Evaluation of Preclinical Development Candidates

Yong X. Wang, MD & PhD
King’s Lab
Shanghai Jiao Tong University School of Pharmacy

yxwang@sjtu.edu.cn
http://kinglab.sjtu.edu.cn
The Drug Discovery Process

12-15 Years and $1.3 billion in 2010
Pharmaceutical Industry R&D

• Research based pharmaceutical Co. spend about 20% of sales on R&D;
• Significantly higher than other industries: electronics, aerospace, automobiles, and computers;
• Since 1980 US pharmaceutical Co. doubled spending on R&D every 5 yrs;
• A steady decline in the number of drugs introduced each year into human therapy
  – 70-100 in the 60s
  – 60-70 in the 70s
  – ~50 in the 80s
  – ~40 in the 90s
• “Innovation Deficit” - between number of NCEs required to be launched in order to accomplish annual 10% revenue increase and the actual number of NCEs introduced in the market by top 10 pharmaceutical Co.;
• Reasons for the innovation deficit:
  – increased demand on safety for drugs;
  – “low hanging fruit” have been picked;
  – Lacking quantity studies
  – Business CEOs intention
• Functional genomics (post genomics) may change it.
An Ideal Preclinical Development Candidate

- Unmet and blockbusted/nichebusted medical needs;
- Validated and differentiated target molecules, including multiple target molecules;
- Druggable and manufacturable new molecule entity;
- Selective, potent, and high efficacy;
- Favorable and targeted pharmacokinetic and metabolism profile;
- Good and defined safety window, toxic organ, and toxicity;
- Predictable and potential human data;
- Patentable and freely-operable intellectual properties;
- Translational pharmacology: cPharmacology as cGMP.
Unmet Medical Needs

• Great achievements:
  – Met medical needs:
    • Hypertension;
    • Congestive heart failure?
  • Peptic ulcers:
    – H$_2$ antagonists,
    – Proton pump inhibitors
      » omeprazole,
      » lansoprazole
      » sexlansoprazole,
      » esomeprazole,
      » pantorazole
      » rabeprazole
      » ilaprazole;
  • Birth control
    – Big and crowd market:
      • Type 2 diabetes;

• Less achievements:
  – Drug-resistant anti-infectious agents
  – Central nervous diseases;
  – Obesity;
  – Cancers-- but crowd;
  – Difficulty in clinical investigation
    • Ventricular arrhythmia;
    • Fibrosis of liver and kidney but not lungs.

• Blockbusted drug;
  – Nichebusted
Proton-Pump Inhibitors

[Diagram of proton-pump inhibition]

- Histamine $H_2$ receptor antagonists
- Muscarinic $M_3$ receptor
- CCK$_2$ receptor
- Acetylcholine
- Gastrin
- Parietal cell
- H$^+$/K$^+$-ATPase
- Proton pump inhibitors
- Acid (HCl)
- Gastric gland lumen

[b] Blood
- Omeprazole

Parietal cell canalculus lumen
- 3 steps
- Sulphenamide intermediate
- Enzyme–inhibitor complex

H$^+$/K$^+$-ATPase
The US Death Rates by Cause, 1950 vs. 2005

*Age-adjusted to 2000 US standard population.

Sources: 1950 Mortality Data - CDC/NCHS, NVSS, Mortality Revised.
Anti-PD-1 and PD-L1 Antibodies for Cancers

Figure 1 | Checkpoint blockade activates antitumour immunity. a, Tumour cells express both...
Durable Tumor Regression by Immunotherapy

→ Durable tumor regression can be achieved by immunotherapy but not targeted therapy.

Maus et al Annu Rev Immunol 2014
Antihypertensive Treatment: Should Natural Medicine Therapy Be Recommended?

- Western treatment of hypertension
  - well-established cheap treatments
  - which reduce risk with few side effects
- Natural medicine treatment of hypertension
  - reasonable evidence for hypotensive action of garlic, sour tea, hawthorn, and tomato extract
  - need for further and larger trials to confirm

Michael Mulvany, PhD
Department of Pharmacology
University of Aarhus
Secretary General of 16th IUPHAR World Congress of Basis and Clinical Pharmacology
Drug Target Molecules

- Macro-biomolecules with physiological and pathological properties, specifically bind with drugs at certain binding sites, leading to pharmacological effects for clinical indications.

- Proteins
  - Enzymes: COX-2 and inflammation
  - Receptors particularly GPCRs: AT1 receptor and heart failure
  - Channels: N and Nav1.7 channels and pain
  - Transporters: Sodium-Glucose Transporter 2 (SGLT2) and type 2 diabetes

- Nucleic acid: a given mRNA, a promoter of DNA;

- Approximately 500 drug targets have been identified and validated;

- One drug Molecule leading to 7-10 new drugs.
Over 60% of the Currently Marketed Drugs Are Targeted to GPCRs

Family A / Class I

Family B / Class II

Family C / Class III

~300

- Adrenergic
- Serotonergic
- Fatty acids
- Neuropeptide
- Glycoprotein hormone
- Protease-activated
- Opioid

~65

- Glutamate
- GABA
- Calcium

~30

Courtesy of Dr. Sexton
The Human Genome Project

- Human gene sequencing determines the order of the nucleotide bases (adenine, guanine, cytosine, and thymine);
- Human Genome Project was completed in 2003, followed in 2006
  - identify 20,000-25,000 genes in human DNA
  - determine sequences of 3 billion chemical base pairs that make up human DNA
  - store this information in databases.
Validation of Drug Target Molecules

• Involves demonstrating the relevance of the target protein in a disease process/pathogenicity and ideally requires both gain and loss of function studies;

• Validation and demonstration
  – Cell models: gene and protein expression;
  – Animal models
    • Disease models;
    • TG/knock in + knock out/knock down.
  – Human investigation.
Methods for Validation of Target Molecules

• Small molecules or peptides to block receptors, enzymes, transporters, and channels
  – Chemical biology;
  – Selection and potency are essential;
• Neutralizing antibodies - cross reaction;
• Oligonucleotides: Antisense, RNAi, DNA methylation - off target;
• Knock-out/knock-down technology - development;
• At least two methods combined.

Functional Genomics: A “Hot” Research Field Post Genomics
Characterization of Ideal Drug Target Molecules

• “Druggability”
  – three dimensional structure will enable the prediction of “druggability” of the protein;
  – Tolerance for agonists.
• Efficacy;
• Signal transduction specificity;
• Tissue specificity: SGLT2
• Disease specificity
  – Upregulation:
    • COX2
• Mechanism of action
  – May be drug target;
  – May not be drug target
    • Signal transduction consequence;
    • Side effects;
    • Toxic effects;
• Traditional Chinese Herbs: “multiple ingredients and multiple targets”
Schematic Representation of the Predicted Structure of the Rat GLP-1R
Significance of Identified Target Molecules on Drug Development

- Human-demonstrated target molecules
  - Less risk;
  - Better (best) in class;
- Animal-demonstrated target molecules
  - High risk;
  - First in class;
  - Leader in business;
  - Renovation reward?
- Predictable druggability
  - Predictable efficacy;
  - Predictable side effects and toxicity;
- Multi-targets.
- Not identified drug target
  - Gabapentin: $\alpha 2\delta$ subunit of calcium channels;
  - Traditional Chinese Herbs.
SGLT2 As a Target Molecule for the Treatment of Type II Diabetes Mellitus

-早在30年代von Mering就发现苹果树中大量存在的根皮苷（phlorizin），在动物实验可以增加糖尿病犬的尿糖排除，并证明根皮苷可以降低糖尿病大鼠的血糖。由于当时的知识局限，认为根皮苷可导致糖尿病并可用于制造糖尿病模型，后者称之为根皮苷糖尿病（Phlorizin Diabetes）;

-自上世纪90年代起，对家族性肾性糖尿患者的基因突变研究。家族性肾性糖尿病人，仅有尿糖增多的临床表现，而其他临床表现包括血糖和肾功能无异常发现。研究表明slgt2基因突变是家族性肾性糖尿的主要原因，提供了一个临床“基因敲除”模型（“gene knockout”）;

-ISIS公司最近研发出针对SGLT2的mRNA的反义寡核苷酸药物ISIS 388626。结果表明，ISIS 388626特异性地抑制SGLT2表达，增加糖尿病大鼠尿糖排泄、降低血糖及HbA1c含量（但不导致低血糖）

“An apple a day keeps the doctor away”
Drug-like Properties Required for an (Orally) Active Drug

- Soluble
- Permeable
- Metabolically stable
- Reaching target
- Sufficient concentration
- Sufficient duration
- Sufficient therapeutic ratio
The “Rule of Five” by Lipinski (Ffizer)

- Poor absorption or permeation are more likely due to:
  - More than 5 H-bond donors
  - The MW is over 500
  - The cLog P (octanol-water partition coefficient) is over 5 (or mLogP is over 4.15).
  - The sum of N’s and O’s is over 10.
- Substrates for transporters and natural products are exceptions.
## Property Trends We Do Not Understand

<table>
<thead>
<tr>
<th>Property</th>
<th>Preclinical</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
<th>Launched</th>
<th>%Changeb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entries</td>
<td>4195</td>
<td>466</td>
<td>700</td>
<td>229</td>
<td>884</td>
<td></td>
</tr>
<tr>
<td>ClogP</td>
<td>3.23</td>
<td>3.00</td>
<td>3.24</td>
<td>2.57</td>
<td>2.50</td>
<td>-23</td>
</tr>
<tr>
<td>% ClogP &gt; 5</td>
<td>22.1</td>
<td>19.3</td>
<td>20.9</td>
<td>16.6</td>
<td>13.7</td>
<td>-38</td>
</tr>
<tr>
<td>% Rule-of-five violations</td>
<td>19.7</td>
<td>14.4</td>
<td>17.6</td>
<td>15.7</td>
<td>10.4</td>
<td>-47</td>
</tr>
<tr>
<td>PSA (Å²)</td>
<td>137</td>
<td>139</td>
<td>129</td>
<td>130</td>
<td>122</td>
<td>-11</td>
</tr>
<tr>
<td>% PSA &gt; 200 Å²</td>
<td>24.1</td>
<td>22.7</td>
<td>20.0</td>
<td>22.3</td>
<td>18.6</td>
<td>-23</td>
</tr>
<tr>
<td>MW (AMU)</td>
<td>393</td>
<td>387</td>
<td>382</td>
<td>361</td>
<td>338</td>
<td>-14</td>
</tr>
<tr>
<td>% MW &gt; 500</td>
<td>22.9</td>
<td>17.8</td>
<td>19.1</td>
<td>14.8</td>
<td>11.2</td>
<td>-51</td>
</tr>
<tr>
<td>No. Rotatable bonds</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>-14</td>
</tr>
<tr>
<td>% Rotatable bonds &gt; 10</td>
<td>24.2</td>
<td>23.4</td>
<td>21.0</td>
<td>20.5</td>
<td>16.2</td>
<td>-33</td>
</tr>
<tr>
<td>Andrews’ BE (kcal/mol)</td>
<td>12.5</td>
<td>11.5</td>
<td>12.8</td>
<td>10.6</td>
<td>10.5</td>
<td>-16</td>
</tr>
<tr>
<td>No. H-bond acceptors</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0</td>
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<tr>
<td>No. H-bond donors</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>-50</td>
</tr>
</tbody>
</table>

*aMedian values listed for each computed property relative to the phase of development.

bPercent change is computed for each property from the values listed in the Launched relative to the Preclinical categories.

<table>
<thead>
<tr>
<th>MWT</th>
<th>393</th>
<th>387</th>
<th>382</th>
<th>361</th>
<th>338</th>
</tr>
</thead>
<tbody>
<tr>
<td>% RO5 viol</td>
<td>19.7</td>
<td>14.4</td>
<td>17.6</td>
<td>15.7</td>
<td>10.4</td>
</tr>
</tbody>
</table>
Non-Drug-Like Properties

- Non-pharmaceutical element
- MW <100 or > 1000
- Total C < 3
- No N, O or S
- Fragments with known toxicity
- But bioactive glass for chronic peptic ulcer?
hERG Activity in Older Drugs

hERG is a gene (*KCNH2*) that codes for a protein known as K\textsubscript{v}11.1, the alpha subunit of a potassium ion channel. This ion channel is best known for its contribution to the electrical activity of the heart that coordinates the heart's beating (i.e., the hERG channel mediates the repolarizing $I_{Kr}$ current in the cardiac action potential).

30% of CNS compounds potentially have HERG issues.
CMC

- Medium-scale manufacturing;
- Entity identification;
- Purity and related substances;
- Stability and pharmaceutics.
Selective, Potent and High Efficacy in Defined Indications

- Potency and efficacy;
- Selective – differentiation
  - Comparison screening;
- In vitro tests and human disease-related animal models.
Principles of Animal Disease Models

• For an effective model, 3 conditions must be met:
  – Full understanding of the animal model;
  – Full understanding of the human disease;
  – The above two cases must be substantially congruent in all important respects.

• Even minor differences in physiology and anatomy can lead to profound differences in disease pathology and treatment effectiveness
  – Right species;
  – Right disease;
  – Two animal model rule;
  – Combined with in vitro activity;
  – Combined with potential human data
# Commonly-Used Animal Models of Pain: Acute Pain

<table>
<thead>
<tr>
<th>Model</th>
<th>Similarities to human disease</th>
<th>Differences from human disease</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail-flick/hot-plate</td>
<td>No equivalent</td>
<td>Does not model any human pathology</td>
<td>Used to study nociceptive modalities and to develop analgesic drugs</td>
</tr>
<tr>
<td>Paw withdrawal</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
</tr>
</tbody>
</table>
## Commonly-Used Animal Models of Pain: Persistent/Central Pain

<table>
<thead>
<tr>
<th>Model</th>
<th>Similarities to human disease</th>
<th>Differences from human disease</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaicin (s.c.)</td>
<td>Skin inflammation by irritants; represents spinal component of pain</td>
<td>A fine model for certain mechanistic components of pain but is of no real relevance to any clinical syndrome</td>
<td>Used as a valuable tool to study selective activation of polymodal nociceptors and 'central sensitization' in the spinal cord</td>
</tr>
<tr>
<td>Formalin (s.c.)</td>
<td>No human equivalent; mechanism is largely unknown</td>
<td>No human equivalent</td>
<td>An extensively used model as it features both peripheral and central components; easy to use with a high throughput capacity; no relevance to chronic pain conditions</td>
</tr>
</tbody>
</table>
# Commonly-Used Animal Models of Pain: Chronic/Inflammatory Pain

<table>
<thead>
<tr>
<th>Model</th>
<th>Similarities to human disease</th>
<th>Differences from human disease</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFA</td>
<td>Rheumatoid arthritis, severe inflammation of joints and soft tissue; it is most relevant to monoarthritis</td>
<td>Symptomatology and pathomechanism is different from human rheumatoid arthritis</td>
<td>Only monoarthritic or localized form is used for pain research; long-lasting, reliable model for inflammatory pain; time course might differ between species</td>
</tr>
<tr>
<td>Carrageenan and turpentine models</td>
<td>Carrageenan and turpentine models</td>
<td>Short duration compared to chronic inflammatory pain in humans</td>
<td>Produce inflammation with a 3–7 day duration and substantial edema formation</td>
</tr>
<tr>
<td>UV-irradiation</td>
<td>Sunburn, moderate burn injury</td>
<td>ot known</td>
<td>Various irradiation periods with UV-B produce skin inflammation with different time courses</td>
</tr>
<tr>
<td>Collagen arthritis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Commonly-Used Animal Models of Pain: Chronic/Neuropathic Pain

| Model                                      | Similarities to human disease                                                                 | Differences from human disease                                                                 | Comments                                                                                                                                  |
|--------------------------------------------|------------------------------------------------------------------------------------------------|
| Bennett model (loose chromic ligature of the sciatic nerve) | Spontaneous pain, allodynia and protective posture all present in this model; pharmacological profile is similar to that of clinical neuropathic pain | Difficult to relate to any particular neuropathic pain condition; nerve compression and direct mechanical neuronal damage might be relevant clinical comparisons | Frequently used model, particularly for behavioural studies; high frequency of autotomy is a disadvantage |
| Seltzer model (partial tight ligation of the sciatic nerve) | Same symptoms as above but less severe; minimal inflammatory process | As above | High reproducibility, easy to perform, excellent for behavioural studies; however, difficult to study changes in the DRG as damaged and undamaged primary afferents are mixed in the nerve |
| Chung's model (tight ligation of one of the two spinal nerves of the sciatic nerve) | General model of neuropathic pain | As above; root compression might be a relevant clinical comparison | Fairly extensive surgery with muscle damage might complicate the pathomechanism; excellent model for *in vitro* use as the damaged and undamaged fibres of the peripheral nerve originate from distinct DRGs |
| Diabetic neuropathy (STZ) | Diabetic neuropathy | Many features and underlying mechanisms differ from diabetes mellitus | Produces severe distress to the animal with deterioration of general condition; difficult to interpret data or obtain clear pain scores; insulin treatment abolishes pain and hyperalgesia |
Knockout Animals

- The first knockout mouse was created by Mario R. Capecchi, Martin Evans and Oliver Smithies in 1989, for which they were awarded the Nobel Prize for Medicine in 2007;
- The p53 knockout mouse is named after the p53 gene which codes for a protein that normally suppresses the growth of tumors by arresting cell division;
- A humouse is an immunodeficient mouse reconstituted with a human immune system;
- Nude mouse-genetically engineered mouse with absent thymus.
Transgenic Mice as Tools

- **Transgenic**: an organism that has had DNA introduced into one or more of its cells artificially.

- **Transgenic mice are often generated to**:
  - characterize the ability of a promoter to direct tissue-specific gene expression, e.g. a promoter can be attached to a reporter gene such as LacZ or GFP;
  - examine the effects of overexpressing and misexpressing endogenous or foreign genes at specific times and locations in the animals.

- **Study gene function**: many human diseases can be modeled by introducing the same mutation into the mouse. Intact organism provides a more complete and physiologically relevant picture of a transgene's function than in vitro testing.

- **Drug testing**

GFP transgenic mouse (Nagy)
## Transgenic Mice Models for Human Diseases

<table>
<thead>
<tr>
<th>strain</th>
<th>disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J Apo-B</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>C57BL/6J Tg NHBV</td>
<td>Hepatitis B</td>
</tr>
<tr>
<td>C57BL/6J Apoe</td>
<td>Alzheimer's</td>
</tr>
<tr>
<td>C57BL/6J sPLA-2</td>
<td>Inflammation</td>
</tr>
<tr>
<td>C57BL/6J TgN (TNF alfa)</td>
<td>Arthritis</td>
</tr>
</tbody>
</table>
Examples of “Valid” Animal Models

- Pain models
  - Acute pain
  - Chronic pain? Kim and Chung model
- Hypertension models;
- Inflammatory models
  - Carrageenan acute inflammation
  - Collagen-induced rheumatoid arthritis
- Relapasing EAE for MS;
- Gastric ulcers (some models);
- Transplantation;
- Food-induced obesity and high lipids and diabetes
  - Glucose tolerance and HbA1C;
  - Blood lipids
Examples of Animal Disease Models Often Failed

- Cancer models
  - “The history of cancer research has been a history of curing cancer in the mouse. We have cured mice of cancer for decades, and it simply didn’t work in humans” Dr. Richard Klausner, Director of the National Cancer Institute 1998.

- Free radical scavengers=Antioxidants

- Endotoxemic models;

- HIV/AIDS
  - Effective in animals (including chimps) and tested in clinical trials: >50
  - Effective in humans: 0 (Bailey J. Biogenic Amines 2005).

- Stroke
  - Effective in animal stroke models: >700
  - Tested in human clinical trials: >150
  - Effective for humans: 0 (Macleod, 2004, 2005)

“This rejection decision was based on the limited clinical validity of data using the MCAO model” from JPET

- Spinal cord Injury: methylprednisolone for treatment of acute spinal cord injury:
  - Cats: mostly positive; dogs: mostly positive; rats: mostly negative; mice: all negative; monkey: positive; rabbit: equivocal; sheep: negative
  - Humans?
Why Animal Experiments Often Fail?

Animal Models ≠ Human Disease
Species Differences in Receptors

Table 2. Receptors (i) ion channels (ii) and enzymes (iii) that are potential or actual targets for therapeutic agents and reported to show species differences in level of expression or affinity and response to agonists or antagonists. The table has been assembled from Smith (1993) and references within, MacDonald *et al.* (1989)*, Chadwick *et al.* (1993)†.

| (i)         | Adenosine         | A1       |
|            |                   | A2       |
|            | Adrenoceptors     | $\alpha_1$, (B,C) |
|            |                   | $\alpha_2$ (A) |
|            | Atrial natriuretic factor | ANF-R1 |
|            | Bradykinin        | B2       |
|            | Cholecystokinin   |          |
|            | Dopamine          | D1       |
|            | Endothelin        | ETB      |
|            | 5HT               | 5HT1 (A,B,D) |
|            | 5HT2              |          |
|            | 5HT3              |          |
|            | 5HT4              |          |
|            | Luteinizing hormone (LH) |          |
|            | Muscarinic        | M2       |
|            | Neurokinin        | NK-1,   |
|            |                   | NK-3     |
|            | P2-Purinoceptors  | P2X      |
|            | Thromboxane       | TA2      |
|            | Vanilloid         | $V_1$    |
| (ii)       | Rapidly activating delayed rectifier $K^+$ channel† |          |
| (iii)      | $Na^+$/K$^+$ ATPase |          |
|            | Renin             |          |
|            | HMG-CoA reductase* |          |
The human and rat GLP-1 receptors differ at 42 amino acid positions.
Drug Receptor Interaction

- Occupation of a Receptor by a drug may or may not result in activation of the receptor;
- \( D + R = DR \) leading to pharmacological effect;
- The active complex (DR) leads to a cellular response that is proportional to the number of receptors occupied;
- A drug has a maximal effect when all receptors are occupied;
- The property of a drug to bind to receptors is Affinity;
- Once it is bound the property to evoke a response is efficacy-intrinsic activity.
Four Parameters of Dose-Response Curve Analysis

- **Slope (Hill coefficient)**
  - usually 1, but varies from drug to drug particularly in *vivo* conditions
  - steepness of slope denotes that a small increase in drug dose leads to large change in response;
  - may represent molecule(s) to binding receptors.

- **Potency**;
- **Efficacy**
  - Minimum Effect;
  - Maximum Effect.
Discovery of EDRF and Nitric Oxide
Acetylcholine and L-NAME: Molecule 1 or 2?
PAP-1 and the Active Metabolites Exhibited Hill Coefficients of 2 Suggesting that 2 or more Molecules Interact with One Kv1.3 Channel
Potency

- The amount of drug needed for evoking a given response depends on its Potency;
- Lower the dose required the more potent the drug;
- In clinical use the dose needed for eliciting 50% response - half effective Dose - ED$_{50}$;
- In experiments as half lethal Dose - LD$_{50}$. 
Efficacy

• Maximal response produced by a drug;
• Depends on the number of Drug Receptor Complexes (DRC) formed;
• Efficiency with which the DRC elicits a cellular response;
• A compound may bind to a receptor without eliciting a response – zero efficacy.

Efficacy: Drug A = Drug C
Drug B has least efficacy
Competitive Antagonists

- Compete for a site on receptor already occupied by an agonist;
- The duration of action of a reversible antagonist is closely correlated with its rate of elimination;
- As it gets eliminated, the concentration at its site of action falls, and there is less of it to compete with the agonist;
- Parallel shifts the DR curve to the right thus making the combination less potent.
Partial Agonists and Non-Competitive Antagonists
Therapeutic Index (TI)

- Ratio of the drug that produces toxicity to the dose that produces the desired clinical effect.
- TI = Toxic dose/effective dose
- Drugs that have small TI are important in leading to toxicity.
- Drugs with large TI are safe.
Discovery of D-Amino Acid Chiral Inversion

![Graph showing the inhibition of NOS and change in MAP](image)

**Left Graph:**
- **L-NA**
- **D-NA**

Log concentration [M]

- EC$_{50} =$ $5 \times 10^{-7}$ M
- EC$_{50} =$ $2 \times 10^{-4}$ M

**Right Graph:**
- **L-NNA** $4.0$ mg/kg
- **D-NNA** $8.9$ mg/kg

Change in MAP (mmHg)

- Graph showing the change in MAP with varying concentrations of N$^G$-Nitro-Arginine (mg/kg).
Pharmacokinetics, Metabolism and Toxicity

- Oral bioavailability
  - > 15%
- PD-PK model;
- Metabolism;
  - Active metabolites
- CYP isoenzymes of different species.

- Target toxic organs;
- Toxicity;
  - LD50?
- TI.
Selective and Potential Human Data

- Plasma stability, human CYP and isoenzymes;
- Human tissues: e.g., pancreatic islets for insulin release;
- Nude or SCID mouse model of human cancers;
- Humice could theoretically be used as novel pre-clinical models of the human immune system, with uses including assessing vaccine efficacy
- Human Apo-B transgenic mice; human TNF transgenic mice;
- Monkey data
  - Biologics;
  - B1 bradykinin receptor antagonists.
GLP-1 Induces Thyroid Cancer in Rats but not in the Human

- Long-term liraglutide causes a rare type of thyroid cancer called medullary thyroid cancer (C-cell hyperplasia) in both mice and rats; FDA said "very rare" for a drug that causes cancer in multiple animal species to be approved;
- GLP-1R was localized to rodent C-cells and its receptor agonists stimulated calcitonin release, up-regulation of calcitonin gene expression, and subsequently C-cell hyperplasia in rats;
- Humans and/or cynomolgus monkeys had low GLP-1R expression in thyroid C-cells, and GLP-1R agonists did not activate adenylate cyclase or generate calcitonin release in primates;
- 20 months of liraglutide (>60 times human exposure levels) did not lead to C-cell hyperplasia in monkeys;
- Calcitonin levels in patients exposed to liraglutide for 2 yr remained at the lower end of the normal range. 5-year human exposure to exenatide showed the same results.
Thank You for Your Attention!