Early Tox Study + Echo
- Dog 14 Day Early Tox Study + JET

Large Animal
CV Study
Recommended Safety Pharmacology Input (e.g. CV) to Stage-Gates

ESD > SDS > LD > CS > Post CAN > Ph I

**Target Safety**
- Is there a CV safety risk associated with the target or pathway (e.g. TKR)?

**Series Selection**
- Are there CV safety risks associated with any of the chemical series of interest?

**Lead Selection**
- Comprehensive CV evaluation of lead compound(s); rank order and differentiation prior to early toxicity rat study

**Required for CAN**
- CEREP full panel
- Pass hERG and in vivo QT risk assessment
- In vivo CV hemodynamic (and ECG) assessment

**Required for FIH**
- GLP Neurofunctional
- GLP Respiratory
- GLP CV dog or GLP JET add on to 1 mo study

**Fit for Purpose Study**
Assays for Evaluating Hemodynamics: In Vitro and In Vivo

Aortic Ring Assay

- Rat thoracic aorta rings excised, placed in Krebs solution
- 37°C Baths aerated with 95/5% O₂/CO₂
- Ring bottom secured to the bottom of the bath, top is tied to transducer
- Basal tension is set to a certain weight

Anesthetized Rat

- Catheters Inserted
  - Jugular
  - Carotid
  - Femoral
- 30 minute stability and baseline period; up to 3 hr
- Anesthesia Caveat: dampened reflexes may result in different profile than conscious

Conscious animals

- Catheters Implanted
- Variety of study designs
- 24 hour data collection
- Standard assay to evaluate single or repeat dose hemodynamic/ECG effects of novel compounds
Langendorff Model: Parameters

- This ex-vivo model can be used to assess direct effects on cardiac function and conduction (ECG intervals and durations).
  - Left Ventricular Pressure (LVP)
  - Contractility (Max. + dP/dT)
  - Perfusion Pressure
  - PR interval
  - QRS duration
  - QT interval
  - MAP

\[ \text{Systolic (mmHg)} \]
\[ \text{Diastolic (mmHg)} \]
\[ \Delta = \text{LVP} \]

\[ \frac{\text{d}P}{\text{d}T} = \text{Contractility} = \text{Force of Contraction} \]
Jacketed External Telemetry (JET) components

(A) Jacket  
(B) T-shirt  
(C) ECG transmitter  
(D) Blood pressure transmitter with paddle receiver  
(E) ECG surface electrodes.

- for oncology JET can be added on to any 2 week early tox study in dog or primate or a 1 month GLP study  
  - default is to add JET on to all dog 2 week early toxicity studies
Oncology Programs that could benefit from use of JET on Early Toxicity Studies – portfolio support

- Oncology small molecules
  - JET add-on Early Toxicity Studies is endorsed for small molecules and provides an opportunity to characterize cardiovascular risk

- Antibody-drug conjugates (ADCs) for oncology
  - JET add-ons for:
    - mAb target with expression in myocardial or vascular tissue
    - Novel Linker/payload system

\[
\text{Antibody} + \text{Linker} + \text{Payload (Cytotoxin)} = \text{ADC}
\]
Antibody Drug Conjugates [oncology therapy]

Target is a cell surface tumor antigen/protein (e.g. CD30, Her2)

- Monoclonal antibody (mAb) framework
- Linker: Tethers the drug/toxin to the antibody
  - Types: Cleavable and noncleavable
- Payload (cytotoxin; ex. Dolastatin family)
  - Is a small molecule drug/toxin with antitumor activity
  - Loading is the number of payload molecules per antibody

- ADC binding to CD30
- Internalization & trafficking to lysosome
- Enzymatic cleavage of linker releases MMAE
- Tubulin polymerization blocked
- Cell cycle arrest & apoptosis

Seattle Genetics, 2007 BioSafe meeting
Example of Rodent CV support – Oncology Small Molecule

- RTK inhibitor
- N=8 rats/group monitored at 5mg/kg and vehicle dosed daily
- Results:
  - Increased blood pressure after day 1
  - Marked fall in heart rate after day 2
  - Decreased ejection fraction and diastolic dysfunction
  - Marked elevations in serum mineralization markers
  - Marked aortic myocardial multi-focal mineralization upon necropsy
- Result = decrease dosage and consider combination therapy approaches
Example of JET Dog support – Oncology small molecule

- Kinase Inhibitor
- Added JET with BP to an dog 14 day Early Toxicity Study
- Monitored for all dose groups 0, 5, 15 and 50 mg/Kg
- JET on Days -2, 2, and 11

Results
- Dose dependent increase in heart rate
  - Max effect on day 11 ~ 22 bpm most prevalent during lights out period
- Dose dependent decrease in diastolic, systolic and mean blood pressure:
  - Max drop ~20 mmHg on day 11 from 1-9 HPD
- Result = triggered stand alone dog cardiovascular study to better understand margins and potential risk
Example of Primate JET support – Oncology ADC

- Dolastatin family Toxin bound to monoclonal antibody
- N=2 monitored for 10 mg/kg cleavable form (TP2C001), 10 mg/kg non-cleavable form (TP2C002) and vehicle
- TP2C001 Results:
  - Increased heart rate, effects starting late in day 2, robust by day 6
  - Increased blood pressure on day 6
  - No noteworthy ECG effects
- TP2C002 Results: no change in BP or HR
- Exposure Results:
  - TP2C001: plasma instability of linker, separation in total Ab versus ADC
  - TP2C002: more stable in plasma

Result = move 002 forward stop 001
CNS Strategy for Oncology NCEs

Strategy #1: No perceived risk

Program with no perceived CNS risk → Appropriately timed clinical observations in toxicity studies

Strategy #2: *Perceived risk

Program with perceived CNS risk → FOB/neurological exam add-on to early toxicity study → FOB/neurological exam add-on to reg toxicity study

* Programs with perceived CNS risk: Indications in which the blood-brain barrier is compromised; Target expressed in central and/or peripheral nervous system; Off target pharmacology
Summary

- Early de-risking is important, especially if high target expression exists in the myocardium, vasculature, brain/nervous system.
  - Literature reviews
  - KO animal information

- Save resource and money using early screening to triage compounds before running larger and more expensive 14 day early toxicity studies
  - Can make translations from in vitro and ex vivo to in vivo studies

- Understanding any potential safety liabilities early will help the research team:
  - Select the best lead candidate with the least liability
  - Be better prepared for IND enabling packages and clinical trial designs

- Additional information on our echocardiography program can be found on poster by Jon Heyen and Michelle Hemkens
- Additional information on our isolated heart applications can be found on poster by David Ramirez and Steve Jenkinson
Additional slides
Early Screens: Cerep & hERG

**CEREPR**

**Candidate Guideline**

- If >50% inhibition at the test concentration then Ki’s will be determined
- Pass = If Ki values are > 100-fold the projected human Ceff free-plasma levels
- Fail = For hits <100-fold then functional activity should be determined
- Functional activity can be determined in vitro and/or in vivo

**hERG**

**Candidate Guideline**

- hERG Pass = <50% inhibition of current at 300x projected human Ceff free-plasma levels
- hERG Fail = If the compound has >50% inhibition of current NOAEL for QT interval/cardiac repolarization in a validated in vivo model should be 30-fold greater than the projected human Ceff free-plasma levels QT hurdle is passed in a validated in vivo model (e.g. anaethetized guinea pig)
Safety Pharmacology Add-Ons to Toxicity Studies

• Safety Pharmacology endpoints typically added to outsourced toxicology studies:
  – Jacketed External Telemetry (JET)
  – Neurofunctional Assessment (NFA)

• Biotherapeutic and oncology programs tend to add JET and NFA to toxicology studies:
  – Stand alone Safety Pharmacology studies are not required for biotherapeutics/oncology
    • typically meet CV and CNS regulatory expectations with surface lead ECG and clinical observations, respectively
  – However, some biotherapeutics and oncology agents carry a higher level of risk based on target expression, MOA, therapeutic indication, and patient population, and need more thorough evaluation:
    • Programs with potential CV risk (can add JET)
      – Diabetes, cardiovascular, or other metabolic indications
      – Projects with targets that are expressed in cardiac and/or vascular tissues
    • Programs with potential CNS risk (can add NFA)
      – Projects with targets expressed in central and/or peripheral nervous system
      – Indications in which the blood-brain barrier is compromised
        » Oncology, central or peripheral inflammatory disorders, diabetes

• Engage Safety Pharmacology early in ESD/SDS to design CV and CNS strategy
  – Allows for development of appropriate, proactive strategy
  – Engagement at ETS or beyond may be too late to design appropriate strategies
  – Will determine if JET or NFA add-ons to toxicology studies are appropriate
    • GSP will partner with STR for integration of add-on into toxicology study design, and data interpretation.
Study design (N values) should be driven by sensitivity required for decision making

- The gold standard assay (internal telemetry standalone study) is powered to detect the changes shown at right
  - Dog studies require N=4, nhp studies require N=8
  - nhp data is inherently more variable than dog, which drives the need for a higher N with this species

- We recommend N=4 for nhp JET studies for Biologics and Oncology programs
  - N=4 is not ideal, but we recognize that this is the best we can do in the context of an ETS

- We recommend N=2 for dog JET studies for Oncology
  - These JET studies are positioned to flag large CV concerns

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<th>Detectable differences, 80% Power, p&lt;0.05</th>
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<tr>
<td></td>
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<tr>
<td>mean BP (mmHg)</td>
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<tr>
<td>HR (bpm)</td>
</tr>
<tr>
<td>QTc ms)</td>
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<tr>
<td>QRS (ms)</td>
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<td>PR (ms)</td>
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Compound Attrition
- Impact Of Cardiovascular (CV) Issues

Cardiac post-approval adverse event reports

- Cardiac arrhythmias
- Coronary artery disorders
- Cardiac disorder signs and symptoms
- Heart failures
- Cardiac valve disorders
- Myocardial disorders
- Pericardial disorders
- Endocardial disorders

Number of AERS reports

Vascular post-approval adverse event reports

- Decreased and non-specific blood pressure
- Vascular hypertensive disorders
- Vascular disorders NEC
- Embolism and thrombosis
- Vascular haemorrhagic disorders
- Arteriosclerosis, stenosis, vascular insufficiency and necrosis
- Vascular inflammations
- Aneurysms and artery dissections
- Venous varices
- Lymphatic vessel disorders
- Vascular injuries

Number of AERS reports
Guinea Pig Langendorff Isolated Heart - Measurable Parameters

Model is ideal for looking at direct effect of compounds on heart
Ex-Vivo Tissue Bath Studies
- Cardiovascular Focus

- The utility of this model as highlighted by the effects of NS5a compounds on various parameters in Langendorff heart preparation correlated with rabbit L-type Ca$^{2+}$ channel blockade.

- The excellent correlation provides compelling evidence that the in vitro effect on rabbit L-type Ca$^{2+}$ channels does translate to a functional endpoint.
Example ETS+JET Study Designs

- 3-4 Jacket acclimation session with 24-48 hour intervals:
  - 4, 24, 36 hours (dog)
  - 4, 24, 36, 36 hours (nhp)
- On study, animals are jacketed afternoon prior to JET monitoring.
- JET Data acquisition (24 hour monitoring):
  - small molecule dog or nhp ETS: typically baseline, day 2, 6 and 13
  - biologic nhp ETS: typically baseline, day 2, 6 and 9 JET monitoring for biologics dosed on days 1 and 8
- Data are compared to baseline control values for assessment of drug effects

Example of use of Main study animals only

<table>
<thead>
<tr>
<th>Group</th>
<th>Males</th>
<th>Females</th>
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<tr>
<td>Control</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Low</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mid</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>High</td>
<td>1</td>
<td>1</td>
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Example of use of Main and Satellite animals to increase power

<table>
<thead>
<tr>
<th>Group</th>
<th>Males</th>
<th>Satellite</th>
<th>Main</th>
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<tbody>
<tr>
<td>Control</td>
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<td>Low</td>
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<tr>
<td>High</td>
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Animals for JET ECG and BP monitoring in red:

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What Makes A Good Payload/toxin?

- Inactive in the conjugate form
- Free drug does not penetrate cell membrane
  - Improved safety profile
  - Lack of bystander activity
- Binds molecular target selectively and with high affinity
  - High intracellular potency
- Lack of activity in normal, non-proliferating, non-transformed cells
- MOA of cytotoxins/payloads
  - DNA damaging: initiate double-stranded breaks
    - Calicheamicin
    - Doxorubicin derivatives
  - Tubulin inhibitors: prevent cell division
    - Auristatins (MMAE, MMAF)
    - Dolastatin (MMAD) and
    - Maytansins (DM1, DM4)
    - Taxanes
CNS Evaluations

- Addition of CNS endpoints to toxicity studies
  - Functional Observational Battery (FOB) in rodents or neurological exam in NHPs
  - Circumvents necessity to run stand alone assessment
- Programs with perceived CNS risk
  - Indications in which the blood-brain barrier is compromised
  - Target expressed in central and/or peripheral nervous system
  - Off target pharmacology

Use of FOB/Neurofunction assessments

- Designed to detect overt effects on general behavior, cranial nerves, and reflexes
  - Analogous to a simple human physical and neurological exam
  - “Is my compound safe to go into humans for Phase 1 testing?”
    - Hazard identification
    - Not a de-risking tool
- Caveats
  - Often detects only marked effects, & Does not detect many CNS AEs
  - Subjective
CNS & General Pharmacology Discipline
Andy Mead-Discipline Lead / Keri Cannon - BTx SME

- Comprehensive safety risk assessments related to the nervous, respiratory, and gastrointestinal systems; Target/Mechanism based

- Develop and execute safety de-risking strategies that assess, but are not limited to:

**SAFETY CONCERNS**

- Seizures
- Auditory deficits
- Anxiety
- Cognitive deficits
- Motor disturbances
- Abuse liability
- Visual disturbances
- Thermoregulation
- Sleep disturbances
- Overt CNS dysfunction
- Ataxia

**CAPABILITIES** (abbreviated list)

- Neurofunctional assessments
- 24 hr locomotor activity
- EEG assessments
- Abuse Potential Liability Studies
  - Self administration
  - Drug discrimination
  - Physical dependence & withdrawal
- Racine scale
- PTZ convulsion model
- Thermal place preference
- Contextual renewal
- OptoMotry
- Plethysmography
- Elevated plus maze
- Hot plate assessment