The Process of Drug Discovery

An Overview

Becky Zheng & Robbie Simmonds

Introduction

- In the past, many drugs were found throughout nature i.e.) Caffeine, Opium, etc. or created through serendipitous discovery - penicillin.
- Now, with advancing technology and practices, like HTS, AI/ML, and Assay creation, we are able to synthetically create drugs in a plethora of ways.
- The Drug Discovery Process has introduced many new methods, databases, and software to increase the efficiency and success of drug design.

What is Drug Discovery?

Drug Discovery is the creation of drugs that can be used to cure and alleviate ailment or illness.

Where a **drug** is a molecule or substance that instantiates a physiological response after consumption. [11]

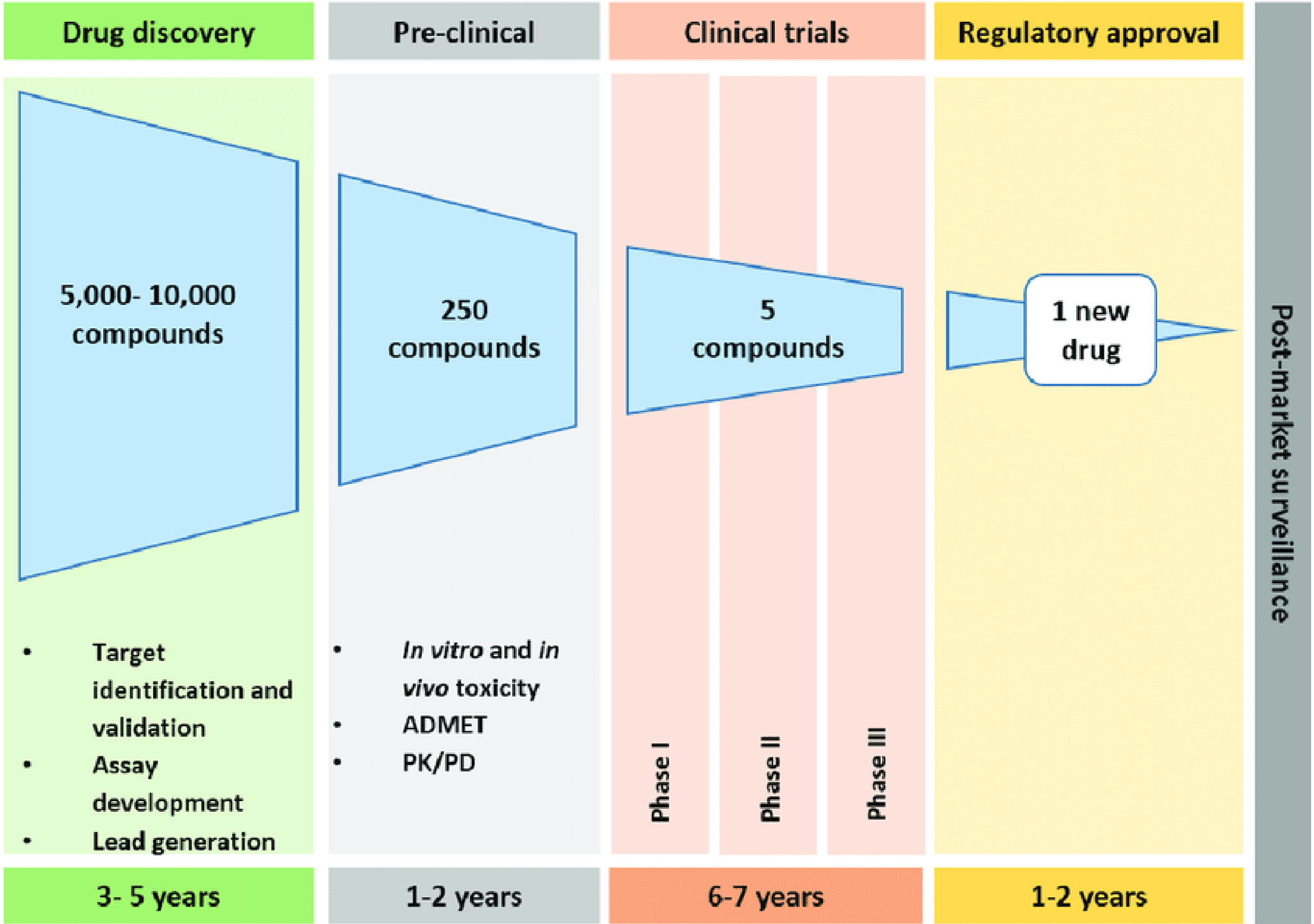
Drug Discovery has been used since the 19th century to create new drugs that can be used by the public.

Problems with Drug Discovery

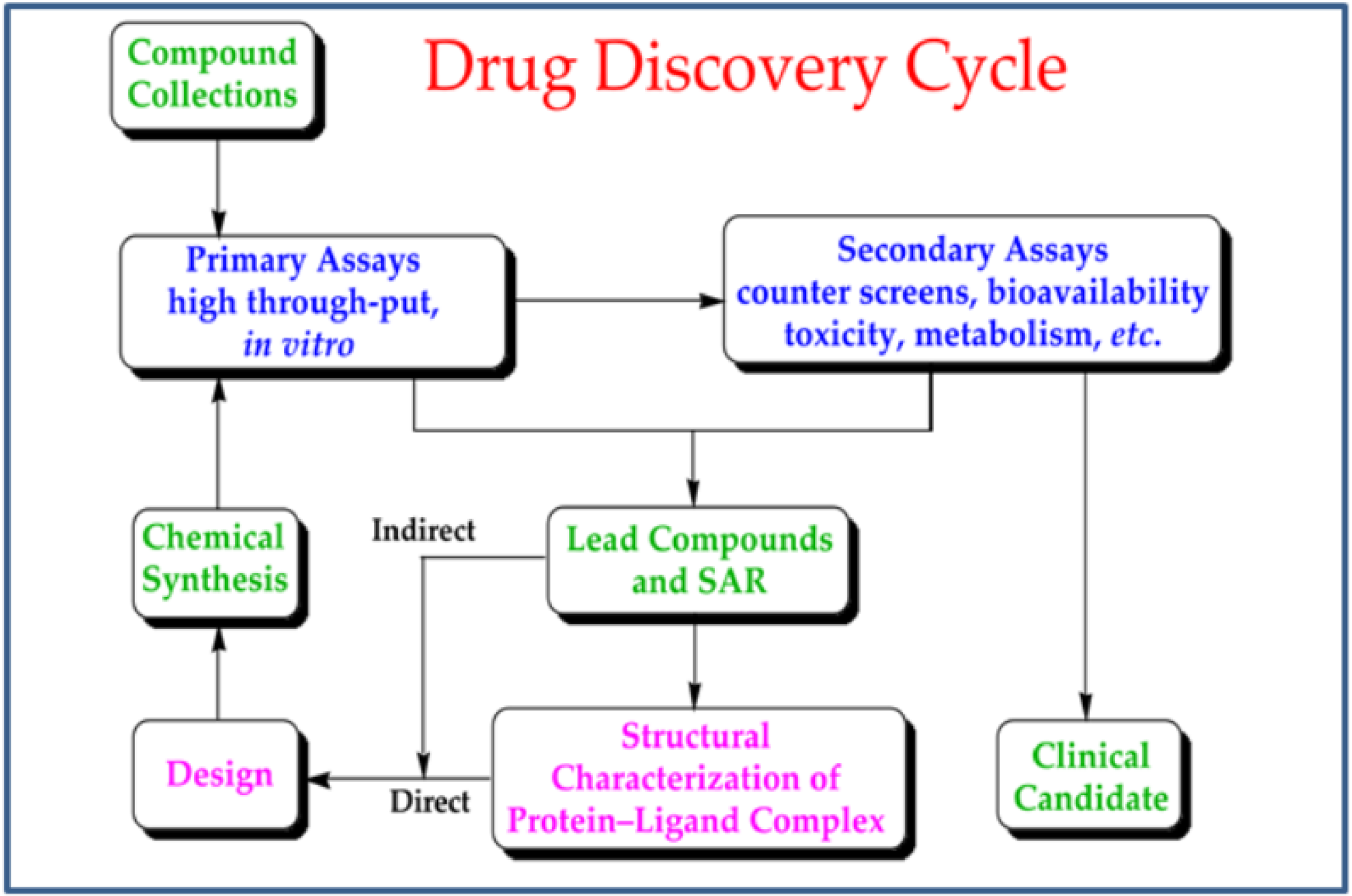
The current issue with Drug Discovery is that it's:

- Costly
- Time Consuming
- Mostly Ineffective

Where each drug discovery can cost over 2 billion USD, take 10 - 15 years from start to completion, and has a < 10% success rate.


Drug discovery
Pre-clinical
Clinical trials
Regulatory approval
Post-market surveillance
5,000- 10,000 compounds
250 compounds
5 compounds
1 new drug
Target identification and validation
Assay development
Lead generation
In vitro and in vivo toxicity
ADMET
PK/PD
Phase I
Phase II
Phase III
3- 5 years
1-2 years
6-7 years
1-2 years

[45]


Drug Discovery Cycle
Compound Collections
Primary Assays high through-put, in vitro
Secondary Assays counter screens, bioavailability toxicity, metabolism, etc.
Chemical Synthesis
Indirect
Lead Compounds and SAR
Design
Direct
Structural Characterization of Protein–Ligand Complex
Clinical Candidate

[30]

Different Approaches to Drug Discovery

1: Targeted Drug Discovery (TDD)
2: Phenotypic Drug Discovery (PDD)
3: Drug Repositioning

Each of these approaches have their pros and cons. Depending on what data is needed, how much information is known prior and resource availability are factors to deciding an approach.

Targeted Drug Discovery

Targeted Drug Discovery relies on the use of a determinate target. Where a **target** is a biochemical entity (protein, RNA, or gene) that is tested against when discovering a drug [11].

In Target Drug Discovery a protein, cell, gene, or etc. is identified and then goes through a screening process where developed compounds that will become drugs are tested against the target.

Targeted Drug Discovery (TDD) Pros and Cons

PROS: Offers a clear understanding of the target and its role in the disease [29]

CONS: Drugs may not effect the given target such that a known result would not be known. [29]

Selective serotonin re-uptake inhibitors(SSRIs) may treat depression by regulating inflammation [28]

Phenotypic Drug Discovery

Phenotypic Drug Discovery doesn't use a target, but instead relies on biological change to develop drugs.

In Phenotypic Drug Discovery a biological or physiological change is identified and then goes through a screening process where developed compounds that will become drugs are tested. This can be done through *in vitro* testing.

Phenotypic Drug Discovery (PDD)

Pros and Cons

PROS: Allows for more wide spread search for molecules that affect the system, rather then only studying a single target.

CONS: Assay flow schemes for PDD programs aren't well defined which creates delays and difficulties for resource planning. [31] i.e.) PDD is more time consuming and has less return than TDD does.

Venlafaxine, an antidepressant drug, was identified through three in vivo animal models of depression, without information about a target. [32]

Drug Repositioning

Drug Repositioning relies on serendipitous discovery to develop drugs, where drugs that are already in use are found to have other therapeutic effects.

In Drug Repositioning the screening process is only used in lead identification and optimization, rather than hit discovery, as any hits (which will be covered later) must be known to follow this path of testing.

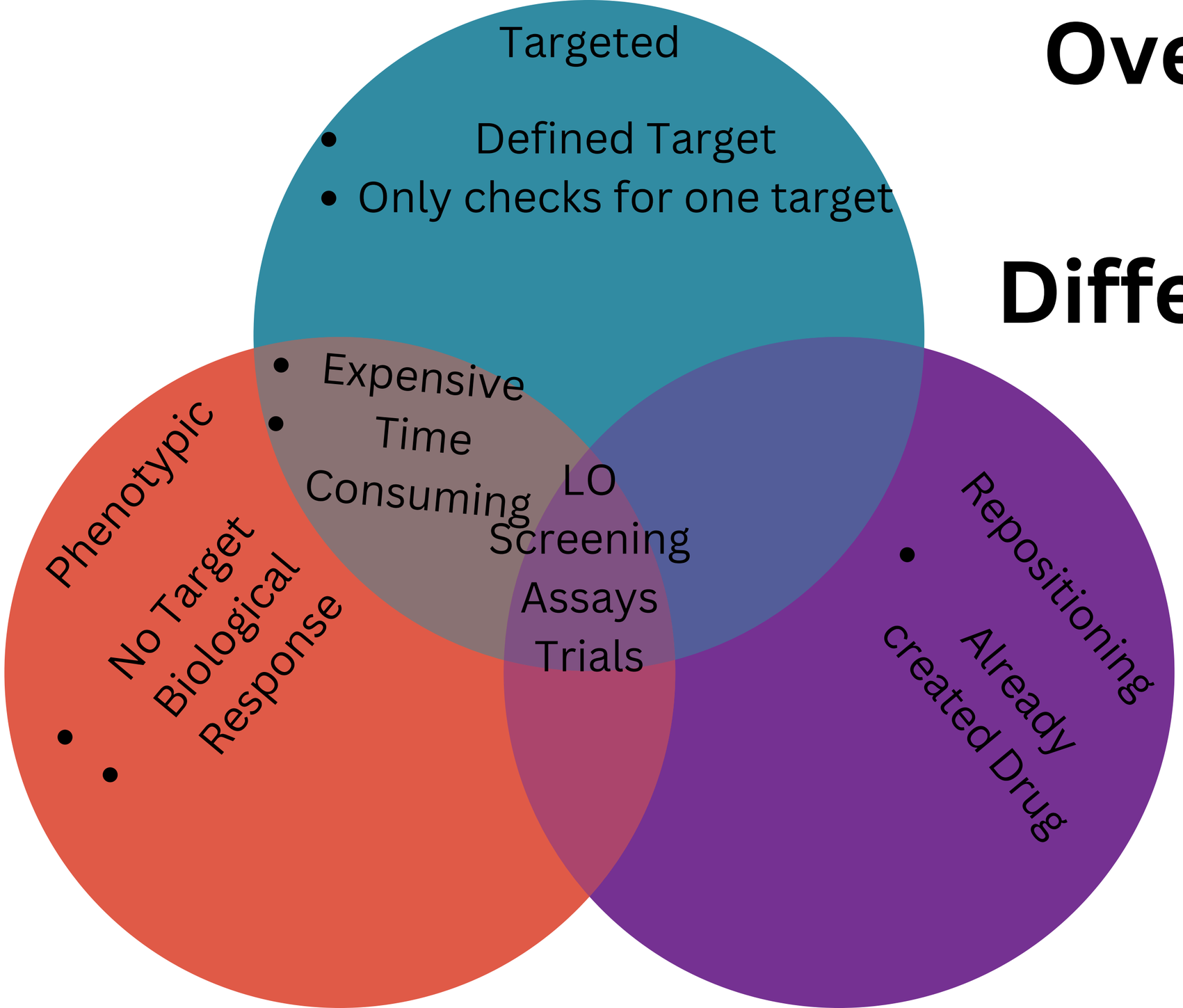
Drug Repositioning Pros and Cons

PROS: Utilizes serendipitous discovery, where known and tested drugs are shown to affect different targets as well as the target they were designed for.

CONS: Requires an already existing drug and may have no positive results after testing.

Aspirin was initially marketed by Bayer as an analgesic and was repositioned in the 1980s as an antiplatelet aggregation drug. It may soon be repositioned again in oncology because it has been shown that daily use of aspirin for at least 5 years can prevent the development of many cancers. [14]

Overview of Differences



What are the Main Steps of the Drug Discovery and Design Process for each Procedure?

Steps of Targeted Drug Discovery

1a: Target Identification

2: Target Selection and Validation

3: Hit Identification

4: Lead Identification

5: Lead Optimization

6: Pre-clinical Trials

7: Clinical Trials

Steps of Phenotypic Drug Discovery

1b: Phenotypic Identification

2: Hit Identification

3: Lead Identification

4: Lead Optimization

5: Pre-clinical Trials

6: Clinical Trials

Steps of Drug Repositioning

1: Drug observation during clinical trials or testing.
2: Secondary Hit Identification **
3: Lead Identification
4: Lead Optimization
5: Pre-clinical Trials
6: Clinical Trials
** Hit identification has already been preformed on the drug, but new hits are identified so that drug can be remodeled to effect that as well.

Step 1a: Target Identification

Used in TDD

In the First Step of Drug Discovery - when using Targeted Drug Discovery, is to identify a target.

Some examples of targets include: Selective serotonin re-uptake inhibitors(SSRIs) [28], H-PGDS [15], G-Protein coupled receptors (GPCRs) [11], and many more.

When looking for targets, you want to ensure they are **druggable** - where the target can be easily modulated by a drug, having a low KI or IC50 as well as not having side effects [26].

Three main ways to Identify Targets:

- **Biochemically** - Based on known target, tested using mammalian cells. Main method for TDD
- **Genetic Interaction** - Less knowledge about target, instead focusing on phenotypic change or reaction. Main method for PDD
- **Computational Inference** - Using AI (Artificial Intelligence), ML (Machine Learning), and NN (Neural Networks) to find targets, used in both TDD and PDD. [20]

AI in Target Identification

SPiDER

SPiDER (self-organizing maps (SOM)-based prediction of drug equivalence relationships) uses a heuristic neural-network system to design target models based on chemically abstract data.

Data comes from the COBRA (collection of bio-active reference analogs) - inSili - to make molecule predictions. [19] [17]

The SPiDER method was trained to mimic target prediction for LOPAC (library of pharmacologically active compounds).

DOGS

DOGS (Design Of Genuine Structures) -another de novo method to identify wanted targets in drug discovery.

DOGS uses 83 reactions - coupling reactions - which were encoded using ReactionMQL. It utilizes virtual organic synthesis utilizing building blocks from Organic Databases. [17]

"The software not only suggests new compounds, but also provides at least one motivated, hypothetical synthesis pathway per ligand candidate structure."

The ZINC database from the Sigma-Aldrich Catalog these compounds were the building block for testing of DOGS. [8]

CATS

CATS (chemically advanced template search) creates Histograms based on atom type frequency. [22]

"As a result of this preliminary analysis in which CATS retrieved a larger number of relevant structures than a conventional fingerprint-based search we concluded that CATS seems to be a useful tool for database mining." - When compared to MERLIN based data mining. [21]

Uses vector math and node distance to find distance-to-seed value, and a ranking list of virtual hits is constructed. Uses the MEDCHEM library.

The CATS system was able to rank 15 out of 29 (52 %) annotated thrombin inhibitors among the top 100 potential hits. [21]

CASP

CASP (computer-assisted synthesis planning) along with MCTS (Monte Carlo Tree Search) has been used to find targets using neural network methods and planning.

A process of tree creation, branching, and iterative processes are utilized using the Reaxys database (containing 12.4 million reactions).

"Top10 and top50 accuracies of 63.3% and 72.5% indicate that the correct transformations are generally ranked highly"

The model starts with a root node, branches out creating follow-up positions, rollout - a system in MCTS where a random search is preformed until finding expected results, and finally updating the root. [23]

Step 1b: Phenotype Identification

Used in PDD

When Utilizing PDD, instead of focusing on a Target, testing is instead done such that a change in phenotype is discovered instead.

"*If we start testing compounds in cells that closely represent the disease, rather than focusing on one single target, then odds for success may be improved when the eventual candidate compound is tested in patients*." [33]

Here more testing is done, and testing may take longer, but it also may yield more results - especially useful when developing drugs for rare monogenic diseases.

Step 2: Target Selection and Validation

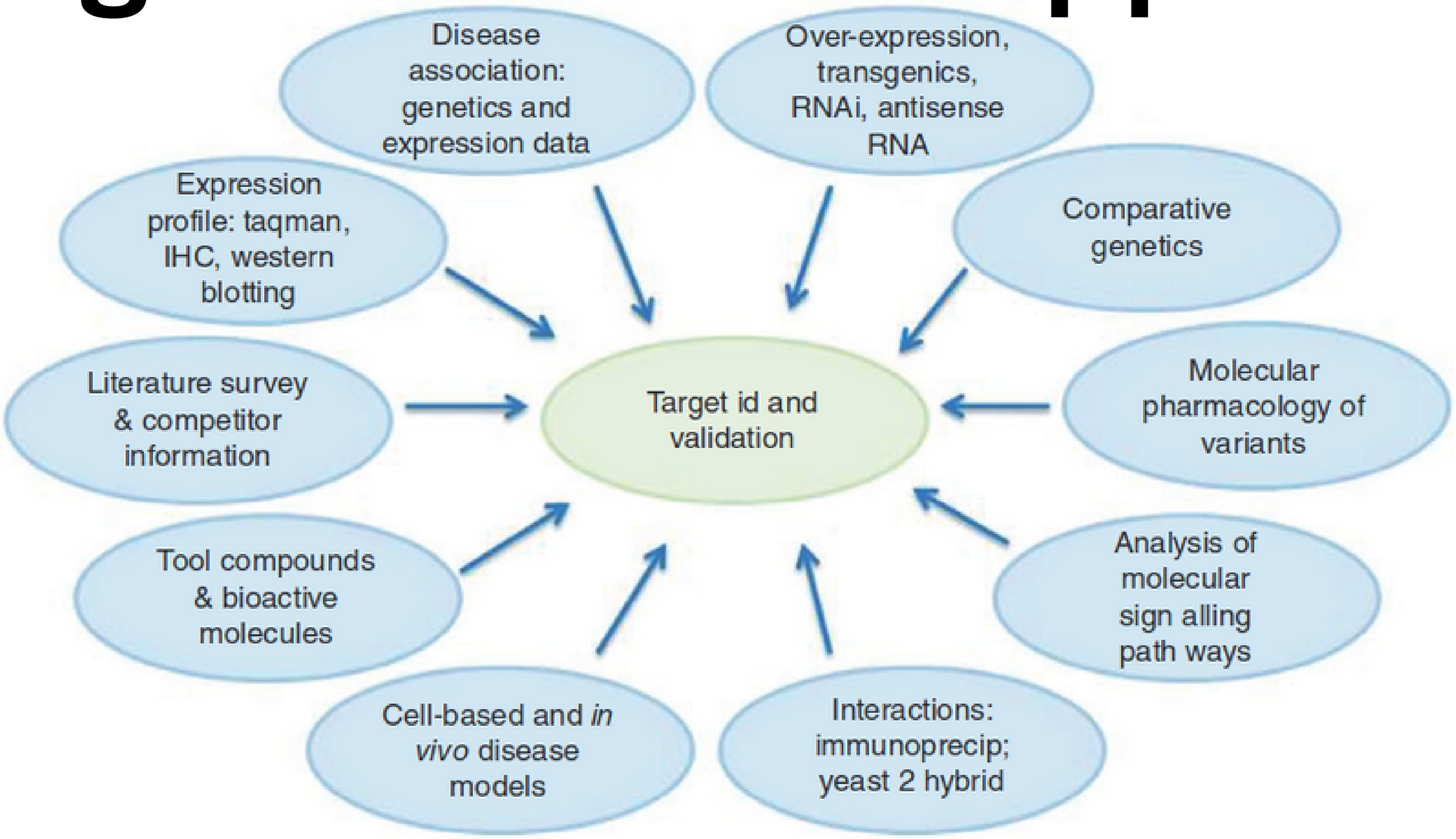
Used in TDD

Targets are chosen on whether or not they meet the requirements for being druggable.

It is during this step when which target inter-connectivity must be understood, as to avoid side effects during drug creation and testing.

For example - "*To verify this result, two HTS experiments were conducted, using a collection of 11,000 drug-like chemicals against the two target candidates. The results showed that 11 chemicals (about 0.1%) targeted H-PGDS while no compounds were effective against HSD, which indicated H-PGDS was a druggable target*" [15]

Target Validation Approaches



[11]

Step 3: Hit Identification
Used in TDD, PDD, and Drug Repositioning

Hit - a compound which has the desired activity in a compound screen and whose activity is confirmed upon retesting.

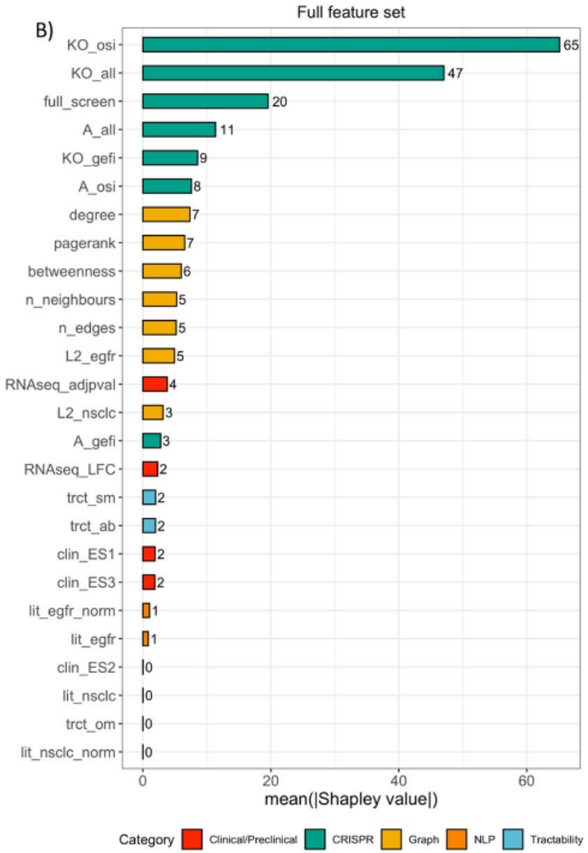
Assays and Screenings - Hits are discovering through Screenings, which are based on created Assays - These will be explained later.

After hits or potential hits are found through screening, the compound (drug) can be optimized. [11]

To optimize in this context means to modify and improve a potential drug candidate to enhance desired properties while minimizing negative effects. [40]

Hit Identification Example

CRISPR has been used for target ID and hit ID, allowing for testing on effects that molecule has.



The following shows gene relation to hit amount, showing that certain genes are more prone to having wanted results. Different models and methods give different results creating a need for more AI and ML used in the Drug Design field. Following is for lung-cancer. [6]

Assays

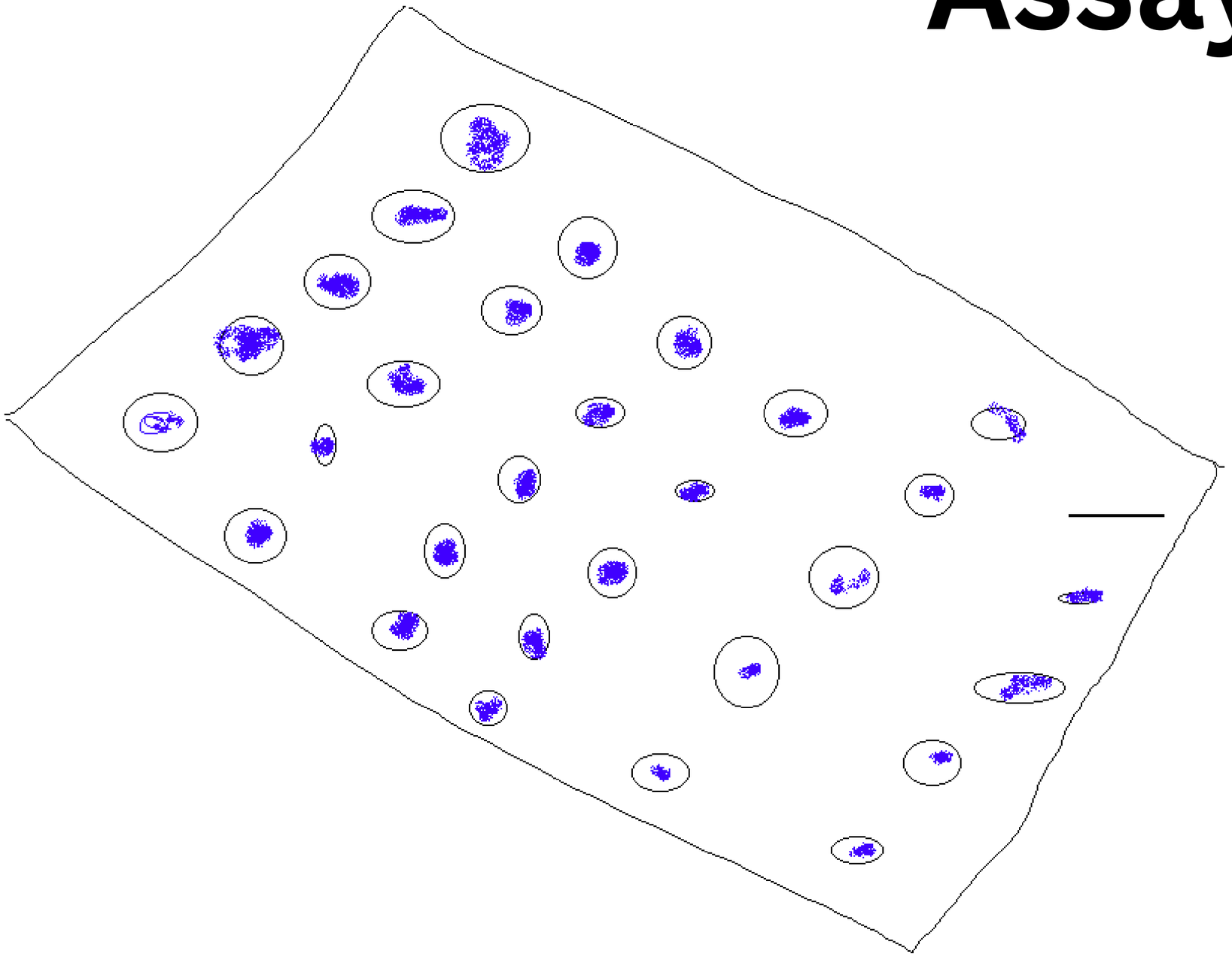
When testing samples, assays are created to be able to identify different characteristics of the hit.

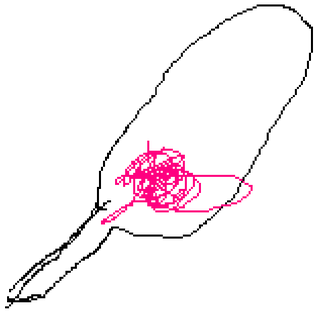
Screening and Assays are used to collect data to be put into databases for later usage and ML and DL prediction.

ML is also used to help optimize assay creating to save both time and money. Optimization is completed by only testing for necessary or wanted characteristics.[11]

There are 2 main types of Assays: **Biochemical** and **Cell-Based**

Assay





Biochemical Assays

- Evaluate and examine target protein and identifying compound that produce desired effect at the target
- Applied to enzyme or receptor targets
- Done *in vitro*

Cell-Based Assays

- Predicts the effect of a drug on an organism by measuring the consequences of a drug's activity within the cells [34]
- Applied to ion channels, nuclear receptors or membrane receptors [35]

Example of Biochemical Assay

ADP Hunter Assay for Kinase Activity

- Measures the accumulation of ADP, a product of kinase enzyme activity [36]
- Incorporates a stop solution to stop the enzymatic reaction which ensures that the ADP levels accurately represent the amt of ADP generated during the assay so that it can be analyzed [36]

Example of Cell-Based Assay

Reporter Gene Assays

- Compound screening assays at G-Protein coupled receptors (GPCRs) have been configured to measure the downstream activation of genes. A signal is given when the compound activates the GPCR and the reporter gene is expressed. [11]

Factors to consider when selecting an assay format:

1. Pharmacological Relevance
2. Reproducibility
3. Costs
4. Quality
5. Effects of compounds

Pharmacological Relevance

Known ligands in the assay should be tested to determine if the assay is able to accurately identify compounds with the desired potency and MOA [11]

Costs

Refers to the expenses associated with performing the assay. The choice of microtiter plates, assay reagents, assay volumes are selected to minimize costs. [11]

Reproducibility

Is the assay consistent and reliable over time? When the same compounds are tested on different plates, it should yield consistent results and should remain consistent across multiple screen days and throughout the entire drug discovery program. [11]

Quality

Assay quality refers to the reliability and robustness of an assay in producing accurate and consistent results and is determined using the **Z' factor**** . [11]

Effects of compounds

The design of the assays should not be affected by the concentrations of solvents used in the assay. [11]

****Z' factor - a statistical parameter that considers the the difference between the high and low signals as well as the variance around them. The Z' factor ranges from 0 to 1 with 1 indicating better assay quality.

Screening

Screening allows for hits to be found and leads to be created in order to start the drug creation process.

Screening is the usage of assays to find hits. This allows for the next step of Drug Discovery

Screening of multiple varieties are used throughout the Drug Discovery Process, both in Hit Identification and Target Identification.

There are multiple screening methods and paradigms depending on resource availability and necessary data collection.
[11]

Types of Screening

High-Throughput Screening

Large numbers of compounds analysed in a assay generally designed to run in plates of 384 wells and above [11]

Used in TDD where targets are known and can be focused on.

Can be expensive and time consuming as well as requiring pre-defined models.

Virtual Screening

Docking models: interrogation of a virtual compound library with the X-ray structure of the protein or, if there is a known ligand, it can act as a base to develop further compounds on [11]

Utilizes computer models to understand protein-ligand interactions.

2 Types:

- Target Based
- Ligand Based

Focused Screening

Compounds previously identified as hitting specific classes of targets (e.g. kinases) and compounds with similar structures [11]

Requires known target, only applicable in TDD.

Less compound testing than in HTS, cheaper and less time consuming.

Types of Screening

Fragment Screening

*Soak small compounds into crystals to obtain compounds with low mM activity which can then be used as building blocks for larger molecules [*11]

Requires a known 3D model of a target.

Fragment screens are ideally suited for assessing whether or not ligands can easily be found for a particular binding site.

NMR Screening

Designing Ligands based on Ligand (Focused) Screening. Using Protein design to determine protein-ligand interactions.

Uses NMR spectroscopy to determine protein shape.

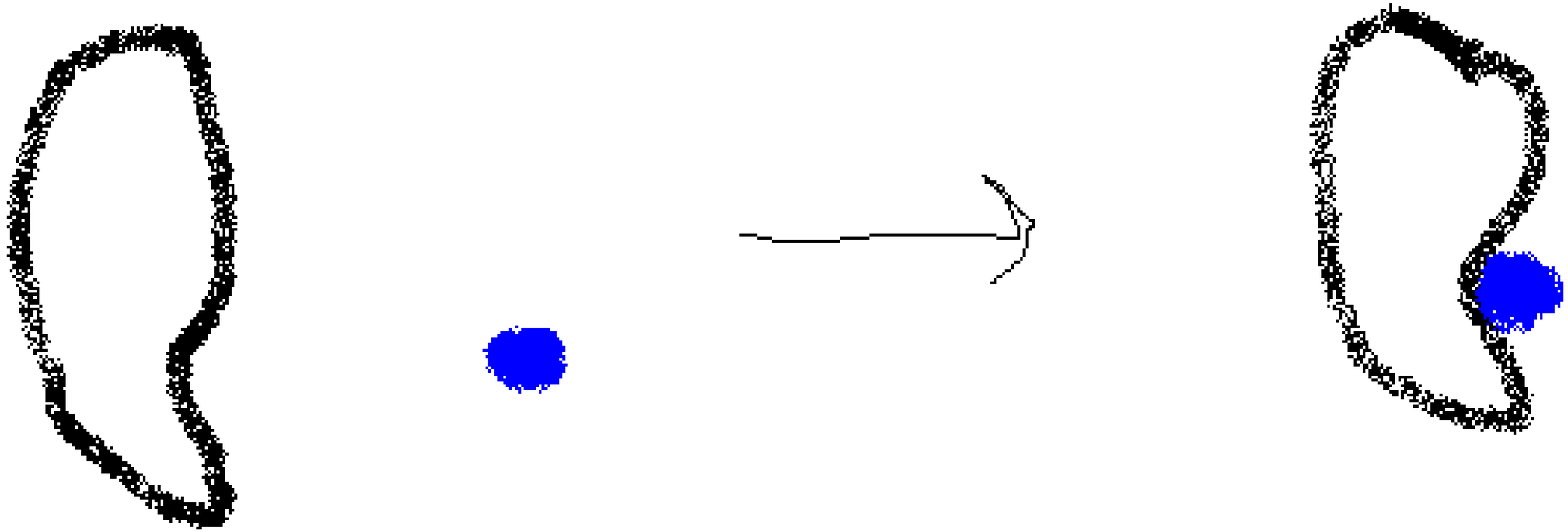
Used in tandem with other screening methods but allows for visual representation and structure for targeted protein. [10]

Physiological Screening

A tissue-based approach for determination of the effects of a drug at the tissue rather than the cellular or subcellular level, for example, muscle contractility [11].

it has become possible to develop high content screening platforms and assays with the ability to measure kinetic parameters of cardiomyocyte function [4]

Ligand (blue) - Protein (Black) Interaction in Hit Discovery



Step 4: Lead Identification

Used in TDD, PDD, and Drug Repositioning

Creating of a library from results of hit identification. Removing guaranteed hits and incomplete hits it becomes a process of simplification.

Leads are any hit which may provide use in the drug creation process.

With AI and ML being used in the screening process to allow for more structural based study with ligand and structural based VS, there is more opportunity for comparison in data collection.

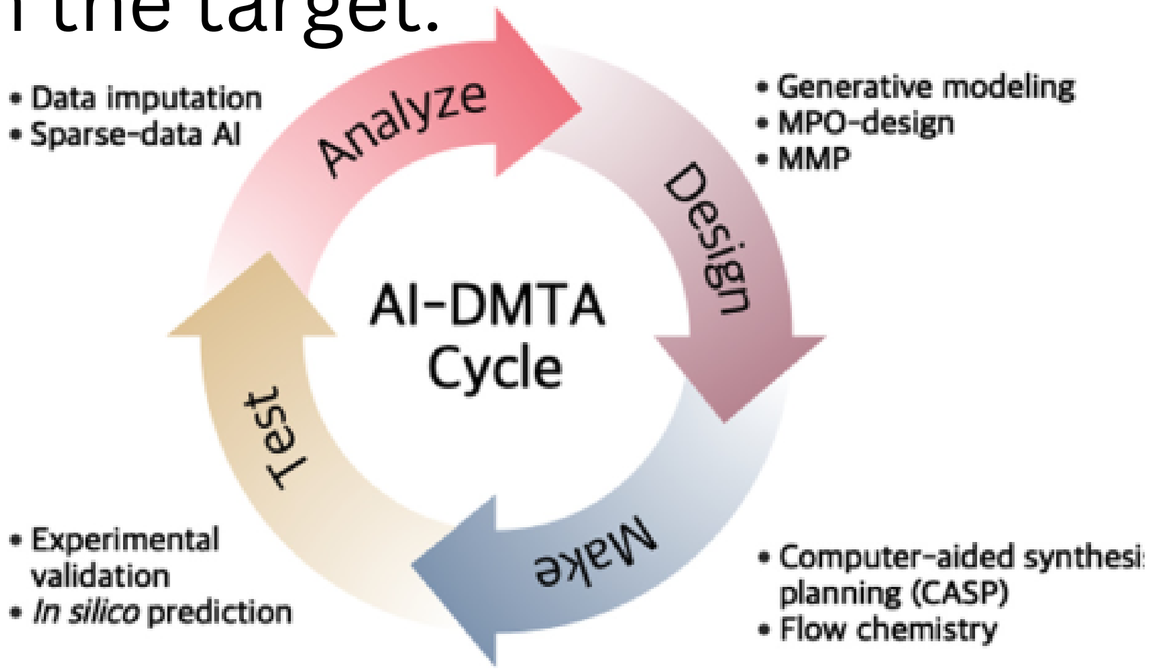
DL and ML are being used to create accurate protein structures that can be used in identifying hits and leads when testing compounds. [25]

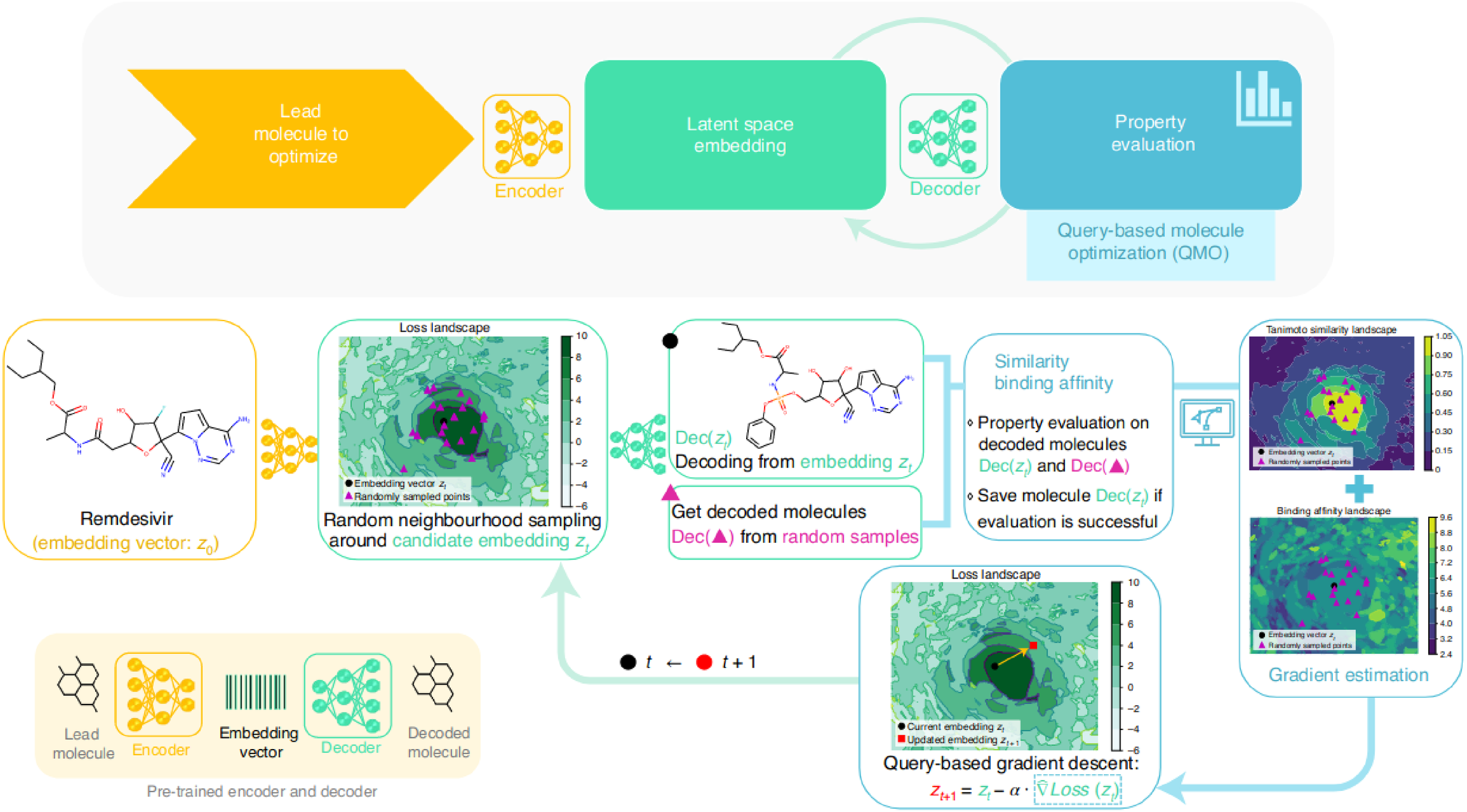
Step 5: Lead Optimization

Used in TDD, PDD, and Drug Repositioning

Uses an iterative process in the form of DMTA (Design - Make - Test - Analyze) to enhance the characteristics of the drug and how it interacts with the target.

A multi parameter optimization method, where compounds are tested and improved iteratively to increase potency and remove toxicity. [25]





[9] proposed QMO (query-based molecule optimization)

Design

Goal - to enhance specific characteristics:
Safety, Specificity, Efficacy, and Pharmacokinetics (PK) properties, while maintaining potency

Generative modeling focuses on calculating various absorption, distribution, metabolism, excretion, and toxicity (ADMET) endpoints

Relies on AI based generative chemistry to model compounds. A good model can reduce the amount of necessary iterations. [25]

Make and Test

Use the models created in the design portion to create drug and compound prototypes.

Computer-aided synthesis planning (CASP) is also beginning to incorporate AI into its design allowing for the automation of compound creation

Organic and Chemical synthesis is used to build optimized compounds that still have potency and interact with the designated target.

The created compounds can then be tested using *in silico* prediction [25] as well as *in vitro*, *in nano*, and occasionally *in vivo*.

Analyze

The results of the testing are then checked to see if improvements have been made.

Since collected data is sparse and non-uniform, it is important to use ML and DL to find associations in the data to improve the *in silico* testing.

These results can be used to find correlations between nodes (as in a Knowledge Graph) as well as build QSAR (quantitative structure activity relationship) models.

Most collected data is noisy or missing so DNN (Deep Nueral Networks) may be in use to find correlations. [25]

Step 6: Pre-Clinical Trials

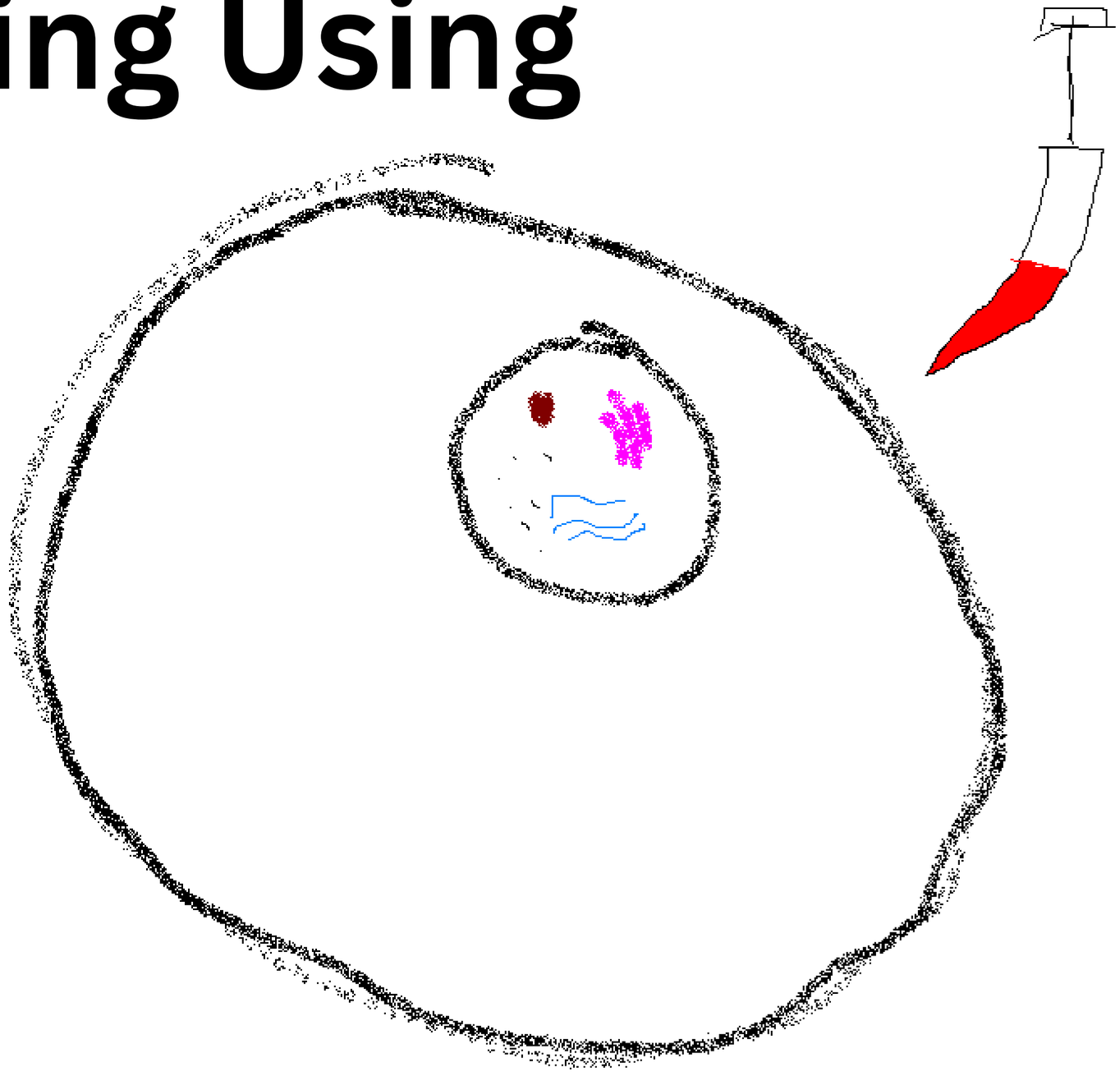
Used in TDD, PDD, and Drug Repositioning

Preclinical trials are done *in vitro* with either mammalian or human cells, rather than a living being (*in vivo*).

Rodents were used as animal counterparts for testing, but swine have more similar cell structure to humans - while more expensive - they also give more accurate results to drug toxicity and potency. [2] [7]

For Example - While testing for drugs suited to combat leukemia, the zebra-fish was used to test effects drugs had on T-cells. [18]

In Vitro Testing Using Animal Cells



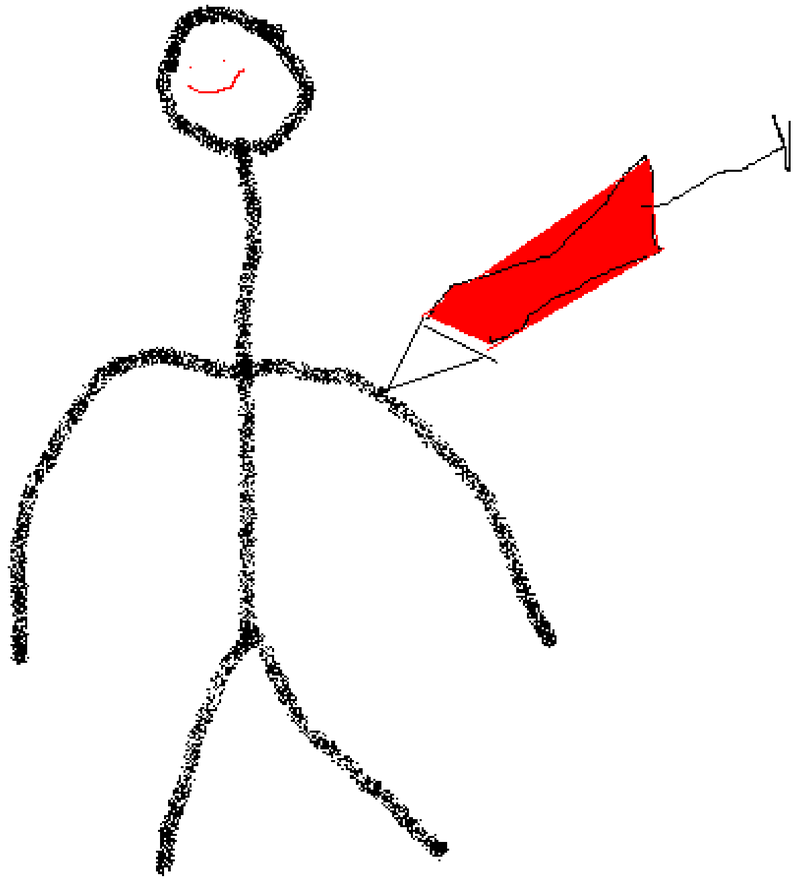
Step 7: Clinical Trials

Used in TDD, PDD, and Drug Repositioning

After completing preclinical trials, the drug then moves to the clinical trials stage. This is where new tests and treatments are studied on human participants with the intention of answering precisely framed questions. [39]

Clinical Trial Phases:
1: Small Scale
2: Larger Scale
3: Population Introduction
4: Marketed

In Vivo - Testing Using Human Patient



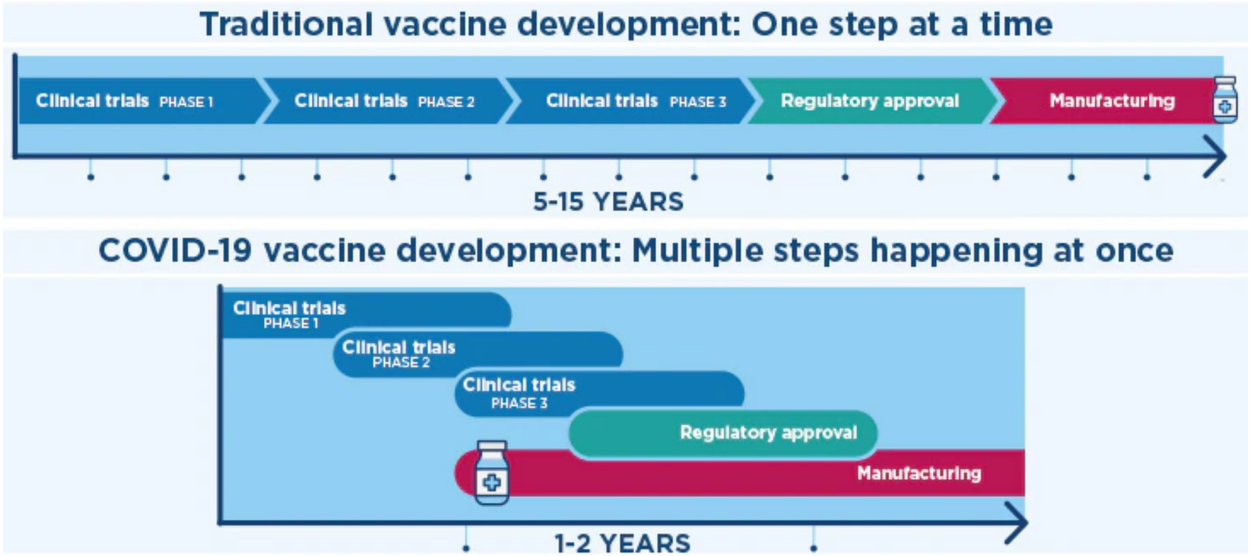
Clinical Trial Guidelines

1) Ethics and patient selection:
The criteria for patient selection should be thought out and defined.
2) Response measurement:
The trial end point should be clearly defined. Side effects should be carefully observed and noted.
3) Experimental design:
Controlled clinical trials must include four safe guards against bias:
a) **double blind technique**: neither participants nor the researcher knows which treatment participants are receiving until end of trial [42]
b) **randomization of treatment**: assigning patients randomly to groups that receive different treatments [43]
c) **matching of patient**: identifying trials that patients may be eligible for based on the eligibility criteria of the trial
d) **cross over techniques**: two or more treatments are provided to patients at different time periods with the sequence of treatment randomized.[44]

CLINICAL TRIAL Example: SARS-CoVid-2

- Phase 1: 100> people ; determines if vaccine is safe, best dosage, and side effects
- Phase 2: few hundred volunteers; how well vaccine works
- Phase 3: thousands of volunteers; compare people who receive vaccine vs people who didn't; determines safety in larger population
- vaccine vs controlled medication [41]

[27]



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Thank you!

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