Target Identification

Target identification is the first and most crucial step in drug discovery.

It makes sure that a specific thing (target) in the body is truly related to the illness, and that working on it could actually help treat the disease.

Finding target, also helps drugs to treat the specific condition and make sure the drugs are free of unsafe side effects.

What can be a target for drug?

- Something that is abundant in the disease state, easily accessible and targeting it will not cause unsafe side effects
- Unsafe side effects to a certain and acceptable degree depend on the drug type
- Examples of Biological Targets : Proteins, Genes, Nucleic Acids (DNA/RNA)

How do you identify a target ?

To identify a target, scientists often look through: Published Research, Gene Expression and Proteomics Data, Genetic Associations, etc.

Phenotypic screening (screening of visual / physical characteristics)

helps identify specific disease-relevant targets based on their relationship with disease phenotypes.

Genetic Evidence (Genes)

is also important in identifying targets. When a target is proven to be genetically related to a disease, it often leads to more effective medicine

Computational Scientists

(aka scientists working with computers) also use bioinformatics databases like ChEMBL, which has data on small and specific molecule activity, and the Open Targets Platform, which combines genetics with other data to help improve finding drug targets

It may take many years to build up a body of supporting evidence before selecting a target for a costly drug discovery program.

E.g., review articles that combine many past published research -> finalizing the causation of diseases such as PKU can take up to 80 years to complete but only some of them are useful for therapies -> good target.

Compound Identification

This step is just identifying compounds that fit and interact with the target from the chemical pool. These compounds are generally known as “Hits.”

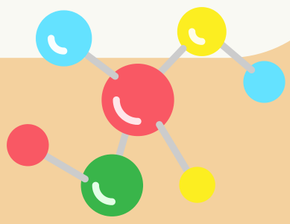
Laboratory-Based Screening

High-Throughput Screening (HTS)

- Often used in this step because it can scan through a massive amount of compounds at once.
- It is designed to indicate whether a compound is a Hit (Yes, the compound interacts with the target) or not a Hit (No, the compound does not interact with the target)
- Now, it is performed using robots/automatic machinery which can increase the amount of compounds being screened at once (more than 100,000 chemicals per day).

Fragment Based Drug Discovery (FBDD)

- Instead of focusing Hits from chemical libraries of large molecules, it uses small fragments which interact with targets more easily.
- Each fragment is a small piece of a final drug, and by slowly adding pieces and seeing if the drug can interact well, you can eventually create the best-fitting drug for the target



Laboratory-Based Screening - Example

Fluorescence-Based HTS

- the target gives off light after a drug interacts with it
- was used to identify hits for one of the targets in PKU -> out of more than 1000 compounds, only 4 were identified as possible hits.

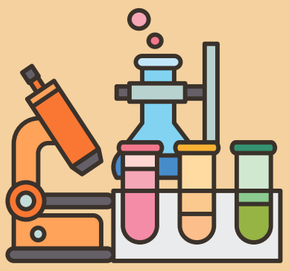
Virtual Screening

Artificial Intelligence (AI) & **machine learning** can also help identify compounds that fit the target.

- they are trained to evaluate the chance that a chemical compound can interact with the target.
- this can provide quick identification of Hits
- some useful tools/software include ChemMapper and the Similarity Ensemble Approach (SEA).

More current and future screening is being done with virtual screening.

Establish Activity



The previous step ensured the target and drug are able to interact with each other, now we have to figure out if that interaction helps treat the disease or if it makes it worse or does nothing.

In other words, we are trying to find a Lead Compound from our Hits.

The Hit's from the last step are organized into different categories based on their structure and inferred biological activity (what they may cause inside the body).

The Hits are analyzed for properties which result in desired outcomes and minimize unwanted side effects

Lead Compounds generally follow the "Rule of five"

1. Its molecular weight is less than 500 g/mol

- The lead compound must be small and light so it can enter the body easily

2. Partition coefficient less than 5

- A measure indicating how hard it is for a drug to cross certain boundaries

3. No more than five hydrogen bond donors and no more than 10 hydrogen bond acceptors

- Minimizing areas on the drug that interact with various things in the body ensure the drug does not accidentally target the wrong thing

We then further improve the properties of the Lead Compounds, shaping them to look more "drug-like"

Clinical Candidate Selection

Once a Lead Compound is improved and appears drug-like, further steps are taken to make it into a Clinical Candidate.

These drugs build on the properties of Lead Compounds and become (theoretically) able to get to the body part where the target is in high quantities without causing any unwanted side effects to any other body part and organ.

To become a Clinical Candidate, Lead Compounds must acquire the following properties:

1. Show expected functional effect on the target

- this was established when it became a Lead Compound, but now we want to ensure it can do this in a cell line (group of cells) rather than just at the target

2. Can selectively bind to the target with high affinity

- its ability to interact with the target was established when it became a Hit, but now we want to ensure it will bind to the target even when other receptors on the cell are around

3. Will not cause toxicity

- this was established when it became a Lead Compound, but now we want to ensure its actions to the inside of a cell do not cause unwanted side effects

4. Can be easily manufactured and stored

- to let the drug be stored in user friendly ways, such as a non-refrigerated pill or as something that dissolves into liquid to drink

5. Proper distribution and metabolism - also known as having a high bioavailability

- we need to make sure the drug will exist in high quantities once inside the body, will be able to move around throughout the desired regions and won't just be excreted (through peeing, pooping, sweating, spitting, vomiting, sneezing, etc.)

6. Proper half-life

- This property ensures the drug will exist in the body for a desired amount of time.
- As sometimes we only want to drug inside the body for a short amount of time, such as caffeine (which only has a 3-5 hour half-life as we don't want to be awake for multiple days in a row) or we want a drug inside the body for a long time, such as Prozac (which is an antidepressant drug that stays in the body for 4-6 days)

7. Will not block or induce cytochrome P450 enzymes

- Cytochrome P450 enzymes mainly exist in the liver and metabolize many compounds in the body.
- Changing the amount it is available may change the way drugs can act. This is one of the most important considerations physicians think about when prescribing you medication (and why they act if you are currently taking any meds)

in vivo Experiments

Up until this point, we have been conducting *in vitro* experiments to improve the quality of our Hits → Lead Compound → Clinical Candidates and shape them into a drug-like item.

In the clinical drug development stages, *in vivo* experiments (animal experiments), are important for testing safety and efficacy of drugs. If severe adverse effects occur, the drug would not go to the next step and the investigators would need to work on other available drugs/compounds.

We use animals to model how a drug behaves in a living being to analyze whether its Clinical Candidate properties remain in practice

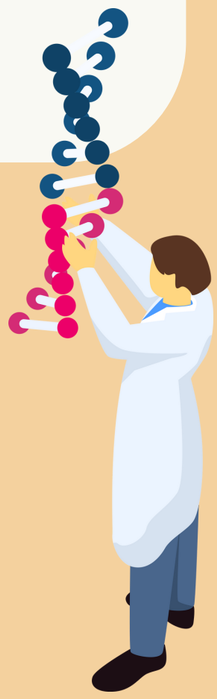
To better explain this we will look at the two currently approved PKU treatment drugs, KUVAN and Palynziq.

KUVAN (Sapropterin Dihydrochloride)

- Approved 2010 in Canada, helps increase signaling proteins, dopamine and serotonin, which are low for those with PKU.
- At the highest tested dose in mice experiments, dopamine and serotonin were NOT increased.
- However, similar signaling proteins, homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), which are related to dopamine and serotonin, are increased which helps with PKU!

Palynziq (PEGylated PAL)

- Approved 2022 in Canada.
- Initial experiments, focusing on using PAL from plants, didn't work so well so scientists tried modifying it to be used as an injection.
- The modified PAL became known as Palynziq. Palynziq, in mice experiments, reduced levels of phenylalanine in the blood and brain for more than 90 days by converting it to something else. (Phenylalanine is something at high levels in people with PKU.) However, after Palynziq use was stopped, these effects were not seen, which means you need to take Palynziq everyday.
- Overall, Palynziq was able to improve quality of life in mice with PKU



Another way scientists have tried to treat PKU was with a special gene editing tool called **CRISPR**.
Using CRISPR to edit a mouse's gene, along with another tool ABE8.8 and some instructions (guide RNAs), they were able to permanently fix the high phenylalanine levels in the mice's blood within 48 hours

Clinical Trial Application

Once all the other steps are completed and the drug / medicine is proven to be effective in treating the disease and the dose is safe in a living creature, labs must submit a Clinical Trial Application (CTA) to the Health Canada.

The CTA is a long document which must include numerous things, but are broadly categorized into 3 main “Modules” (or sections)

Module 1: Administrative / Clinical Information

This is the longest of the three modules.

Labs must report information about their drug and the logistics of their clinical trial.

Subsections examples:

[1.2.1] Drug Submission Application Form:
The form requesting permission for experimental drugs use in humans, which contain signatures of the important workers on the trial (senior medical/scientific officer and senior executive officer).

[1.2.3] Investigator’s Brochure:
Supplementary information about how much of the drug is needed (drug dose), how often you take the drug (dosing interval), how the drug is given (administration route) and what side effects to look for (safety concerns).

[1.2.6] Informed Consent Document(s):
Samples of the consent forms and informational documents given to participants are reviewed to ensure they follow Health Canada’s ethical guidelines in terms of participant safety and compensation.

Module 2: Common Technical Document Summaries
- Quality (Chemistry and Manufacturing) Information

This module involves the submission of specific information pertaining to the chemical and manufacturing properties of the drug.

The main subsection **[2.3] (Quality Overall Summary)** requires additional information as a drug proceeds through the clinical trial phases.

Some items required for all three phases include:

Drug Information: Labs must provide the drug’s scientific name and company name, formula, its drug family, dose, administration, and more.

Clinical Trial Summary: Labs must state the clinical trial’s name and a brief description including; goals, design, how long the trial will last, locations, amount of people, and more.

Manufacturing Details: Labs must express the location of the drug maker's factory, what company is handling the drug, what information will be on the drug packaging, and more.

Clinical Trial Application cont’

Module 3: Quality - Additional Supporting Quality Information
This module is used as a support if more information is needed in Module 2.
What information is being supported here must be referenced in Module 2 so the reader understands it better.

Past Scientific Knowledge (Literature) about the drug family, target, disease being treated, and more are also included here in the form of references to allow for the readers to understand the background knowledge further

The time labs generally face when waiting for approval can range from 1 to 4 years, depending on the nature of the drug and clinical trial application. Some would take even longer due to Health Canada requesting additional information from labs.

Application review may also be stalled due to the sheer volume of applications (More than 100,000 applications submitted yearly).

Success rate for the initial CTA into **Phase I Clinical Trials** is roughly **1%**.

One famous example of a drug being approved quickly is the **COVID-19 vaccine**.

The main reason for its rapid development was governmental and pharmaceutical policies being shifted to prioritize the analysis of COVID-19 vaccine development and CTA review at the governmental level.

Thus, it was not due to rushed scanning of the application, but making COVID-19 related applications very important, which led to its clinical trials beginning quickly.