Drug Discovery: Methods and Principles

Prof M Kaur

Dug discovery background

- Over the years, many new treatments have been found
- e.g. HIV-AIDS
  - Virus identified- 1983
  - 1987- AZT (Retrovir, azidothymidine) proved
  - Mechanism- first reverse transcriptase inhibitor
  - Over 3 decades- other drugs developed-
    - Viread (Tenofovir), Viracept (Nelfinavir), Crixivan (Indinavir) (HIV Protease inhibitor)

    - Improved treatment with HAART (highly active antiretroviral therapy)- cocktail treatment
      - All-in-one Complera and Stribild

Modern drug discovery changed the course of AIDS epidemic in <30 years

Cancer

	Breast	Prostate	Melnoma
5-Year Survival rate	60-91%	43-99%	49-93%

Chemotherapeutic drugs
Natural products and their derivative:

Taxol (Paclitaxel), Velban (Vinblastine), Adriamycin (Doxorubicin)

Small molecule kinase inhibitors:

Gleevac (Imantinib), Tasigna (Nilotinib), Tarceva (Erlotinib)

Cardiovascular

- Hypertension (the silent killer):
  - Diuretics (Midamor (Amiloride)
  - Beta-blockers (Tenoretic (Atenolol))
  - Angiotensin-converting enzyme (ACE) inhibitors (Capoten (Captopril)

Other class:

HMG-CoA reductase inhibitors

STATINS by blocking cholesterol production

Lipitor (Atorvastatin)

Zocor (Simvastatin)

Alzheimer's

- Most common form of dementia
- Described in 1906
- >110 years gone, still no treatment
- Potential targets are:
  - Beta-secretase (BACE)
  - Gamma-secretase
  - Glycogen synthase kinase 3beta (GSK3beta)
  - Cyclin-dependent kinase-5 (CDK5)

Challenges faced over time

Confidence: we have drugs to treat all bacterial infections (quinolone, tetracyclines, macrolide antibiotics)

What happened:

- MRSA (methicillin-resistant *Staphlococcus aureus* (MRSA)) in 1980s and 1990s.
- Methicillin (Staphcillin) introduced in 1959- to treat penicillin-resistant bacteria
- 2 years later- resistant strains in European hospitals.
- By 1980, it spread across the globe and costs of bacterial infection treatment in US alone were- $3-$4 billion annually.

Lesson: it is learned that drug discovery must go on….

Pharmaceutical companies are backing away from a growing threat that could kill 10 million people a year by 2050

Just two years after Novartis announced it would embrace the challenge of searching for cures for life-threatening infections known as superbugs, the drugmaker said last week it would exit antibacterial and antiviral research.

Novartis' retreat follows a growing trend of big pharmaceutical companies — including AstraZeneca, Sanofi, and Allergan — that are exiting from this type of research because of a lack of profit.

That leaves Merck, Roche, GlaxoSmithKline, and Pfizer as the remaining pharmaceutical companies with active antibiotic programs, according to Nature Biotechnology. Only 12 antibiotics have been approved since 2000.

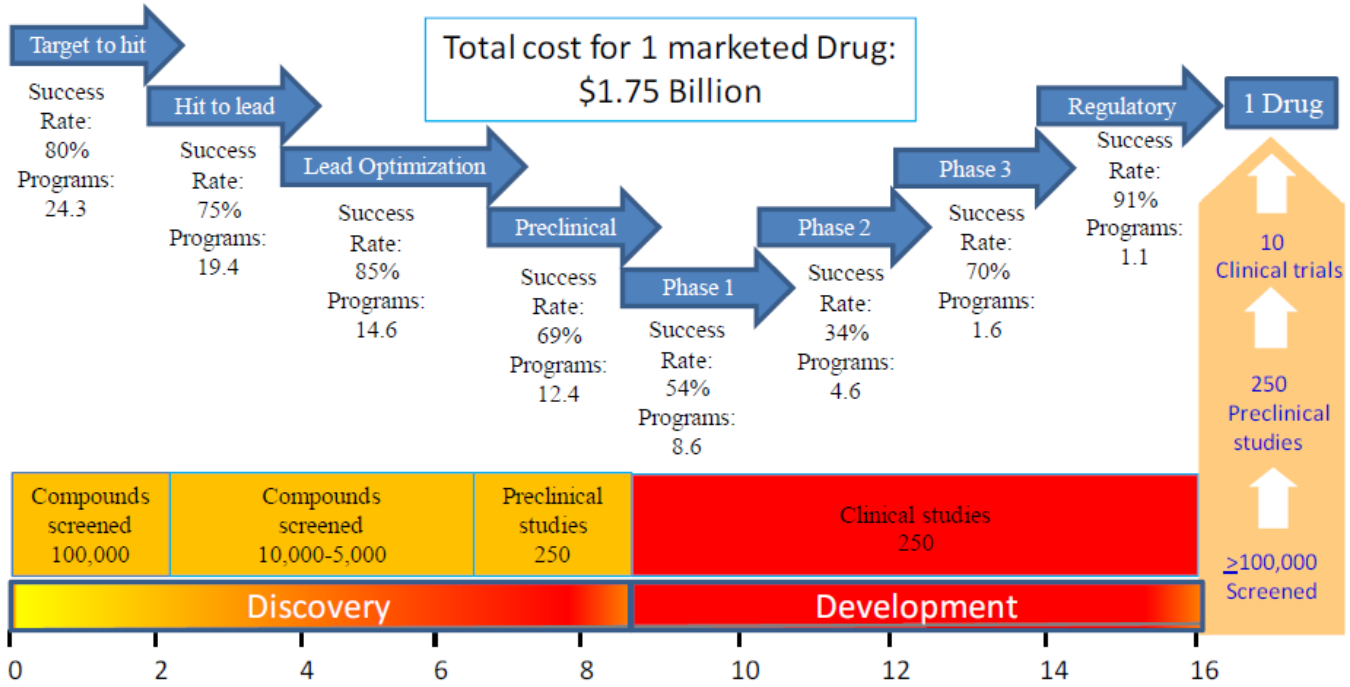
July, 2018

https://www.businessinsider.com/major-pharmaceutical-companies-dropping-antibiotic-projects-superbugs-2018-7?IR=T

Challenges with drug discovery process

Out of millions of possible compounds

- Understand disease or infectious agent biology
- Toxicity to normal cells
- Physiological effects
- Safe delivery
- Which compounds pharma would like?
- Cost of development



Multidisciplinary field requires expertise from various research areas

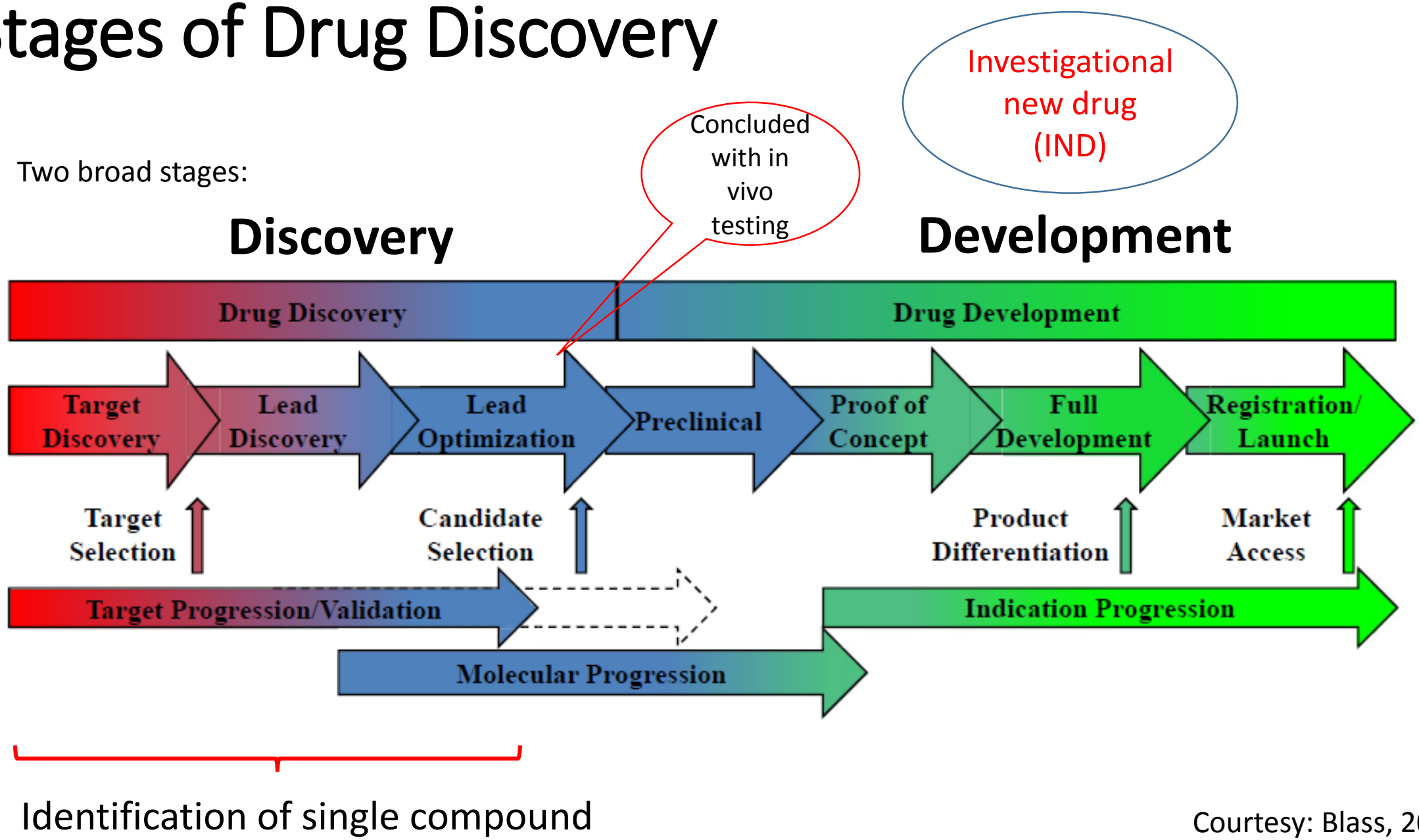
Key is 'collaboration'

1 of 10 clinical candidate will reach market with overall success rate 0.001%

Courtesy: Blass, 2015

Stages of Drug Discovery

Two broad stages:



Identification of single compound

Courtesy: Blass, 2015

Stages of Drug Discovery: CLINICAL TRIALS

- **Phase I** – Human Pharmacology (Healthy volunteers – 20-50 subjects)
- **Phase II** - Therapeutic Exploration (patients – 50- 400)
- **Phase III** – Therapeutic Confirmation (large scale multi-centre; 250-1000)
- **Phase IV** - Therapeutic Use (post-registration monitoring)

{Phase 0 , Microdosing}

34% pass rate

Safety margins for further progression
Maximum tolerated dose

IIA – Therapeutic dose set

IIB – Overall efficacy

Efficacy in standard of care
New drug may be approved here by FDA

Post marketing surveillance
Detect rare side effects
Lead to alteration in labels- safety profile, use with another drug

After Phase IV- drug can be removed from market, e.g, COX-2 selective NSAID Vioxx (Rofecoxib)- increased risk of ischemic events

FDA warns of serious genital infection link with diabetes drug

SEPTEMBER 5TH, 2018 WEEKLY ROUNDUP

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The **US Food and Drug Administration (FDA)** has warned that a serious genital infection has been reported in patients taking a certain class of diabetes drugs, with one death and 11 others hospitalised. ***Reuters Health*** reports that the warning pertains to a class of medicines called SGLT2 inhibitors, first approved in 2013 to lower blood sugar in adults with type 2 diabetes.

The US health regulator has also called for including this risk in the drugs' labelling. The SGLT2 inhibitors approved by the FDA include **Johnson & Johnson's** Invokana, **Eli Lilly & Co's** Jardiance, as well those from **Bristol-Myers Squibb, Astra Zeneca, Merck & Co** and **Pfizer**.

The report says the companies did not immediately respond to a request for comment.

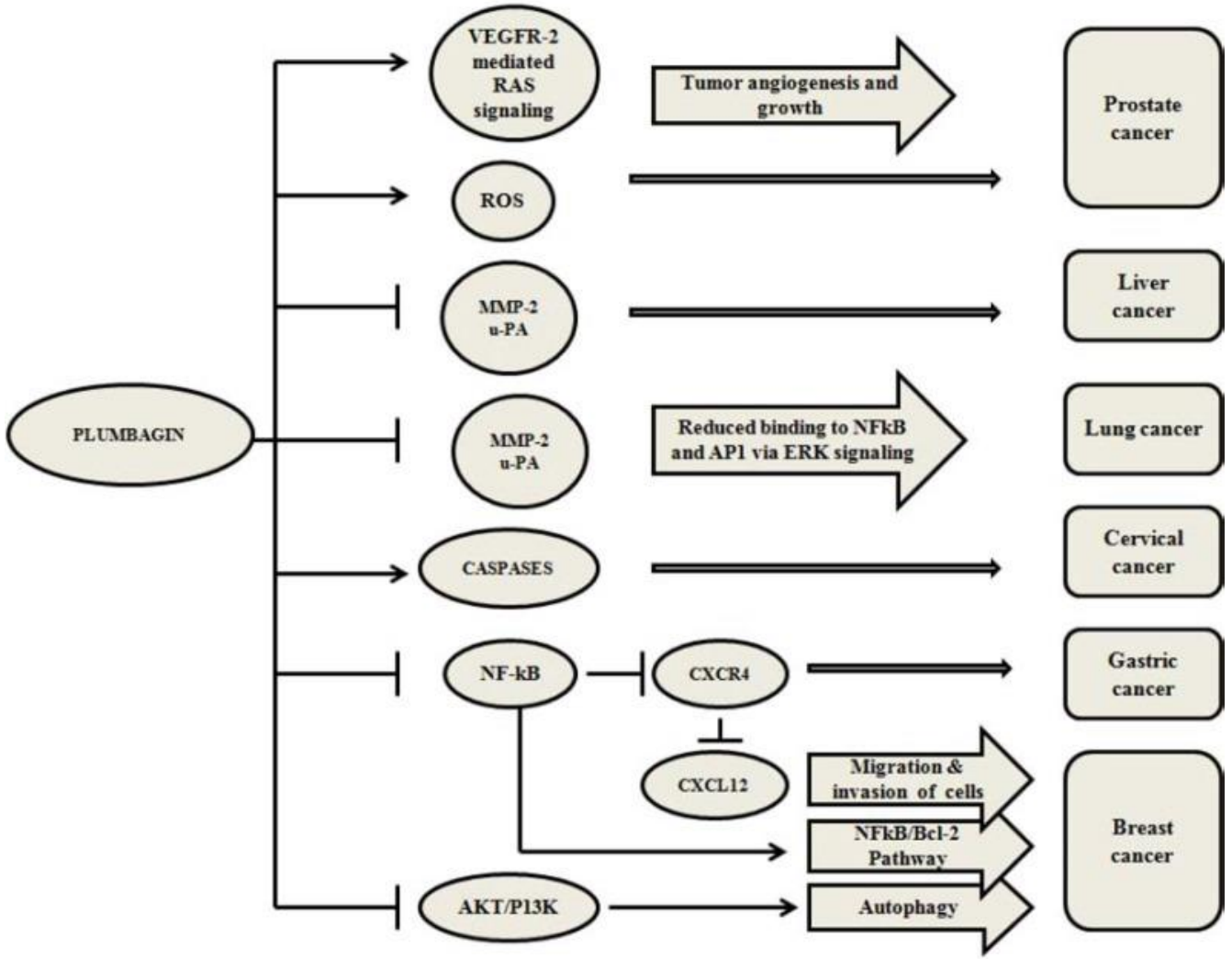
Patients are at risk of the infection known as Fournier's gangrene, an extremely rare but life-threatening bacterial infection of the tissue under the skin that surrounds the genital area, the FDA is quoted in the report as saying.

The bacteria usually enter the body through a cut and quickly spread. Having diabetes is a risk factor for developing Fournier's gangrene.

The FDA said it identified 12 cases of Fournier's gangrene – 7 in men and 5 in women – between March 2013 and May 2018. One patient died, while some required multiple disfiguring surgeries and developed complications, the agency said.

https://www.medicalbrief.co.za/archives/fda-warns-serious-genital-infection-link-diabetes-drug/

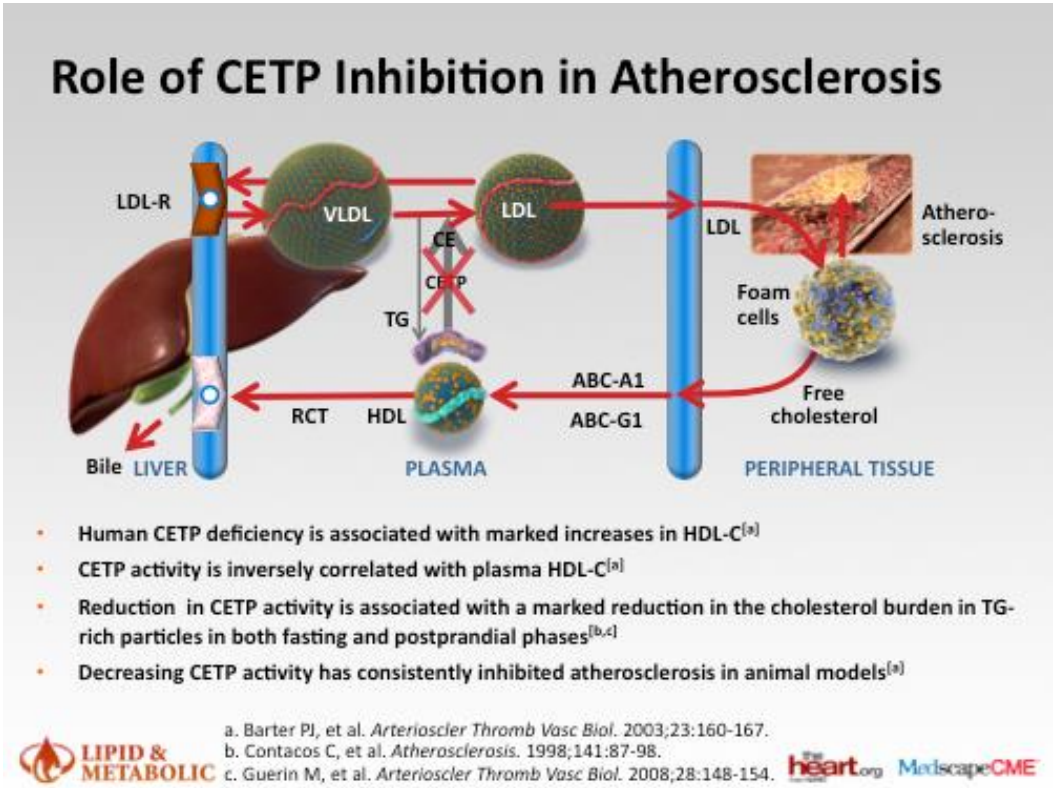
Single molecule can have multiple targets



Anticancer Agents Med Chem. 2014 Jan;14(1):170-80.
Cytotoxicity and apoptosis induced by a plumbagin derivative in estrogen positive MCF-7 breast cancer cells.
Sagar S, Esau L, Moosa B, Khashab NM, Bajic VB, Kaur M.

Target selection: The first step

- Prioritize disease
  - Scenario 1: e.g Alzheimer's disease- but no target
  - Scenario 2: target is linked theoretically to disease
    - CETP (Cholestryl Ester Transfer Protein)



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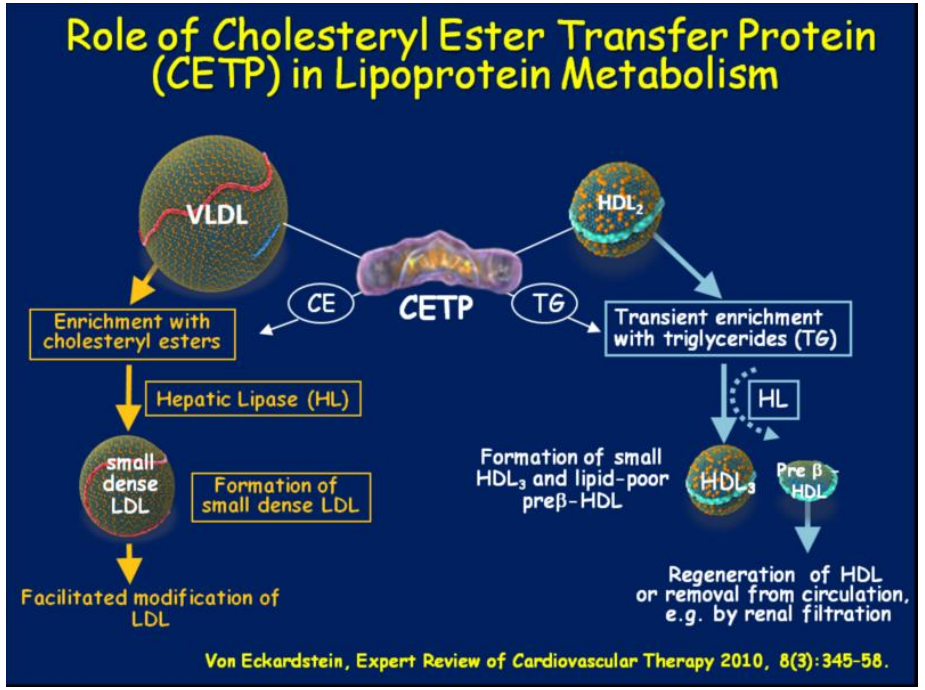

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↑ HDL ↓ LDL

Torcetrapib
Dalcetrapib
Ancetrapib

None approved

- Not a viable drug target
- Compounds have off-target effects
- Pharmacokinetic issues

Torcetrapib- increase in BP and Mortality
Terminated in 2006

Other contributors to target selection

- Financial profit to corporates
- Target selection sets the stage for all future research programs
- Rare diseases (Amyotropic lateral Sclerosis (ALS)- a progressive degeneration of the motor neurons of the central nervous system, leading to wasting of the muscles and paralysis (Courtesy: google search).
  - 20,000- 30,000 patients in USA
  - Life expectancy 3-5 years
  - No therapy available

Hit Identification: the starting point

- A compound that modifies selected target.
- Exceptionally complex and multifaceted process
- CAS (Chemical Abstract Service Database)- http://www.cas.org/content/cas-databases
  - Has 143 million small molecules (organic and inorganic)

- Guidelines for useful drug-like biological molecules
  - Lipinski's rule of 5
    - Molecular weight < 500
    - logP < 5
    - < 5 H-bond donors
    - <10 H-bond acceptors
    - < 10 rotatable bonds

Lipinski's rule: examples and exceptions

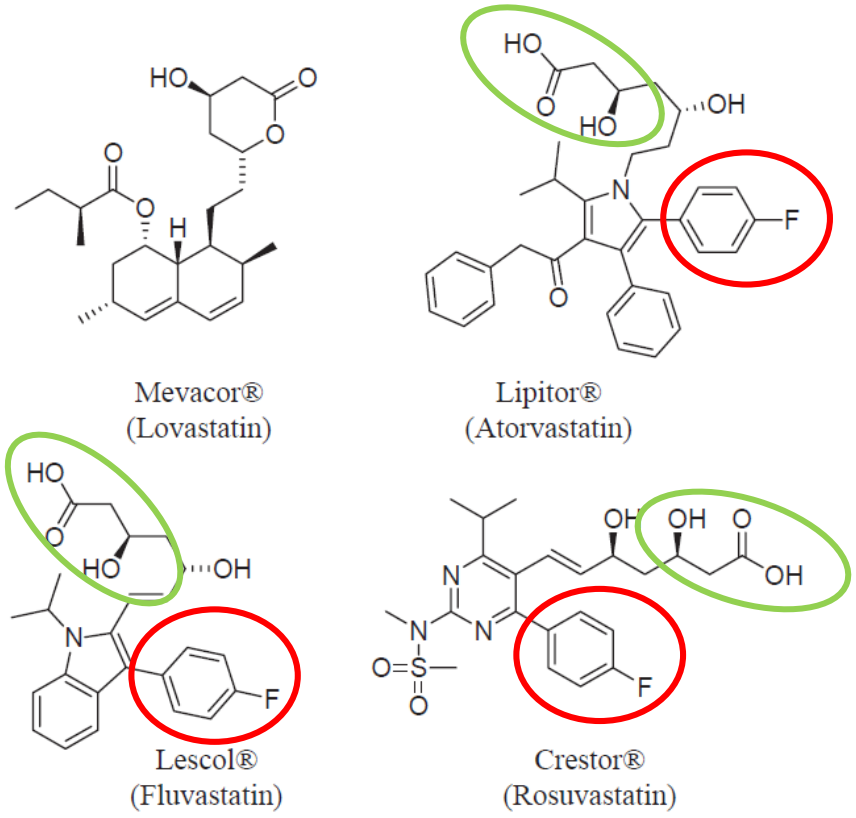


FIGURE 1.12 The HMG-CoA reductase inhibitors Mevacor® (lovastatin), Lipitor® (atorvastatin), Lescol® (Fluvastatin), and Crestor® (rosuvastatin) have some structural similarities, but there are a number of differences that make each unique.

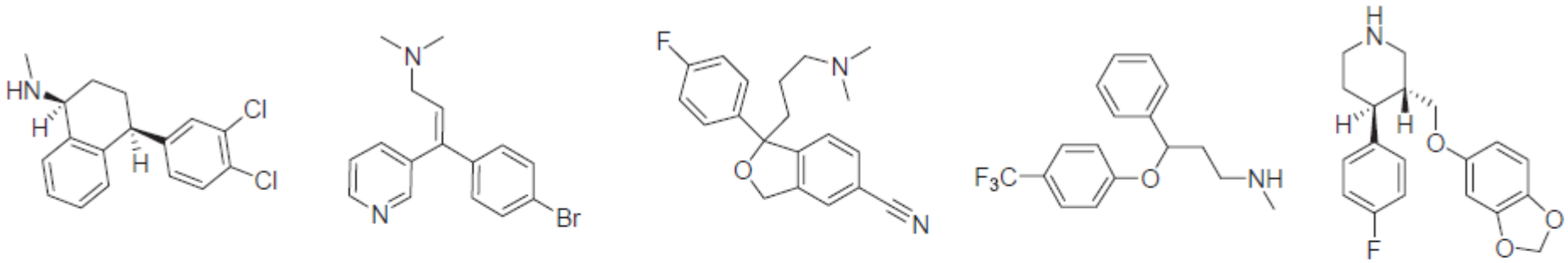
- Lipitor, Lescol and Crestor have similarities- para-flourbenzene ring and 1,3-diol-carboxylic acid side chains- otherwise compounds are quite different to each other.
- Mevacor- entirely different class of compounds

All above are HMG-CoA reductase inhibitors

(3-hydroxy-3-methyl-glutaryl-**coenzyme A** reductase)

Courtesy: Blass, 2015

Exceptions: SSRI's (Selective serotonin reuptake inhibitors)



Zoloft®
(Sertraline)

Zelmid®
(Zimeldine)

Celexa®
(Citalopram)

Prozac®
(Fluoxetine)

Paxil®
(Paroxetine)

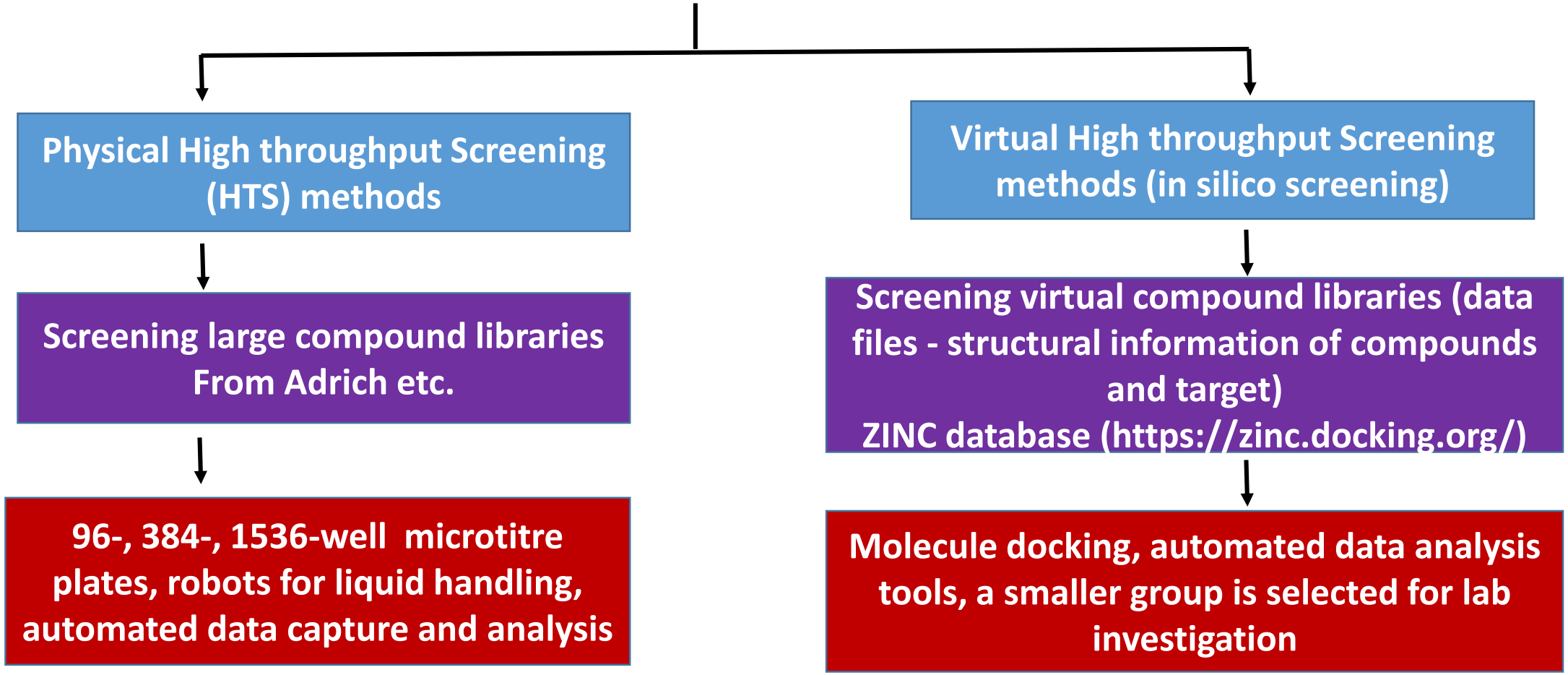
FIGURE 1.15 Zoloft® (Sertraline), Zelmid® (Zimeldine), Celexa® (Citalopram), Prozac® (Fluoxetine), and Paxil® (Paroxetine) are all selective serotonin reuptake inhibitors (SSRIs) useful for the treatment of depression, but structural similarities are limited.

Structurally very different compounds with same biological target

Courtesy: Blass, 2015

Finding an initial chemical lead- Challenge no 1

Strategies to overcome challenge



Limitation of HTS methods

Physical HTS

- Sensitivity (False positives) and specificity (False negatives)
- Due to reagent handling, sample degradation
- Require repetition for ‘hit’ compounds

Virtual HTS

- Remember- results are predictions not real (e.g, structure through X-ray crystallography (as a crystal) but in real life, biological molecules are either dissolved or membrane bound
- Validation required in laboratory

Nonetheless, this exercise identifies ‘hit’ compounds for lead discovery step

Lead Discovery

- To examine if ‘hit’ compounds need follow-up efforts or not

500,000 compounds → Screening → 500 (0.1%) compounds → Different structural classes of compounds

e.g aminonaphtoquinones

Information from previously known active structurally similar compounds

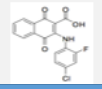
Structure-activity relationship

Short-listed compounds

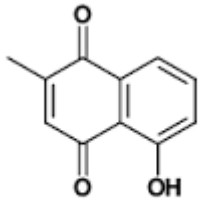
Synthesize analogues

2-(hydroxymethyl) naphthalene-1, 4-diol (Triol)

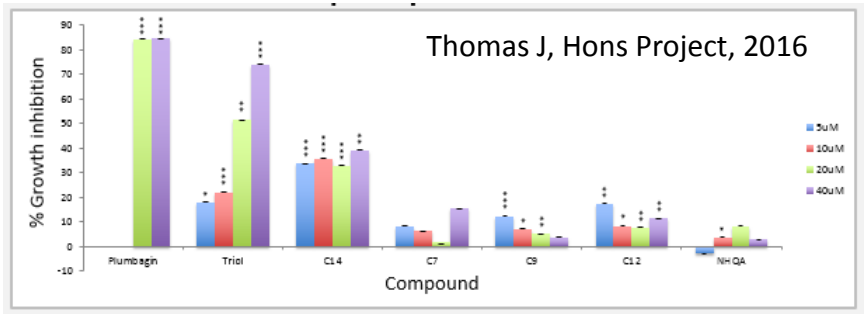
3-[(4-Chloro-2-fluorophenyl)amino]-1,4-dioxo-1,4-dihydronaphthalene-2-carboxylic acid (C14)



Biological testing



Plumbagin



Lead Optimization

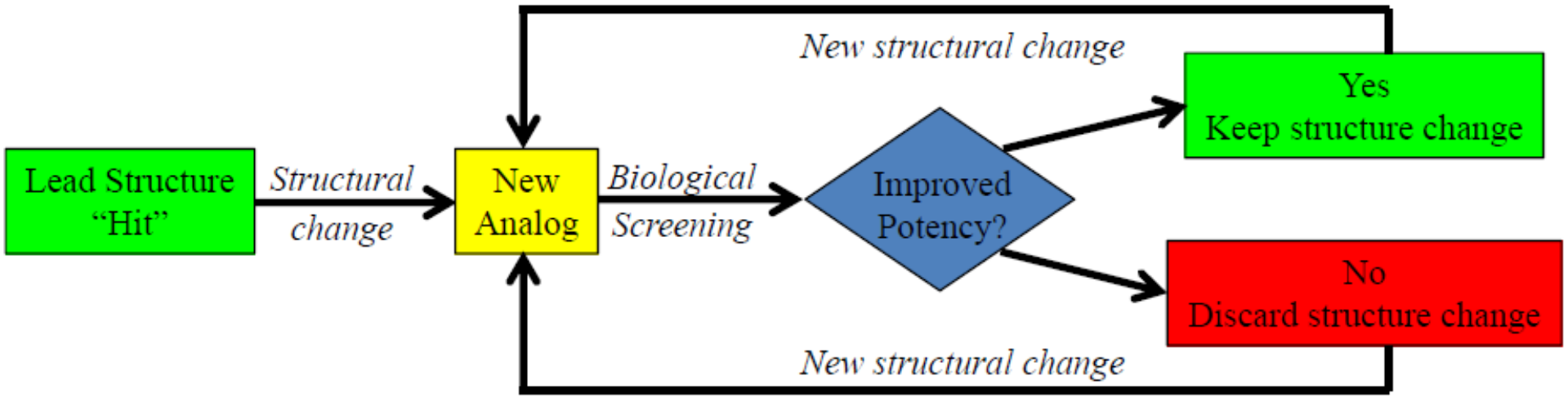


FIGURE 1.16 The lead optimization cycle begins with the identification of a lead structure ("hit") in a relevant biological assay. New analogs with structural modifications are prepared and screened in the biological assay. If the assay results improve, then the changes are kept and the cycle is repeated. If the changes are detrimental, then the changes are discarded and the cycle is repeated. This process continues until a candidate compound with the desired properties is identified.

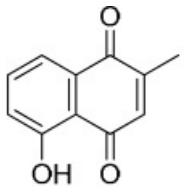
Courtesy: Blass, 2015

Anticancer Agents Med Chem. 2014 Jan;14(1):170-80.

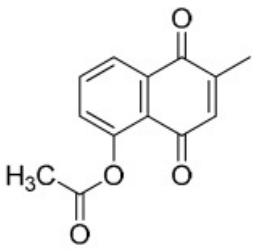
Cytotoxicity and apoptosis induced by a plumbagin derivative in estrogen positive MCF-7 breast cancer cells.

Sagar S, Esau L, Moosa B, Khashab NM, Bajic VB, **Kaur M**.

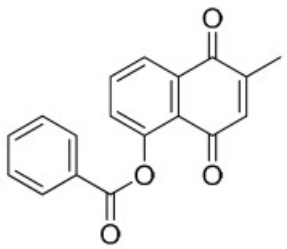
An example-



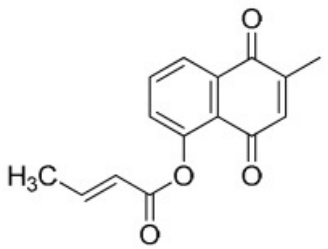
Plumbagin (PL)



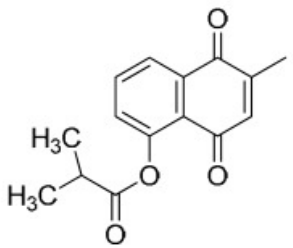
Acetyl Plumbagin (AP)



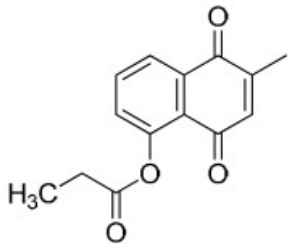
Benzoate Plumbagin (BP)



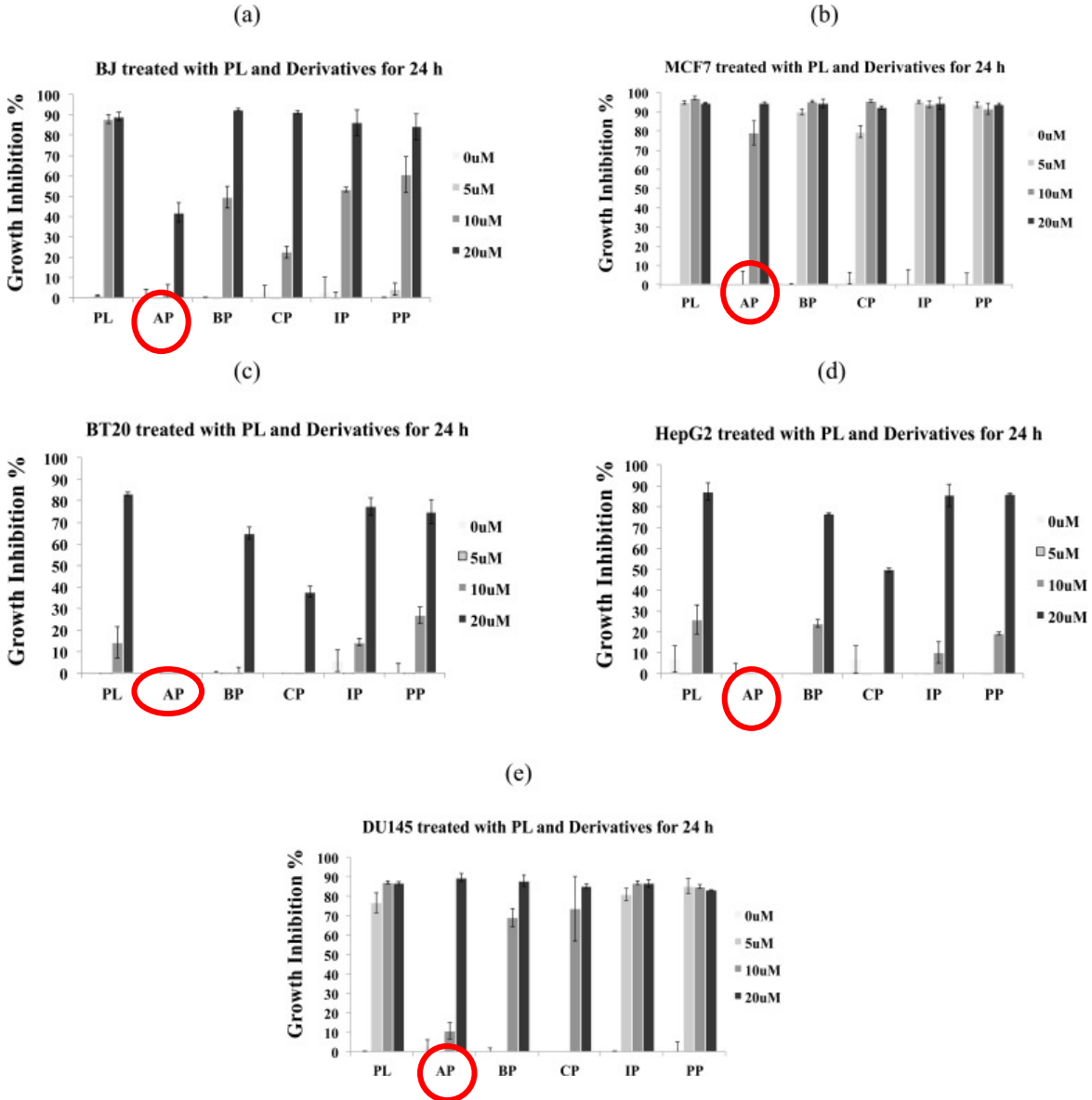
Crotonate Plumbagin (CP)



Isobutyrate Plumbagin (IP)



Propionate Plumbagin (PP)



AP for clinical evaluation?

A clinical candidate: Juggling the properties

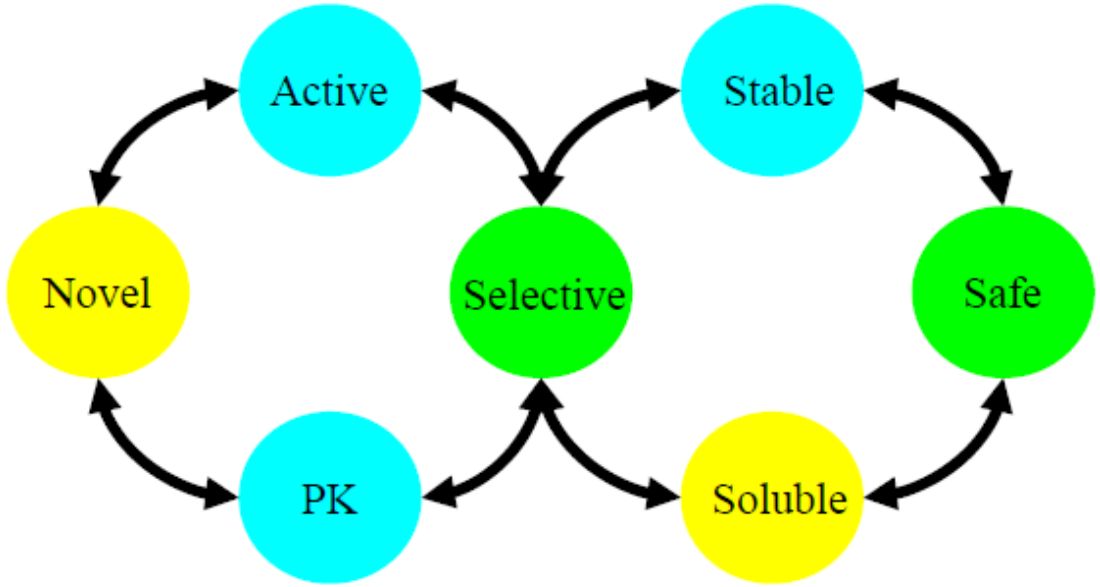


FIGURE 1.17 The identification of a clinical candidate requires consideration of a variety of properties beyond activity at the biological target of interest. Drug discovery and development programs seek to optimize as many of these properties as possible in order to identify the best opportunity for success.

Screening must go on…….

Courtesy: Blass, 2015

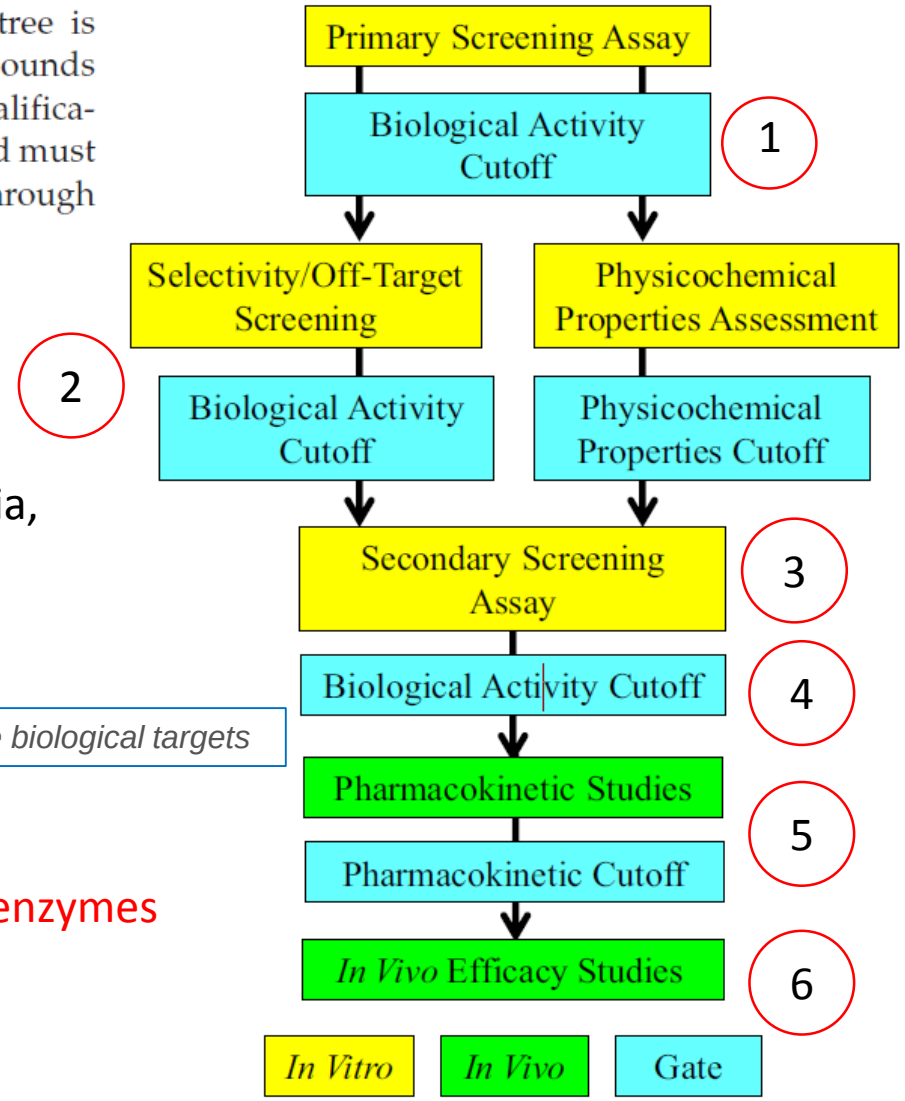
pharmacodynamics is the effect that drugs have on the body, pharmacokinetics is the study of the way in which drugs move through the body during absorption, distribution, metabolism and excretion.

Screening Cascade to reach clinical trials

To decrease the number of compounds at each level (a filtering process)

in vitro to *in vivo*

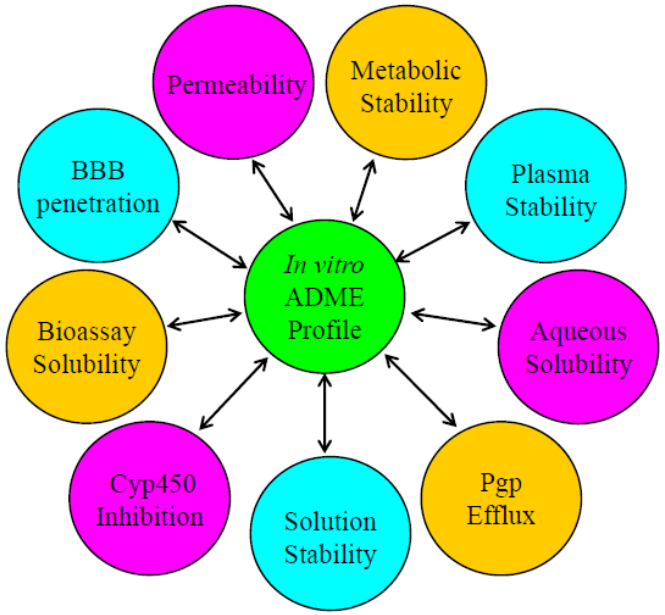
FIGURE 1.18 A screening tree is designed to identify lead compounds by establishing a series of qualifications or "gates" that a compound must surpass in order to advance through the process.



1. Potency- Determine low dose (e.g. 5nM or 5 micromolar)
2. Selectivity and physiochemical properties
   Example of Kv1.5 voltage-gated potassium channel for atrial arrhythmia, matches with 70 other channels to varying drees, e.g. hERG channel- causes sudden cardiac death
3. Promiscuous compounds- potent on a variety of other targets-
   risk of side effects *Definition- specific interaction of a small molecule with multiple biological targets*
4. ADME- Absorption, distribution, metabolism, and extraction
   Drug-like properties- potent, selective, soluble, penetrate biological membranes, BBB, metabolized in body, CYP enzymes
5. PK studies- how much in circulation, how rapidly metabolized, freely distributed or confined to an organ
6. In vivo complete testing along with safety studies

Courtesy: Blass, 2015

Conclusion



- Drug discovery scientists walk on the edge of several precarious slopes in attempting to identify potential new therapeutic entities. Balancing the needs of potency, selectivity, solubility, stability, pharmacokinetics, safety, and novelty is critical to the success of any project, and failure to deliver in any one of these areas can terminate the forward progression of a test compound.

- Another major driving force- Patents

Courtesy: Blass, 2015