The Value of Phenotypic Screening to Drug Discovery: Historical Perspective

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Big Sky, Montana

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Take home messages

**Phenotypic (empirical)** approaches are more successful for first-in-class medicines.

Molecular mechanisms of action (MMOAs) that provide a useful therapeutic index are *a priori* difficult to predict.

**Target** is not the exclusive definition of mechanism.

Need to redefine what mechanism means which regards to drug discovery and development.
Outline

Historical perspective
   Part 1. How medicines work

   Part 2. How medicines are discovered

Future perspective
   Part 3. How to realize the full value from phenotypic drug discovery?
Artemisinin-discovered with phenotypic screening

- 284–346 AD- *Artemisia* extract described to have antimalarial activity
  - “A Handbook of Prescriptions for Emergencies” by Ge Hong (Jin Dynasty).
- early 1970s You-You Tu, at the Institute of Chinese Materia Medica, China Academy of Traditional Chinese Medicine found a promising degree of inhibition against parasite growth
- extracts were evaluated in mouse malaria model for *plasmodium berghei*.
- Artimisinin approved for Malaria 2006
- 2015 Dr Tu awarded Noble prize in medicine

**Omeprazole**-discovered with phenotypic screening

- **Indication:** GERD (gastroesophageal reflux disease)
  - 1972 **dogs** used as the initial screening model & started with toxic anti-secretory molecule developed by Servier
  - Initially **optimized for accumulation of weak bases in the acidic compartment of parietal cell close to proton pump**
  - Later mechanistic studies showed acid mediated conversion to the active species
    - activation to reactive intermediate by low pH
    - irreversible inhibition of $\text{H}^+,\text{K}^+\text{ATPase}$

We look for knowledge to provide a blueprint for discovery and initial use of the medicines.
Pharma Productivity has decreased while the number of NMEs are unchanged.
Ten First-in-class medicines approved per year

Improved Measures of Drug Innovation
(2013) Health Affairs 15, 1433
Is the current drug discovery process optimal to discover innovative new medicines?
Drug action begins with binding
‘Corpora non agunte nisi fixata’
A *substance will not work unless it is* bound
-Paul Ehrlich, 1913

Molecular Mechanism of Action (MMOA)

- MMOA: mechanism through which specific molecular interactions between the drug and its target result in an effective and safe pharmacological response.
  - Includes binding kinetic and conformational changes that specifically provide a therapeutically useful response.

MMOA-pharmacological hot spot
Aspirin and Ibuprofen: Two Medicines, One Target, Different Molecular Mechanisms, Different Uses

• Aspirin has anti-platelet activity whereas NSAIDs do not
  ➢ Effective for prevention of atherothrombotic disease

• Both bind to the active site of cyclooxygenase 1 and 2
  ➢ Aspirin irreversible inactivation via acetylation of Ser530
  ➢ Ibuprofen and other NSAIDS are reversible

• Irreversible action of aspirin in platelets leads to long lasting anti-thrombotic effects
  ➢ Platelets do not have the capacity to resynthesize new protein
Estrogen Receptor Modulators
One Target, Different Molecular Mechanisms, Different Uses

Ligand induced conformational changes recruit coactivators and corepressors in a context specific manner.

Differentiated therapeutic use

**Estradiol** agonist
- postmenopausal hormone deficiency

**Tamoxifen** SERM (selective estrogen receptor modulator)
- breast cancer

**Raloxifen** SERM
- osteoporosis

ER ligand binding domain

Topoisomerase/gyrase inhibitors
Bind to transient conformational state.

Quinilone anti-bacterials
-trap transient conformational intermediate.

- MMOA:
  - topoisomerase form intermediate cleavage complex with DNA
  - Quinilones bind to intermediate complex
  - Block movement of replication forks and transcription complexes

Leo JBC, 280, 14252 (2005)
• Communication of information as an analogy of MMOA

  – Proximity is rarely sufficient for effective sharing of specific information
  – MMOA is a language to communicate specific information.

  ‘Pharmacological hot spots’
MMOA provides a mechanism for safety and selectivity.

- **Physiology**
  - spatial and temporal controlled of physiologic responses

- **Drug Discovery**
  - flood the system with drug.
  - Endeavor to maintain drug concentrations above IC\textsubscript{50} per dosing interval.

**Mechanisms of approved drugs 2001-2004**  
Swinney *CTMC* 6, 461, 2006
PART 2

How medicines were discovered.
Drug discovery is an iterative process starting with an unmet medical need and an idea to address that need.

- **Medical Need**
  - Discovery strategy
  - Translation biomarker

- **Drug Candidates**
  - Discovery
    - Lead identification
    - Pharmacological mechanism
  - Drug discovery uses an iterative learn and confirm cycle

- **Basic research**
  - Starting pt (assay/target)
  - Discovery strategy

- **Drug Approval**
  - Drug approval
    - Phase 3 Efficacy
  - Development
    - Proof of concept
    - Clinical safety
  - Discovery
    - Lead optimization
Types of drug discovery

• Phenotypic (PDD)
  – start with a functional assay such as a cells, tissue or animal
  – measure activity against a surrogate/biomarker related to the disease
  – the assays do not require prior understanding of underlying mechanism
  – empirical

• Target based (TDD)
  – start with a molecular target such as an enzyme, receptor, channel
  – apply molecular and chemical knowledge to investigate specific molecular hypotheses

• Mechanistic-informed phenotypic drug discovery
• Natural substance- enzymes, modified peptides and cofactors
• Biologics- mabs
Conclude the value of phenotypic assays is to discover new MMOAs which are difficult to \textit{a priori} predict

The majority of small molecule \textbf{first in class} medicines were discovered with \textbf{phenotypic} strategies (28 to 17)

\textbf{-followers} were discovered with \textbf{target-based} strategies (83 to 30).

NMEs approved FDA 1999-2008
- 259 total
- 183 small molecules
- 20 imaging agents
- 56 therapeutic biologics

75 first in class
164 followers

\textbf{NME- new molecular entity}
First in class medicines discovered with phenotypic screening

28 discovered by phenotypic screening

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<th>Phenotypic screening</th>
<th>Serendipitous discoveries</th>
<th>Intentional targeting of specific phenotype</th>
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<td>Daptomycin*</td>
<td>Azacitidine*</td>
<td>Memantine</td>
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<td>Ezetimibe</td>
<td>Caspofungin*</td>
<td>Sinecatechins*</td>
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<td>Linezolid</td>
<td>Cilostazol</td>
<td>Vorinostat</td>
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<td>Nateglinide</td>
<td>Cinacalcet</td>
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<td>Pemirolast</td>
<td>Docosanol*</td>
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<td>Rufinamide</td>
<td>Levetiracetam</td>
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<td>Screening of random compound library</td>
<td>Lubiprostone*</td>
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<td>Miglustat</td>
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<td>Nelarabine*</td>
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<td>Sinecatechins*</td>
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<td>Vorinostat</td>
<td>Varenicline</td>
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Ezetimibe-predecessors discovered in animal models

Cinacalcet predecessors discovered using a library of phenylalkylamines in a bovine parathyroid cells

Vorinostat: the effect of DMSO on cells due to inhibition of HDACs

Varenicline mechanism driven approach to reduce toxicity by looking for partial agonists
First in class medicines discovered with target-based screening

17 discovered by target-based screening

Phenotypic screening was essential for many of the discoveries

MIPDD
Conclusions

• First-in-class medicines discovered with empirical strategies
• Majority of resources on reductionist target-based strategies

mechanistic paradox

- the knowledge of mechanism (e.g. how a drug works) is very helpful to discover and precisely use medicines
- the knowledge available is rarely sufficiently complete to provide a blueprint for discovery and initial use of the medicines.
Definitions of phenotypic drug discovery

• **Knowledge based definition**-Empirical- knowledge of specific molecular mechanism of action (MMOA) not assumed.

• **Process definition**-testing of a large number of compounds in a systems-based approach using a target-agnostic assay that monitors phenotypic change.
First in class approvals by US FDA

78 were from TDD and only 8 from PDD
1999 to 2013
Eder *et al* NRDD 2014

28 from PDD and 17 from TDD
1999 to 2008
Swinney & Anthony NRDD 2011
The discovery of first-in-class drugs: origins and evolution
Eder, Sedrani & Weismann NRDD 13, 577, 2014

- phenotypic screening: testing of a large number of compounds in a systems-based approach using a target-agnostic assay that monitors phenotypic change.
  - assumes that no mechanistic information is available.
- “chemocentric” was used to categorize systems-based approaches in which an active component had been identified previously such as isolation of aspirin from willow bark.
PART 3

How to get the most value from PDD?

TDD - most effective when mechanistic knowledge is complete

PDD – Does not require complete mechanistic knowledge
Why is there such strong support for TDD?

- when it works (when the target/MMOA are validated) it provides a rational approach, analogous to engineering.
- aligns with the potential for genetics to inform the cause of disease and provide biomarkers.
- enables better selection of patients for clinical trials and increases the probability of success.
- use structure based design to a specific target allows optimization of efficacy and drug like properties in a rational way.
- used by clinical pharmacology to set doses related to target occupancy in order to maximize the therapeutic index.
- provides a metrics to communicate how the drug works to the stakeholders across the value chain, from basic researchers to chemist to developers to funders.
strengths of PDD and TDD compliment their respective weaknesses.

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<tr>
<th>STRENGTHS</th>
<th>TDD</th>
<th>PDD</th>
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<tr>
<td>Knowledge based</td>
<td>Knowledge based -structure based design -PK/PD predictions -Patient selection</td>
<td>Empirical -System-based -Identification of MMOA -Early safety evaluation</td>
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<td>WEAKNESS</td>
<td>-Most available knowledge is incomplete -Not systems based -Target selection -Identification of MMOA</td>
<td>Difficult to use empirical findings with -Structure based design -PK/PD predictions -Patient selection</td>
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Identification MMOA “pharmacological hot spot” is required for reductionist, engineering approach.

Molecular, target-based strategy

identify target, MMOA and networks of first-in-class

Target is biomarker for disease

first in class

‘pharmacological hot spot’

mechanistic

empirical

Unmet medical need

Translational biomarkers Clinically relevant

phenotypic assay that translates to human disease

Screen with limited mechanistic assumptions-

Swinney, CPT 93, 299 2013
Challenges

• Do not know mechanism/target?

• Can not optimize using structure?

• How do you get funding?
Mechanistic paradox - mechanism has different meaning and value depending on context

• Target
• MMOA
• Effect on a phenotype or biomarker
  – Cholesterol-lowering
  – Anti hypertension
• Physicians and prescribers think of mechanistic differences within drug class-
  – Avoid resistance
• Mechanism is not important for drug approval
  – Janet Woodcock, Head of FDA
• Patients rarely are concerned with mechanism

Change the definition of mechanism
PDD provides opportunity for innovation

• Need and opportunity to further refine a discovery process for first in class medicines that
  – Is appropriate for PDD
  – That is not held hostage by the need to identify an exact mechanism
  – That redefines what mechanism means in terms of identification of new therapies for patients.
How can we do things differently to increase the number of medicines approved and patients?

**Precision medicine initiative**

- **Access of available knowledge**
  - Genomics, translational biomarkers
  - Predictive models pharmacology
  - New innovative medicines for patients

**Modeling and simulation of systems pharmacology**

**Quantitative Systems Pharmacology**

*When quality beats Quantity*

Scannell & Bosley, PLOSone 2016
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Need to redefine what mechanism means which regards to drug discovery and development
Institute for Rare and Neglected Disease Drug Discovery, aka iRND3

Non-profit 501c3 drug discovery organization
Well equipped laboratory Mountain View, CA, USA
Experience drug discovery team with many years of Pharma experience
www.irnd3.org

Mission
iRND3's mission is to discover new medicines for rare and neglected diseases

Vision
-to utilize our understanding of how successful new medicines are discovered to implement and execute drug discovery strategies that supply our growing pipeline of new candidate medicines for rare and neglected diseases.

-to evolve an innovative, socially responsible operational model for successful collaborations between the non-profit, private and public to translate scientific discoveries to a continuous source of new medicines.