“If mice were only people....”

Re-thinking Pre-Clinical In Vivo Models To Increase the Probability of Clinical Success

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Pharma Industry is Challenged

- Many analyses that probe and propose reasons for the decrease in R&D productivity.
- Consensus is that drug discovery needs to change to be able to deliver novel drugs in the current environment.
Target Discovery

Lead Discovery

Identify a cellular or molecular structure involved in the pathology of interest.

Druglike Properties

5,000-10,000

Cost

Preclinical

Clinical

Ph I

Ph II

Ph III

Ph IV

Preclinical Safety/Tox

Investigational N

Purpose: demonstrate and has pharmacologic

New Drug Application

Sponsor’s Purpose: seek approval to market.

Purpose: Clinical data support labeling claims humans.

Cost to bring a drug to market was $2.56 billion in 2013 dollars. (Tufts Center for the Study of Drug Development).
Drug Discovery—Convergence of Disciplines

- Combinatorial Chemistry
- Synthetic Chemistry
- Patent Law
- Project Management
- Medicine
- Epidemiology
- Enzymology
- Biochemistry
- Immunology
- Pharmacology
- Toxicology
- Genetics
- Behavior
- Pathology
- Physiology
- Information Technology
- Modelling
**Problem:** Drugs discovered in the Preclinical stage frequently fail to translate into clinical success (~10% overall success).


**2000’s**

- Preclinical
- Ph 1
- Ph 2
- Ph 3

**But why doesn’t the preclinical work translate to humans?**
Animals ≠ Humans

- Genetic
- Surgical Manipulation
- Mimic a chronic condition in an acute time-frame.
- Apply animal behavior to a human behavior.

- Effects in preclinical in vivo disease models are poor predictors of efficacy in the clinic.
Pathological Process

PHARMACOKINETICS

**Drug** → **Target Exposure**

- **C_p** (Plasma)
- **C_e** (Target site)

**PHARMACODYNAMICS**

**Target Engagement**

- Target Occupancy
- Target Mechanism
- Biological Process

**Transduction to Efficacy/Safety**

- Pathological Process
Think about it Differently

1. An integrated and quantitative understanding of the PKPD relationships and how these translate to humans.
   - Human PK – can we get enough drug to where it needs to be?
   - Human Target Engagement – can we modulate the target with the right intensity and duration?

2. Confidence in target-disease linkage.
   - Human genetics
   - Validated preclinical model

3. Identify and test assumptions
   - Integrate data to reduce assumptions
   - Identify critical assumptions and assess impact

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**PHARMACOKINETICS**

- Dose → $C_p$ (Plasma) \(\rightarrow\) $k_{eo}$ \(\rightarrow\) $C_e$ (Target site) \(\rightarrow\) $k_{on}$ \(\rightarrow\) Target Engagement

**PHARMACODYNAMICS**

- Target Occupancy \(\rightarrow\) Target Mechanism \(\rightarrow\) Biological Process \(\rightarrow\) Pathophysiology
  - Outcome
  - Transduction to Efficacy/Safety
Translational Biomarker Bubble Diagram

Human

In-Vitro Assays

Drug Concentration (PK)

Target Engagement Biomarker

Target Mechanism of Action Biomarker

Physiological Response Biomarker

Pathophysiology or Disease Process

Efficacy / Outcome

Preclinical Species

In-Vitro Assays

Drug Concentration (PK)

Target Engagement Biomarker

Target Mechanism of Action Biomarker

Physiological Response Biomarker

Pathophysiology or Disease Process

Efficacy / Outcome
Inhibit CETP \rightarrow \text{Increase HDL} \rightarrow \text{Protect from CVD}
What do we need to ask? / What do we know? / What do we have?

1. What is the target? → CETP
2. What do we want the drug to do? → Inhibit CETP
3. What is the effect of inhibiting CETP? → Increases HDL

- Commercial CETP Inhibitor Screening Kit
  - Human → Yes
  - Blood → Yes

- Commercial HDL Screening Kit
  - Human → Yes
  - Blood → Yes

- Measure of clinical effect = Decreased CVD related deaths

- Able to measure drug levels in the circulation (Pharmacokinetics)

- Human CETP Transgenic Mouse
  - CVD

- Mice do not develop CVD naturally.
  - Mice ≠ Humans
Translational Biomarker Bubble Diagram: CETP Inhibitor Program

**Proof of Mechanism**
- Drug Concentration (PK)
- Target Engagement Biomarker
- Target Mechanism of Action Biomarker
- Physiological Response Biomarker

**Proof of Concept**
- Pathophysiology or Disease Process
- Efficacy / Outcome

**Human**
- In-Vitro Assays
- CETP inhib. w/ Kit

**CETP Mouse**
- In-Vitro Assays
- CETP inhib. w/ Kit

**PK Assay**
- CETP inhib. w/ Kit
- CETP inhib. From human blood
- HDL

**Arrow** = Can be measured both in preclinical experiments and in human clinical trials

CETP inhib.

PK Assay

HDL

CV Deaths

Diabetes, Athero, HT, etc.
Case Study
Case Study
Anti-Thrombotic Drug
For Stroke and AFIB
What are the Main Cause and Effects of Thrombotic Events

1. **Cause**: Atrial Fibrillation
2. **Effect**: Stroke(s)

What is the **Human** cost?

1. Lives
2. Annually, Death rate is nearing 300,000 in the USA
3. More than 1,500,000 hospitalizations annually

What is the **Financial** cost?

1. Personal
2. Broad Economic losses (work related)
3. Forty Billion ($40,000,000) dollars!
Atrial Fibrillation - AFIB

Disease of the heart characterized by irregular and often faster heartbeat.

Causes of an Irregular Heartbeat

- Hypertension, Diabetes, Congestive heart failure, Dehydration, Hyperkalemia, Mitro-valve Prólapse, Poisoning, (cocaine, amphetamine, digitalis…), Anaphylaxis, hyper & hypo-thyroidism, Cardiomyopathy

Indications

- Abnormal electrical discharges (signals) that generate chaotically throughout the upper chambers of the heart (atria).
- Reduction in the Atria to pump blood into the ventricles
  - Response is the heart to beat too rapidly.
- AFIB causes turbulence of the blood which causes clot formation.
- [https://youtu.be/fxUITWjrhh](https://youtu.be/fxUITWjrhh)
- Management: Rate Control, Maintenance of normal rhythm, stroke prevention

Cost

- Prevention: Pharmacological and/or surgical (Cardio conversion, Catheter Ablation, surgical ablation, atrial pacemaker)
- Annually more than 130,000 deaths in the USA.
- More than 750,000 hospitalizations occur annually.
- AFib costs the United States about $6 billion each year, diagnosis and treatment.
Strokes

Sudden death of brain cells from lack of oxygen.
  • Caused by blockage of blood flow or rupture of an artery to the brain.
    • Ischemic stroke (part of the brain loses blood flow, bleeding from periphery)
    • Hemorrhagic stroke (brain bleed, Transient Ischemic Attack, TIA >24 hours)

Indications
  • Sudden loss of speech, weakness, or paralysis of one side of the body can be symptoms.
  • Confirmation by scanning the brain with special X-ray tests, such as CAT scans.

Costs
  The death rate and level of disability resulting from strokes can be dramatically reduced by immediate and appropriate medical care.
  • Prevention involves minimizing risk factors, such as controlling high blood pressure and diabetes.
  • Stroke kills about 140,000 Americans each year—that’s 1 out of every 20 deaths.
  • Annually 795,000 people in the United States have a stroke. About 610,000 of these are first or new strokes
  • About 87% of all strokes are ischemic strokes.
  • Stroke costs the United States an estimated $34 billion

Animal Models: AV Shunt
Project A: Anti-Coagulant Drug Discovery Program: Intrinsic Pathway Inhibitor
Coagulation Factor Targets of Currently Approved SOC or Anti-Coagulant Drugs

ELIQUIS (apixaban)
PRT-054,021 (betrixaban)
SAVAYSA(edoxaban)
XARELTO(rivoroxaban)

Vitamin K antagonists (warfarin)
What do we need to ask?/What do we know?/What do we have?

1. What is the target? \(\rightarrow\) FIXa
2. What do we want the drug to do? \(\rightarrow\) Inhibit excess thrombotic activity
3. What is the effect of inhibiting factor 9? \(\rightarrow\) Decreased in thrombotic events

- **Commercial FIXa Inhibitor Screening Kit**
  - Human \(\Rightarrow\) Yes
  - Blood \(\Rightarrow\) Yes

- **Commercial aPTT Screening Kit**
  - Human \(\Rightarrow\) Yes
  - Blood \(\Rightarrow\) Yes

- **AVS Model**
  - Able to measure drug levels in the circulation (Pharmacokinetics)

- **Human AVS Model**
  - Long time period
  - Measure of clinical effect = Decreased CVD related deaths

- **Rabbits**
  - \(\bullet\) Rabbits do not develop CVD naturally.
  - \(\bullet\) Rabbits \(\neq\) Humans

- **FDA APPROVED**
aPTT: A Routine Clinical Test and a Biomarker of Intrinsic Pathway Coagulation

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

TIME TO CLOT

Inhibitors of the Intrinsic Pathway $\rightarrow$ $\uparrow$ APTT
Arteriovenous (AV) Shunt in Rabbits: Model to assess anti-coagulation.

**Method:**
- Connect an artery and vein to a shunt.
- A thrombogenic stimulus (e.g. thread) is inside the shunt.
- As blood flows through the shunt → clot will form on the thread.
- Weigh the clot.

**Anti-Coagulation** = clot weight, blood sampling

**Issues:** AVS is a mixed arterial venous antithrombotic model.

**Needs:** To enhance our confidence in the predictive value of our preclinical models, translational, or creating a plan to further characterize activity of SoC, define target engagement levels etc.
Arteriovenous (AV) Shunt in Rats: Model to assess anti-coagulation.

- **Method:**
  - Connect an artery and vein to a shunt.
  - A thrombogenic stimulus (e.g. thread) is inside the shunt.
  - As blood flows through the shunt → clot will form on the thread.
  - Weigh the clot.

- **Anti-Coagulation** = clot weight
- Can take blood samples from the AVS rats.
Work Sheet
Tranlsational Biomarker Bubble Diagram:

Human

In-Vitro Assays

Drug Concentration (PK)

Target Engagement Biomarker

Target Mechanism of Action Biomarker

Physiological Response Biomarker

Pathophysiology or Disease Process

Efficacy / Outcome

Model

In-Vitro Assays

Drug Concentration (PK)

Target Engagement Biomarker

Target Mechanism of Action Biomarker

Physiological Response Biomarker

Pathophysiology or Disease Process

Efficacy / Outcome

Arrow = Can be measured both in preclinical experiments and in human clinical trials
Explanation of Bubbles

**In vitro assay**: Measure of in vitro activity, such as potency or affinity

**Drug concentration**: The pharmacokinetics of the compound typically measured as unbound plasma concentrations and/or target site exposure

**Target engagement biomarker**: Measurement of compound binding to target, such as with PET or measuring antibody bound to antigen.

**Target Mechanism of Action Biomarker**: A proximal biochemical or proximal physiological (eg electrophysiological) response as a result of compound interaction with the target

**Physiological Response Biomarker**: A physiological or tissue response driven by compound activity at the target, but not directly linked to pathophysiology

**Pathophysiology or Disease Process**: A biochemical response involved in the disease process or activity in an animal model or on a clinical endpoint that serves as an index of the disease process

**Efficacy/Outcome**: Activity in an animal model of disease that has been demonstrated to predict clinical efficacy or positive effect on a clinical endpoint endorsed by regulators as sufficient for approval
**Case Study: Translational Biomarker Bubble Diagram**

**Human**
- **In-Vitro Assays**: aPTT
- **Drug Concentration (PK)**
- **Target Engagement**
- **Ex vivo aPTT, PT**
- **Target Mechanism of Action**
- **Physiological Response**
- **Pathophysiology or Disease Process**
- **Proof of Concept**
  - Phase II: Thrombotic events; Stroke, TIA, Pulmonary
  - Phase III: CVD Related Deaths

**Rabbit**
- **In-Vitro Assays**: aPTT, Plasma
- **Drug Concentration (PK)**
- **Target Engagement**
- **Ex vivo aPTT**
- **Physiological Response**
- **Pathophysiology or Disease Process**
- **Proof of Concept**
  - X

**Green shading** = Biomarkers that are translational and/or support IVIVC and have been validated/reduced to practice
**Blue arrow** = Need for mathematical translational modelling across species
* Indicates assay in development