Development and Use of Quantitative Adverse Outcome Pathways:

Lessons Learned from Application to Cardiotoxicity

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Funding
National Institute of Health
P42 ES027704
T32 ES026568

U.S. Environmental Protection Agency:
STAR RD83516602
RD83580201

Society of Toxicology (Grimm):
Colgate-Palmolive and Syngenta Fellowship Awards
Outline

• Background – Cardiotoxicity of xenobiotics

• qAOP for QT/QTc prolongation as a case study integrating in vitro, in silico, and clinical data

• Lessons learned

Cardiotoxicity Hazards of Xenobiotics

Pharmaceuticals: YES

<table>
<thead>
<tr>
<th>Phase</th>
<th>Non-clinical</th>
<th>Phase I</th>
<th>Phase II-III</th>
<th>Phase III/ post-approval</th>
<th>Post-approval</th>
<th>Post-approval</th>
<th>Post-approval</th>
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</thead>
<tbody>
<tr>
<td>Information</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</td>
<td>Withdrawal from sale</td>
</tr>
<tr>
<td>Sample size</td>
<td>88 CDs stopped</td>
<td>1,015 subjects</td>
<td>82 CDs stopped</td>
<td>1,136 drugs</td>
<td>21,298 patients</td>
<td>121 drugs</td>
<td>47 drugs</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>12%</td>
<td>9%</td>
<td>10%</td>
<td>13%</td>
<td>12%</td>
<td>9%</td>
<td>4%</td>
</tr>
<tr>
<td>Hepatotoxicity</td>
<td>8%</td>
<td>7%</td>
<td>10%</td>
<td>13%</td>
<td>0%</td>
<td>12%</td>
<td>4%</td>
</tr>
</tbody>
</table>

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

Environmental Exposures: ??

- Air Pollution: YES
- Other exposures: Maybe
  - Little data beyond epidemiologic studies of a few chemicals (air pollution, lead, environmental tobacco smoke,...)
  - Not routinely tested for in experimental animal studies
QT interval at a biomarker of cardiovascular risk

• Genetic and drug-induced QT prolongation known to increase risk of sudden cardiac death.

• Emerging (last 3-5 years) literature on baseline QT as a risk factor in the general population:
  – Sudden cardiac death (e.g., Deo et al. 2016);
  – Major cardiovascular event or death (e.g., Shah et al. 2016);
  – Stroke, independent of atrial fibrillation (e.g., O’Neal et al. 2015).

\[\text{Tissue} \quad \text{Organism} \quad \text{Population}\]

- QT interval prolonged \rightarrow Increased likelihood of torsades de pointes \rightarrow Increased likelihood of myocardial infarction \rightarrow Increased incidence in the general population
Current Drug Testing Strategy for Cardiotoxicity Focuses on QT prolongation

- Multi-million dollar clinical trial – the “Thorough QT/QTc” (TQT) study – required even without preclinical concerns.
- Threshold of regulatory concern = “upper bound of the 95% confidence interval around the mean effect on QTc of 10 ms.”
- Highly successful in reducing cardiotoxicity in approved drugs.

Current Chemical Safety Testing Strategy for Cardiotoxicity ... *Does Not Exist*

- Rodents are fed low fat diets, and are not monitored for cardiotoxicity beyond pathology.
- Main preclinical models (e.g., dog) are not routinely used for non-pharmaceuticals.
- Most data on cardiovascular effects of chemicals is from epidemiology – effects may already be occurring in the population.
- *How can mechanistic data help inform cardiotoxicity?*
Current Chemical Safety Testing Strategy for Cardiotoxicity ... *Does Not Exist*

<table>
<thead>
<tr>
<th>Human Epidemiology</th>
<th>Experimental Animals</th>
<th>Mechanistic Data</th>
</tr>
</thead>
</table>
| • Data available only for a few well-studied chemicals | • Rodents are fed low fat diets, and are not monitored for cardiotoxicity beyond pathology.  
• Main preclinical models (e.g., dog) are not routinely used for non-pharmaceuticals. | • In vitro channel blocking assays are not routinely used for non-pharmaceuticals.  
• iPSC-derived cardiomyocytes           |
Potential of iPSC-derived cardiomyocytes

- Low cost (compared to clinical trials)
- Potential for population-level and patient-specific testing
- Phenotypically-relevant
  - Beat synchronously *in vitro*
  - Exhibit expected response to congenital and drug-induced cardiotoxicity
- Reproducible resource (unlike primary cells)
Potential Cardiotoxicity from Environmental Chemicals?

- Cardiomyocytes *in vitro* reproduce *in vivo* phenotypes for cardiotoxic drugs.
- Environmental chemicals also affect beating rhythm and other parameters *in vitro*.

Proof of Principle Recently Published

Potential role of (q)AOPs in systematic review of mechanistic data

• Organizing framework for scoping and problem formulation (NASEM 2017 Report; Smith et al. 2016).

• Relating in vitro concentrations associated with mechanistic data to evidence on internal and external doses associated with health effects in vivo in animals and humans (beyond “standard” IVIVE).

• Relating the evidence from short-term mechanistic studies to outcomes after longer term exposure in animals and humans.

QT/QTc prolongation as a case study
Outline

• Background – Cardiotoxicity of xenobiotics

• qAOP for QT/QTc prolongation as a case study integrating *in vitro, in silico*, and *clinical* data

• Lessons learned

*Thorough QT/QTc in a Dish: An In Vitro Human Model That Accurately Predicts Clinical Concentration-QTc Relationships.*
Why quantitative AOP?

- Most AOPs present a deterministic series of events in homogenous populations
- Most AOPs can only inform Hazard ID
- Quantitative AOP enables incorporation of stochastic events and population variability
- Quantitative AOP predicting exposure-response
  - Provides greater confidence in Hazard (a la “Hill” criteria/GRADE, etc.)
  - Can also inform dose-response assessment
(q)AOP for QT/QTc prolongation

**ADME**
- Absorption
- Protein binding
- Hepatic Metabolism
- Renal Excretion

**Molecular**
- Chemical blocks ion channel
  - Chemical
  - Extracellular
  - Intracellular
  - OR Other mechanisms

**Cellular**
- Action potential prolonged

**Tissue**
- QT interval prolonged
- Increased likelihood of torsades de pointes

**Organism**
- Increased likelihood of cardiovascular morbidity
- Increased likelihood of death

**Population**
- Increased incidence of cardiovascular morbidity and mortality in the general population

*In vitro-in silico TK model*  
*In vitro-in silico TD model*  
*In vivo effects at individual and population levels based on clinical and epidemiologic data*  

Incorporate population variability
(q)AOP for QT/QTc prolongation

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Population-based in vitro testing + Bayesian concentration-response modeling

Accuracy confirmed by comparing to clinical in vivo drug data
Study Design

Chemicals (drugs) with corresponding \textit{in vivo} clinical data

Positive for \textit{in vivo} QTc prolongation
- Cisapride
- Citalopram
- Disopyramide
- Dofetilide
- Moxifloxacin
- N-acetylprocainamide
- Quinidine sulfate
- Sematilide
- Sotalol
- Vernacalant

Negative for \textit{in vivo} QTc prolongation
- Cabazitaxel
- Lamotrigine
- Mifepristone

Blanchette et al., 2018; Sirenko et al. 2013, 2017
Establishing **qualitative and quantitative** *in vivo* and *in vitro* correspondence

**Qualitative Comparison**

- **Normal ECG**
  - *In vivo*: Normal beating
  - *In vitro*: Normal beating

- **QT Prolongation**
  - *In vivo*: ↑ Decay/Rise Ratio
  - *In vitro*: ↑ Decay/Rise Ratio

- **Torsades de pointes**
  - *In vivo*: Notch formation
  - *In vitro*: Notch formation

- **Quiescence**
  - *In vivo*: ↓ Amplitude + ↑ BPM
  - *In vitro*: No beating

**Quantitative Comparison**

- **In vivo**: use published PD modeling results for concentration-response relationships for QTc
- **In vitro**: conduct Bayesian population PD modeling (Chiu et al. 2017) of decay-rise ratio
- **Compare in vivo and in vitro** concentration-response relationships (e.g., median and their CI)
Establishing **qualitative and quantitative** *in vivo* and *in vitro* correspondence

**In Vivo**

- **Common dose metric**
  - Literature-based values for free fraction in serum used to re-scale total concentrations to free concentrations

- **Common effect metric**
  - Study-specific values for baseline QTc used to re-scale responses to percent change from baseline

**In Vitro**

- **Common dose metric**
  - Free fraction measured in serum and cardiomyocyte media using Rapid Equilibrium Dialysis
  - Media free fraction results compared to those from mass-balance model

- **Common effect metric**
  - Reparameterized Hill directly predicts percent change from baseline

*Model predictions restricted to concentrations ≤ study-specific Cmax.*
Thorough-QTc Study in a Dish

1. Baseline QTc in Relevant Patient Population
2. In Vitro Concentration-Response Model
3. Conversion from Media to Plasma Concentrations
4. Predicted Probability of $\Delta$ QTc $\geq$ 10 ms

NHANES Population C-QTc Equilibrium Dialysis
Results:

Model Development and Evaluation

• All 10 positive control drugs exhibited
  – Increased decay-rise ratio in multiple donors
  – Notch formation in multiple donors

• For 3 negative control drugs
  – Some donors exhibited increased decay-rise ratio
  – No donors exhibited notch formation

• Population concentration-response model accurately fit experimental data
Results:
Qualitative Predictions (Hazard)

- **In vivo hazard for QTc prolongation** can be predicted from *in vitro* data

- *In vitro* model correctly predicted observed effect/no effect at *in vivo* free Cmax
  - Known positive compounds:
    - Predicted effects from 1% to 46% at *in vivo* free Cmax
  - Known negative compounds:
    - Predicted effects < 0.01% at *in vivo* free Cmax
    - Upper confidence bound estimates of <0.5%
Results:
Quantitative Predictions

Positive Control

Disopyramide Percent Change Peak Decay Rise Ratio

Free Concentration (uM) vs. Decay Rise Ratio (Percent Change)

- Standard Donor (1434)
- in vitro pop median
- in vivo Hill
- in vivo Linear
- in vitro random indiv CI

Highly Consistent Concentration-Response Relationships in vitro to in vivo!

Negative Control

Cabazitaxel Percent Change Peak Decay Rise Ratio

Free Concentration (uM) vs. Decay Rise Ratio (Percent Change)

- Standard Donor (1434)
- in vitro pop median
- in vivo Linear
- in vitro random indiv CI
Results:
Quantitative Predictions

Typical prediction error of < 3-fold!

Population-based prediction more accurate and more precise than using a single donor
Results: Clinical Translation

- Clinical translation of *in vitro* C-QTc modeling results involves determining the probability that clinical $\Delta$QTc($x_{\text{plasma}}$) > 10 ms
- All the positive controls except moxifloxacin, clearly fail the regulatory safety threshold at $C_{\text{max}}$.
- All negative controls except lamotrigine clearly satisfy the regulatory safety threshold.
- For moxifloxacin and lamotrigine, results more ambiguous, with different conclusions at population versus individual level (consistent with clinical literature).
Summary of Results

The combination of a population-based *in vitro* model and *in silico* pharmacodynamic modeling can accurately predict the results of the *in vivo* clinical TQT study:

- Concentration-QTc relationship
- Range of clinical concentrations that satisfy the regulatory threshold (<10 msec at 95% confidence)
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- Increased likelihood of torsades de pointes
  - EAD

**Organism**
- Increased likelihood of cardiovascular morbidity
  - Mortality

**Population**
- Increased incidence of cardiovascular morbidity and mortality in the general population

Population RTK (IVIVE) Models

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Accuracy confirmed by comparing to clinical in vivo drug data

Published Clinical Epidemiology-Based Cardiovascular Risk Models

**Concentration (µM)**

<table>
<thead>
<tr>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>100</td>
</tr>
</tbody>
</table>

**Liver**

**Richly perfused tissues**

**Poorly perfused tissues**

Accuracy confirmed by comparing to clinical in vivo drug data

Figure: Calibration plot of the National Health and Nutrition Examination Survey (NHANES) ECG-derived AF burden score and the stimulation of the atrial ischemic event rate
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Why does it work?
Building a better AOP with biomarkers

Molecular initiating event (MIE) is not necessary or sufficient to build a qAOP

Quantitative/predictive biomarker such as QTc can serve as the critical link from cell/tissue \(\rightarrow\) individual \(\rightarrow\) population

Advantages:
\begin{itemize}
  \item Enables evaluation of qualitative and quantitative correspondence between model system (e.g., iPSC-based organotypic cultures) and \textit{in vivo} human effects
  \item Takes advantage of \textit{clinical biomedical literature} on biomarkers and risk prediction
  \item Naturally focuses efforts on endpoints with \textit{human relevance} and \textit{public health impact}
\end{itemize}