



Drug Discovery from Target Identification to Lead Optimization

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Disclaimer

The views and opinions expressed are solely those of the speakers and do not represent those of our current or former employers

Course Outline

- **Small molecule drug discovery** – 50 min
 - **Target identification:** Evidence that modulating a biomolecule will be therapeutically beneficial
 - **Hit finding:** Identify molecules that modulate target in a specific manner
 - **Hit-to-lead:** Confidence in target and in chemical matter to ungate significant investment
 - **Lead optimization:** Deliver candidate molecules to progress to preclinical development
- **Q&A, break** – 10 min
- **Large molecule drug discovery** – 50 min
 - **Target identification**
 - **Choosing a drug modality**
 - **Antibody discovery**
 - **Newer molecule modalities**
- **Q&A, break** – 10 min
- **Case studies in SM and LM drug discovery tag team** – 50 min
- **Q&A, wrap up**



Small Molecule Drug Discovery



About Me



Undergraduate
in University of Pittsburgh

Chemistry



Stanford
University

Graduate school at Stanford
University

Ph.D. in Organic chemistry

Palladium-catalyzed reactions
and total synthesis



Neurodegenerative Diseases

Multiple therapeutic programs

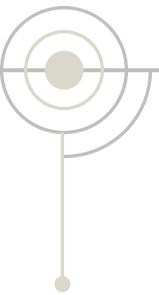
Small Molecule Drug Discovery



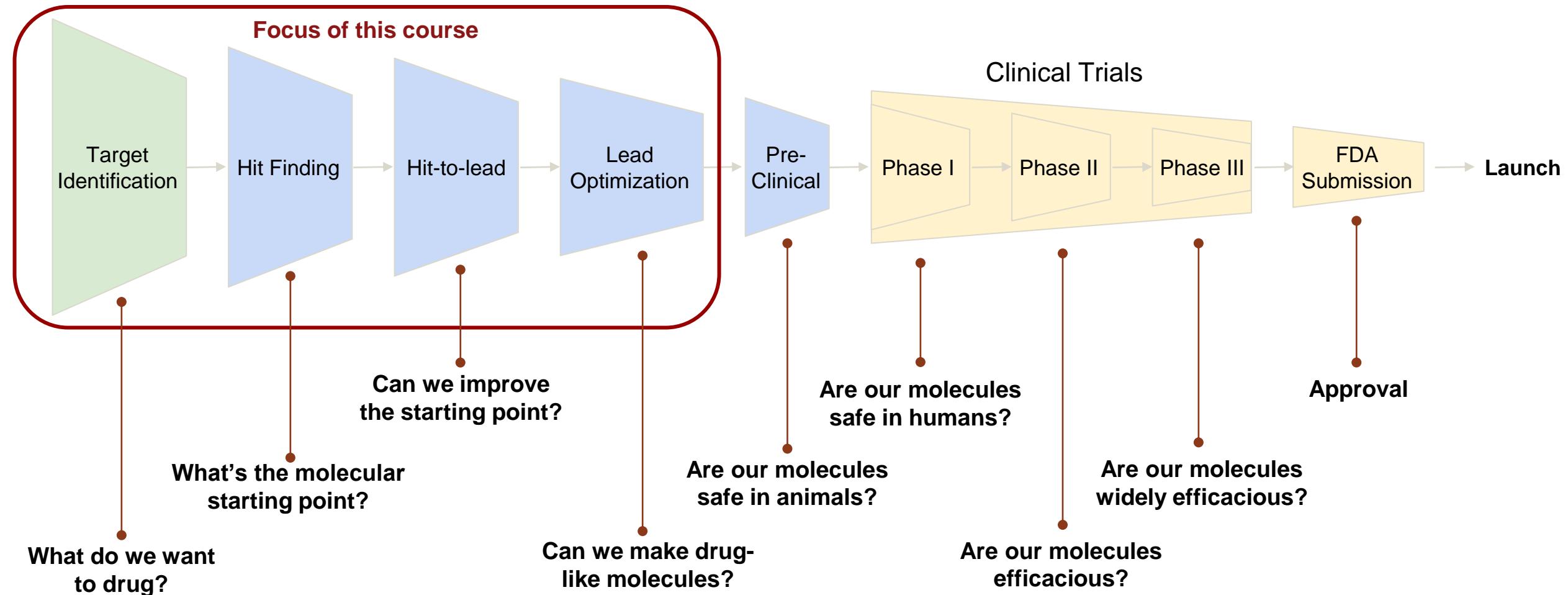
Immuno-oncology

IDO1 inhibitors

Medicinal Chemistry

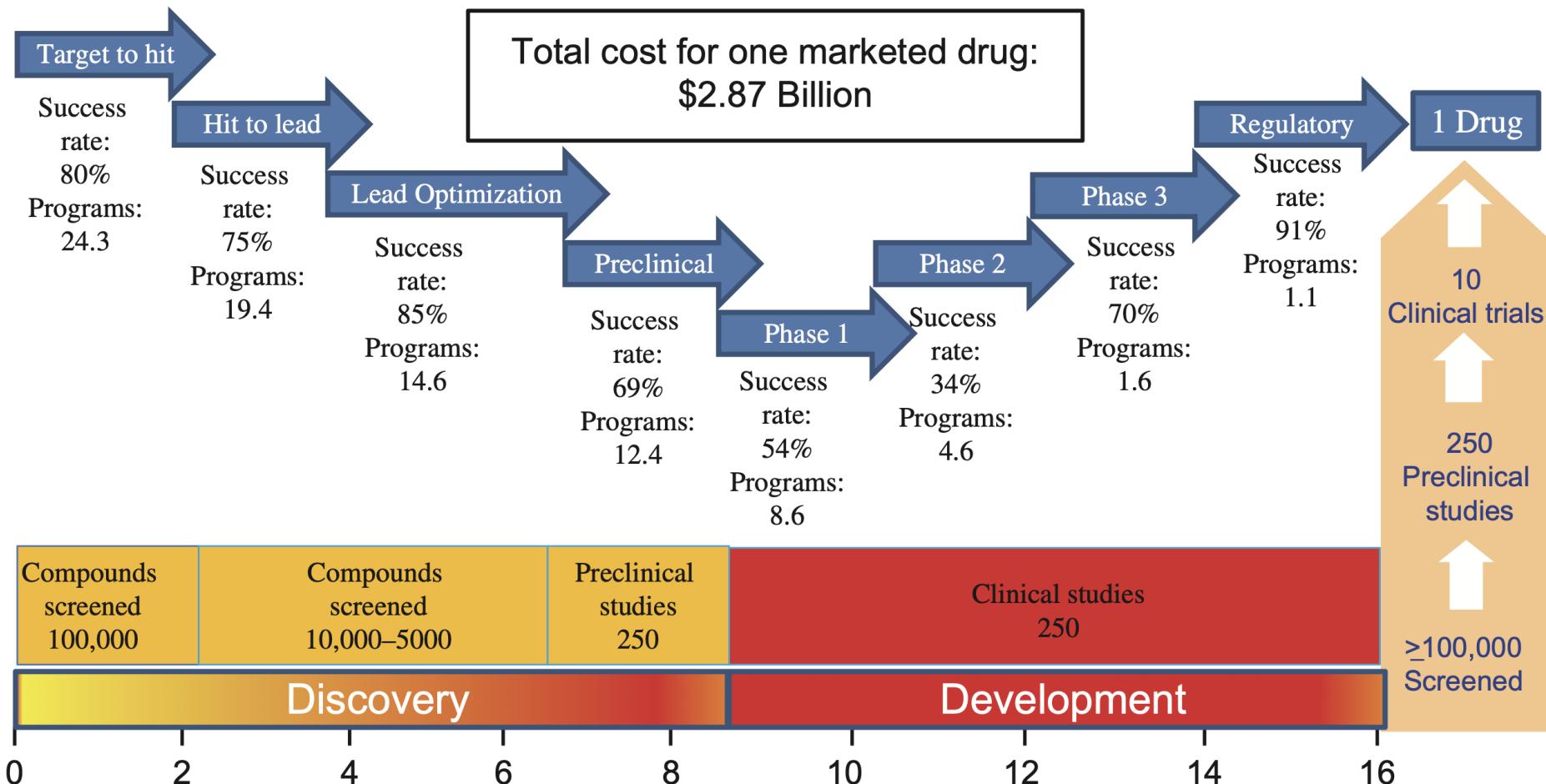


The Drug Discovery Process Has Stages With Key Questions



^a [Measuring the return from Pharmaceutical innovation 2022](#); ^b JAMA, 2020, 323(9):844-853; ^c BJP, 2011, 162, 1239; ^d *PharmacoEconomics*, 2021, 39,1243. ^e Future Med. Chem. 2020, 12, 939

The Drug Discovery Process is Long and Challenging

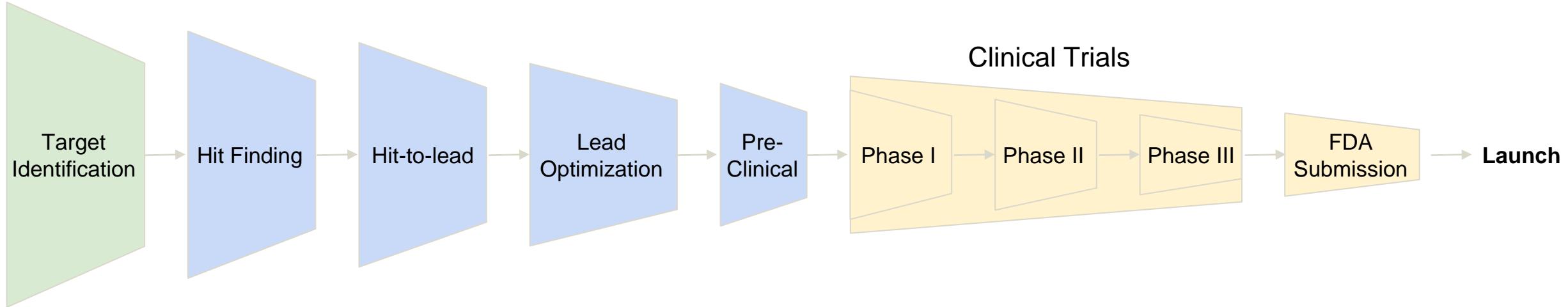


^a Blass, B. *Basic Principles of Drug Discovery and Development.*; 2020, Ch1; ^b JAMA, 2020, 323(9):844-853; ^c BJP, 2011, 162, 1239; ^d *PharmacoEconomics*, 2021, 39,1243; ^e [Measuring the return from Pharmaceutical innovation 2022](#) ^e Nat. Rev. 2015, 14, 475



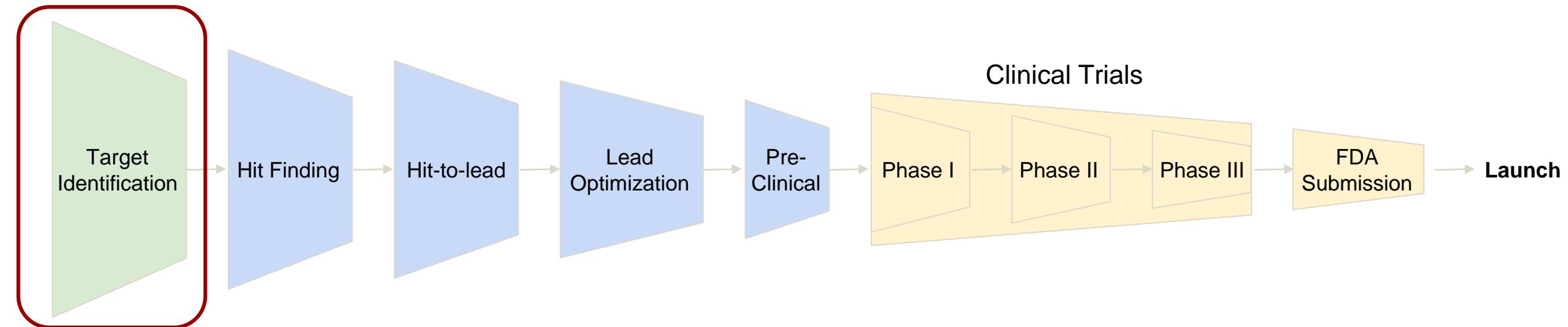
Target Identification

Poll 1: At What Stage Should You Know The Target?



- Target ID
- Hit Finding
- Hit-to-lead
- Lead Optimization
- Pre-Clinical
- Phase I
- Phase II
- Phase III
- The drug works, I don't care

Target Identification



Key Question: What do you want to drug and does it make sense?

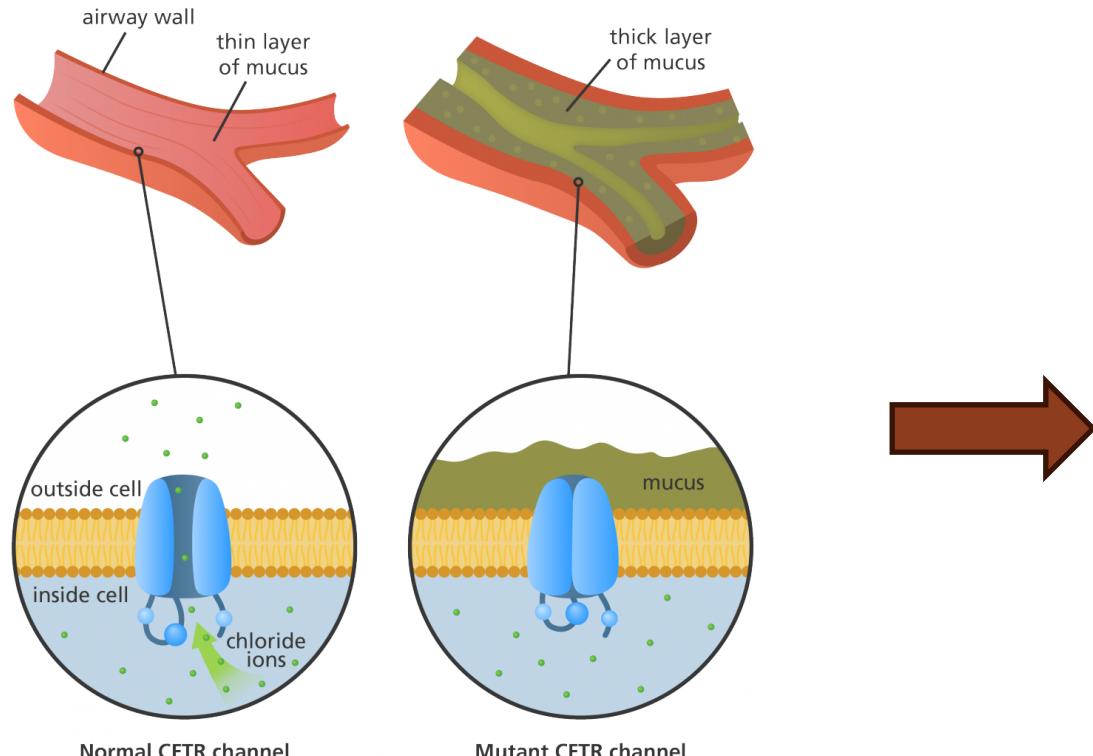
- Identifying the biological origin of a disease and candidate targets for therapeutic intervention, is the first step in drug discovery
- Essential for the long-term success of pharma/biotech industry
- Once identified, candidate targets must be validated by showing that they are directly involved in a disease process, and that their modulation in cells or preclinical models is likely to confer therapeutic benefits
- Target validation involves in-depth vetting and adherence to a variety of stringent criteria to minimize risks and gain confidence the target is worthy of further investigations/investments

Target Identification Criteria

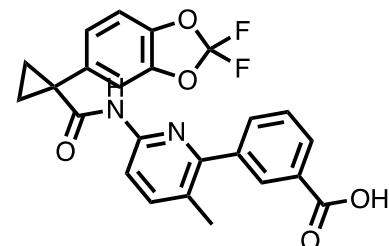
- Identify what (target or phenotype) you want your small molecule drug to act on
- Formulate the therapeutic hypothesis: activation, inhibition, or other target modulation will treat disease or improves outcomes
- Generate POC data for therapeutic hypothesis: demonstrate that your target does what you think it does in some model system
- Propose therapeutic modality: e.g. small molecule, large molecule or gene therapy
- Enable progression to Hit Generation: e.g. development of cellular assays for small-molecule screening

Drug Target Connection To Disease: Sometimes It's Clear

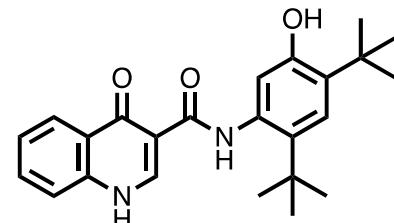
Cystic Fibrosis (CF) – Cystic fibrosis transmembrane conductance regulator (CFTR)



Lumacaftor



Ivacaftor



MOA: CFTR corrector

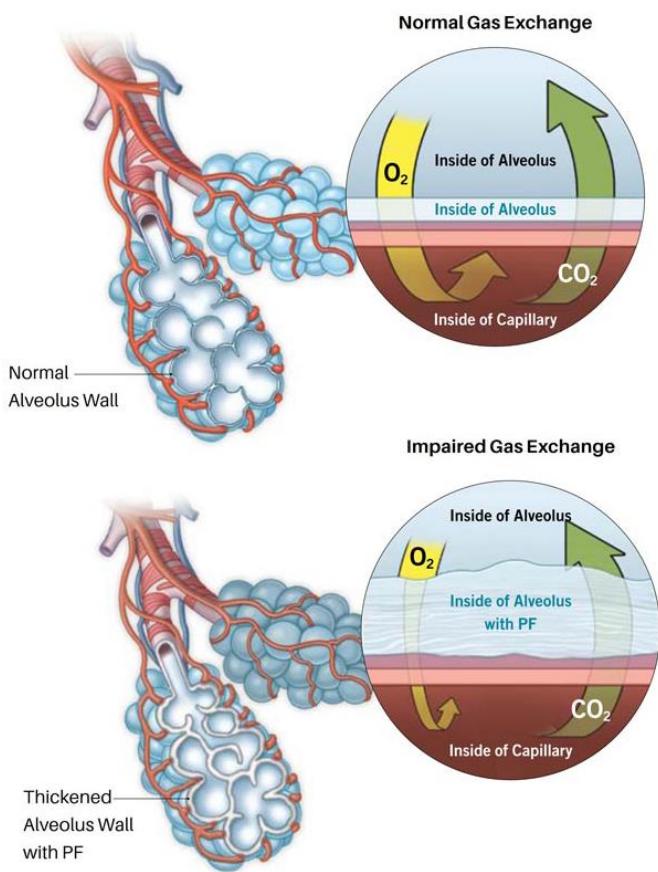
- CFTR mutations reduce the channel's ability to traffic to the cell surface
- Lumacaftor increases CFTR trafficking

MOA: CFTR potentiator

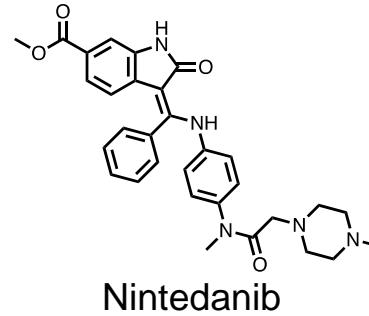
- CFTR mutations reduce the channel's ability to open
- Ivacaftor increases the open probability of CFTR

- Mutations and loss of function in CFTR leads to CF
- Single target – single disease

Drug Target Connection To Disease: Sometimes It's Unclear



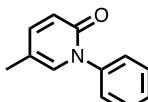
Idiopathic Pulmonary Fibrosis (IPF)



Nintedanib

MOA: inhibitor of receptor and non-receptor tyrosine kinases

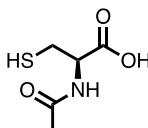
- Interferes with fibrotic processes: fibroblast proliferation, differentiation and laying down extracellular matrix
- Improves quality of life with no survival benefit



Pirfenidone

MOA: anti-fibrotic agent with anti-inflammatory properties

- No single target known
- Improves patient survival



N-acetyl cysteine

MOA: anti-oxidant and glutathione precursor

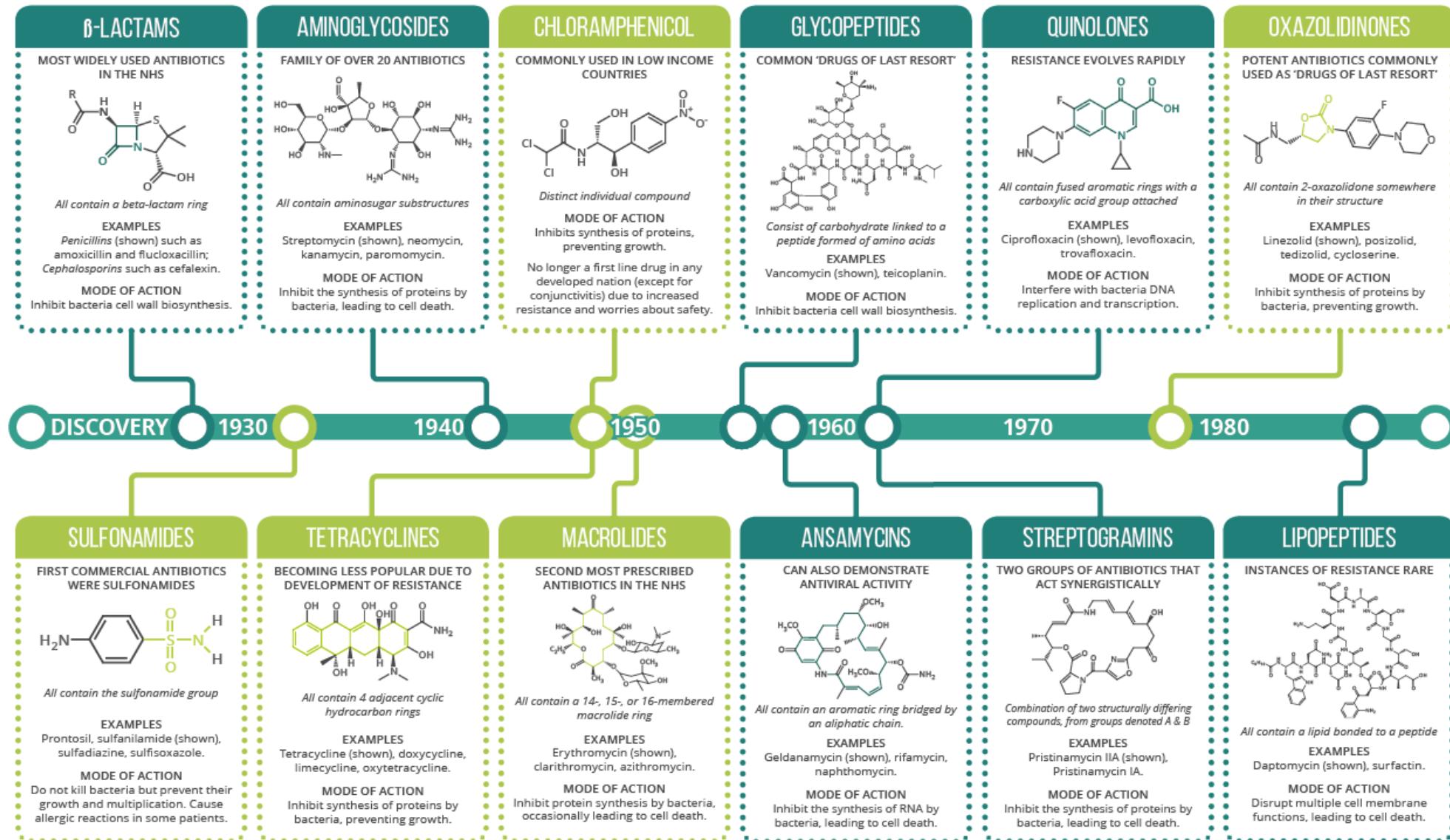
- No single target known
- Improves patient survival

- Cause in unknowns – risk increases with environmental factors
- No direct drug target linked to pathology

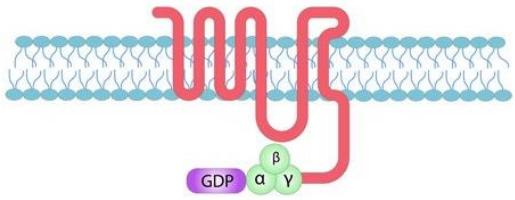
What Is A Small Molecule Drug?

An organic compound with a molecular weight below 1500 Da that can interact with a biological target to elicit an effect

Key: ● COMMONLY ACT AS BACTERIOSTATIC AGENTS, RESTRICTING GROWTH & REPRODUCTION ● COMMONLY ACT AS BACTERICIDAL AGENTS, CAUSING BACTERIAL CELL DEATH

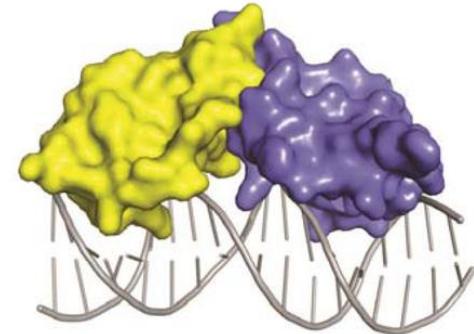
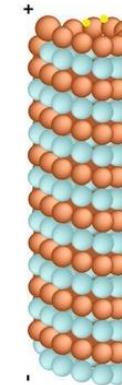
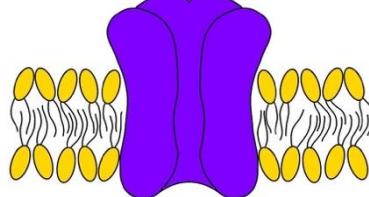
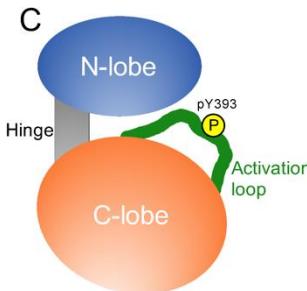
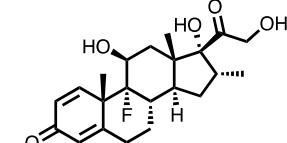
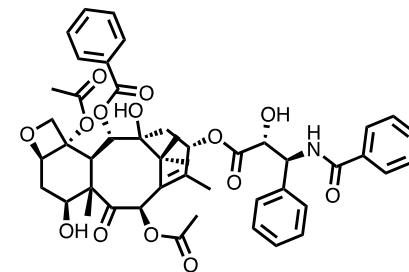
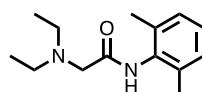
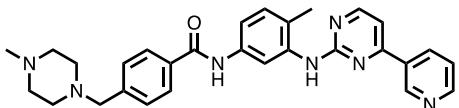
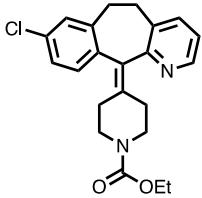


What Are Common Small Molecule Drug Target Classes?



Receptors:

- GPCRs ~ 50% of all drug targets
 - Loratadine (Claritin) is an inverse agonist of the H1 Histamine receptor for allergies

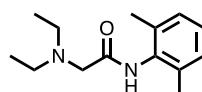


Enzymes.

- Kinases, proteases, esterases
 - Imanitib (Gleevec) is Bcr-Alb kinase inhibitor for oncology



- Lidocaine is a sodium-channel blocker used as a local anesthetic



Structural proteins:

- Paclitaxel (Taxol) is a tubulin binding agent that is used as a chemotherapeutic agent for oncology

Nuclear Receptors:

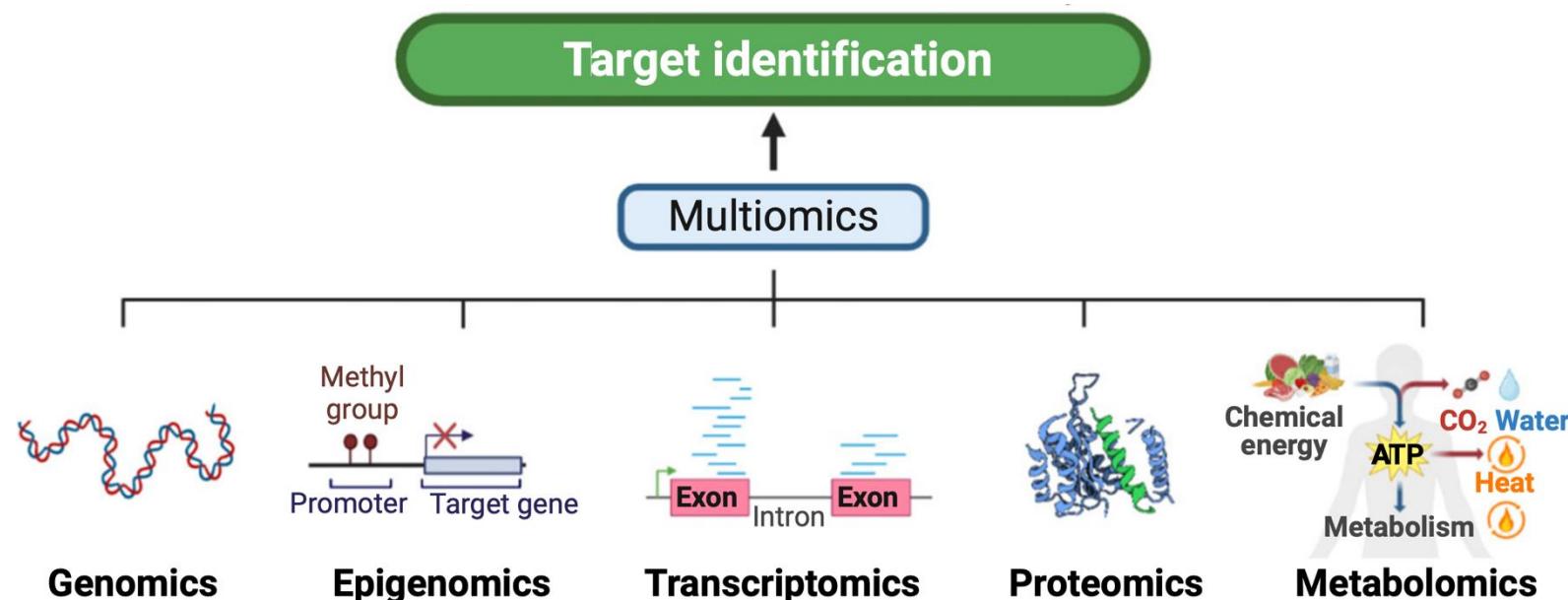
- Dexamethasone is an anti-inflammatory agent used for a wide range of disorders

Omics Technologies Have Accelerated Target Identification

Omics: characterization and quantification of specific biomolecules to understand how they translate to structure, function, and disease state in an organism

Generic Example:

- Take healthy and diseased cells.
- Characterize and quantify entirety of proteins in both
- Look for differences between healthy and diseased cells
- Utilize computational methods to process and analyze the data to identify targets of interest implicated in disease
- Similar workflows can be used for disease model animals looking at multiple omic biomolecule classes



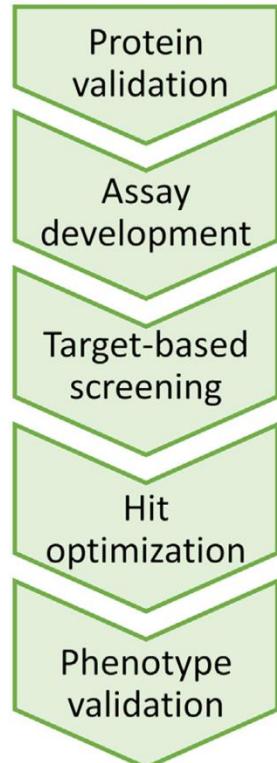
^a Trends in Pharm. Sci., 2023, 44, 561

Target Selection Has Many Caveats That Determine Success

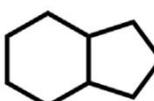
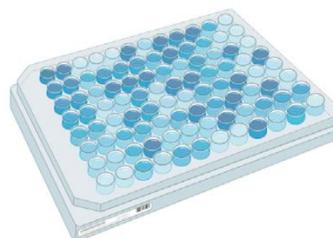
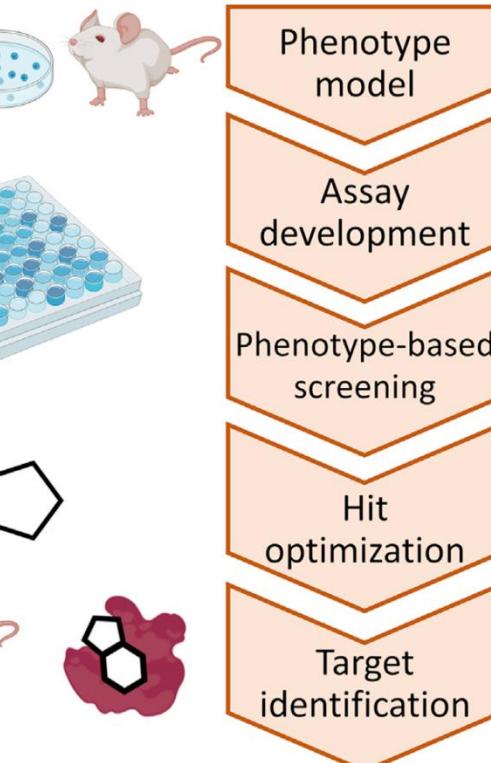
- **On-target safety:** Is it safe to modulate the target of interest?
- **Druggability:** Small molecules are better modulating some targets than others
- **Efficacy:** Does modulating a given target lead to a measurable therapeutic effect
- **Therapeutic MOA:** Agonism | Antagonist | Binding | Others
- **Unmet medical need:** How much of a need is there? How many patients are there?
- **Commercial need:** First in class. Best in class. Do we need another ABC inhibitor on the market

Phenotypic Screening Looks For A Specific Effect: Target Agnostic

Target-based approach



Phenotype-based approach



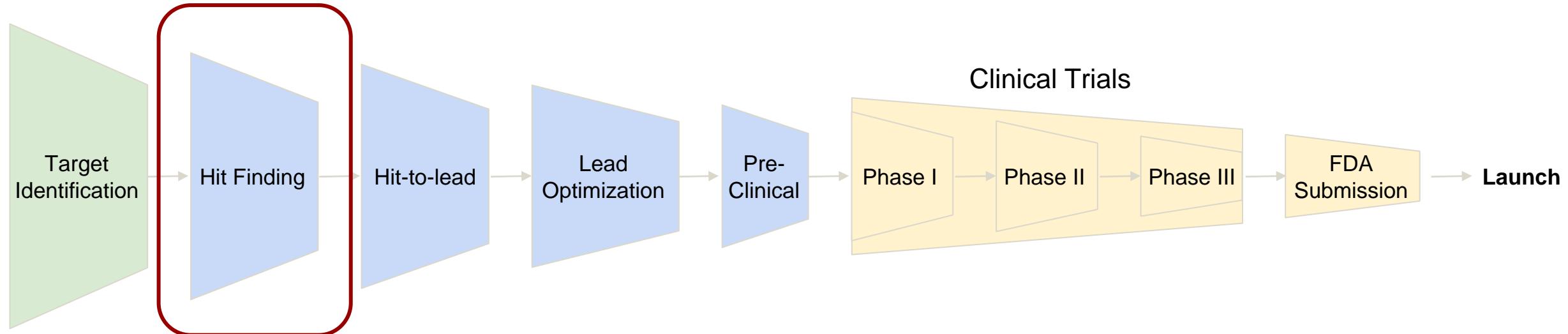
- Phenotypic approaches were the way medicines were discovered historically
 - Herbal medicines
- Advances in molecular biology led to a shift to target-based approaches
- Recent resurgence in phenotypic approaches but now with target deconvolution

^a *Cell Chem. Biol.*, 2021, 28, 394 ^b *ACS Med. Chem. Lett.*, 2020, 11, 1820. ^c *Med. Chem. Commun.*, 2016, 7, 788. ^d *Nat. Rev. Drug Disc.* 2022, 21, 899

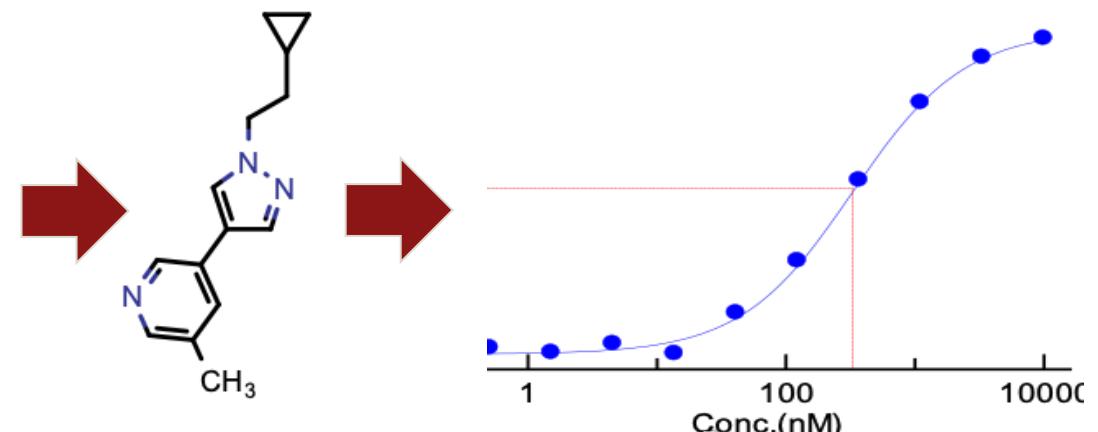
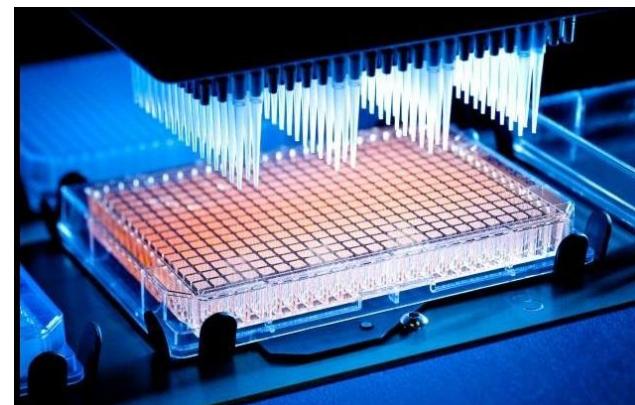


Small Molecule Hit Finding

Hit Finding: How Do You Find A Small Molecule Starting Point?



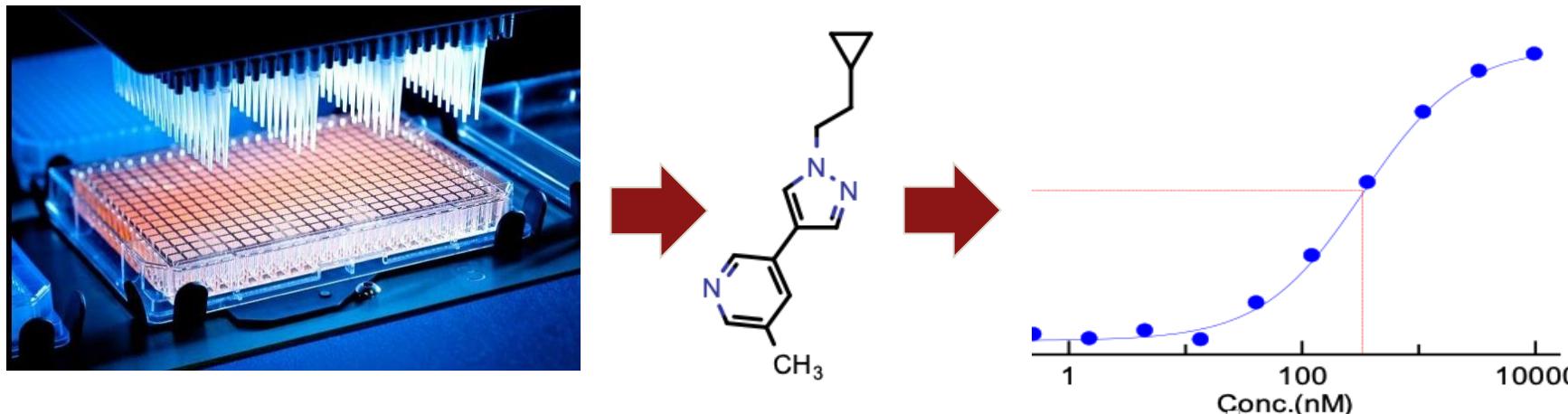
Key Question: What chemical structure do I start with and how do I find it?



^a BJP, 2011, 162, 1239;

Poll 2: Which Hit-Finding Approaches Have You Used?

- High-throughput screening
- Fragment-based screening
- DNA-encoded library screening
- Information-driven approach
- Virtual screening
- None of the above



Small Molecule Hit Finding Approaches

High Throughput Screening (HTS)

- >100,000 compounds screened in a single concentration
- Utilizes biochemical or cellular assay

DNA-Encoded Library Screening (DEL)

- $>10^9$ compounds screened as mixtures across multiple conditions
- Protein binds hits which are decoded by DNA sequencing and confirmed by hit re-synthesis

Fragment-based screening (FBS)

- <1000 compounds screened by a biophysical method
- Lead compounds built from hits in a modular way and supported by structural information

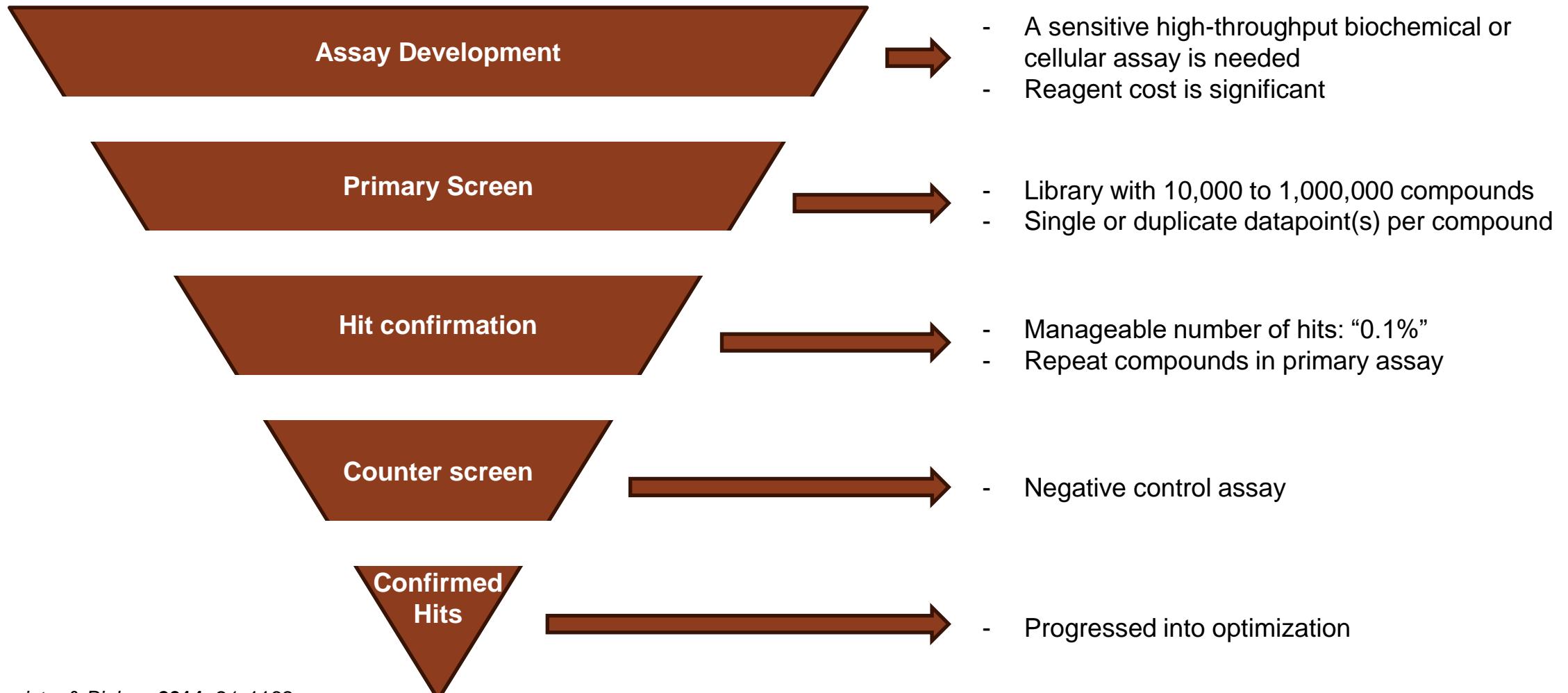
Virtual Screen (VS)

- Starts from X-ray structure or homology model
- Computationally dock small molecules to the binding site

Information-driven approach (Follow on)

- Uses literature, patents, or known drug as starting point
- IP space can be challenging
- Typically, a best-in-class approach

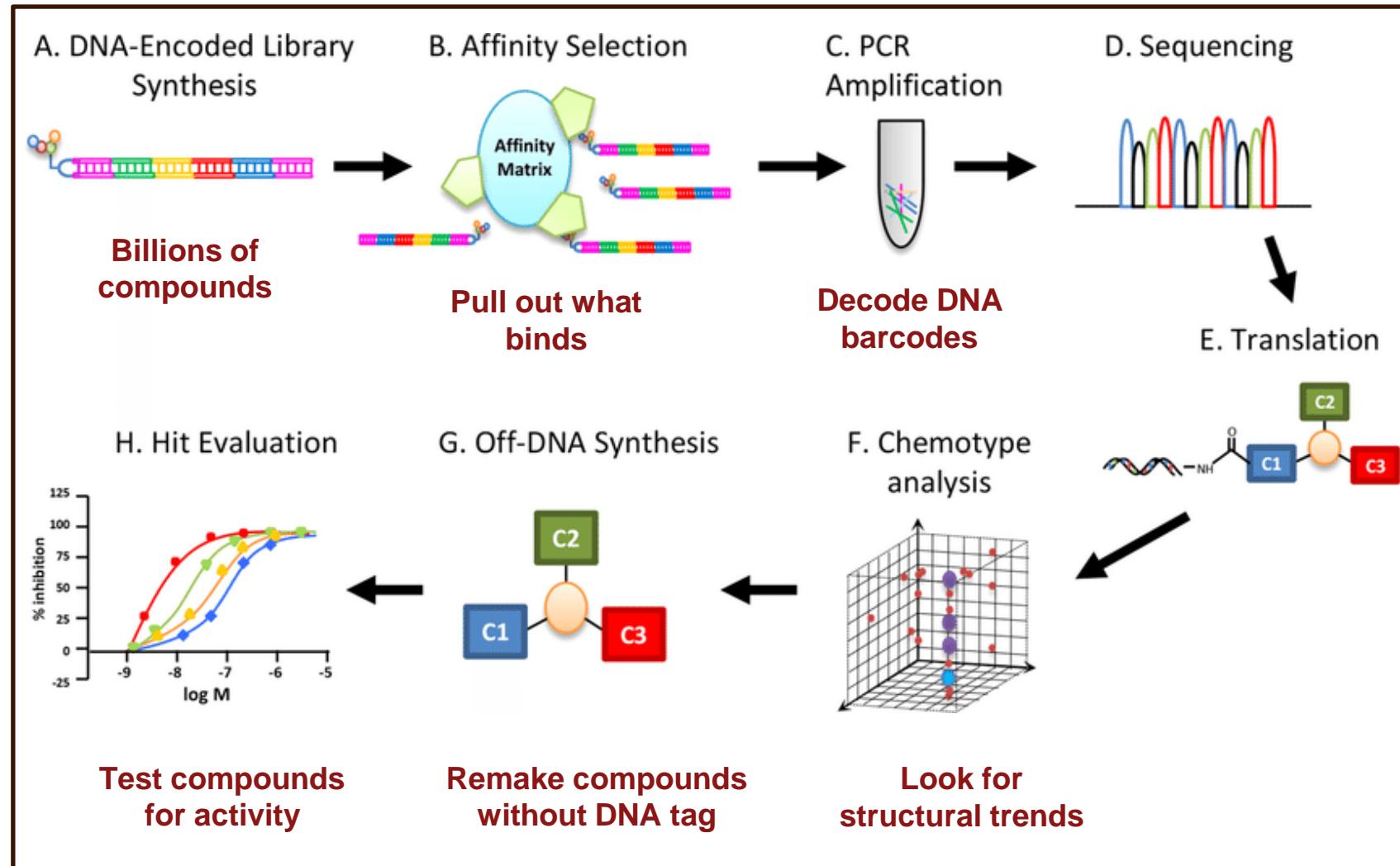
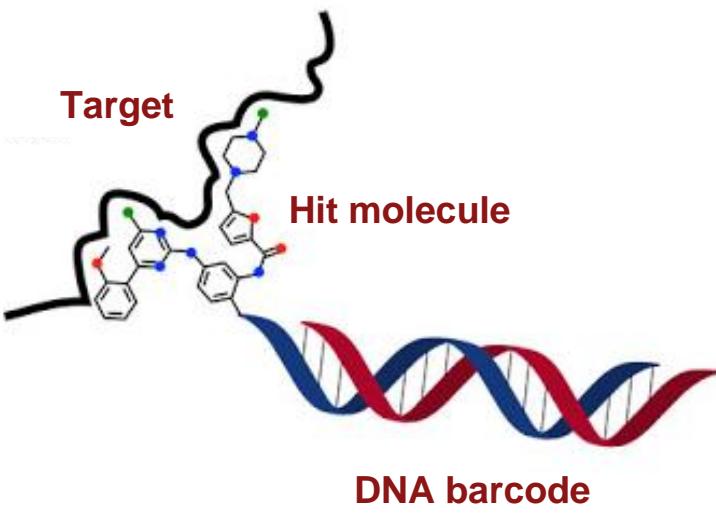
High Throughput Screening: Finding Novel Starting Points



^a *Chemistry & Biology* 2014, 21, 1162

DNA-Encoded Library Screening

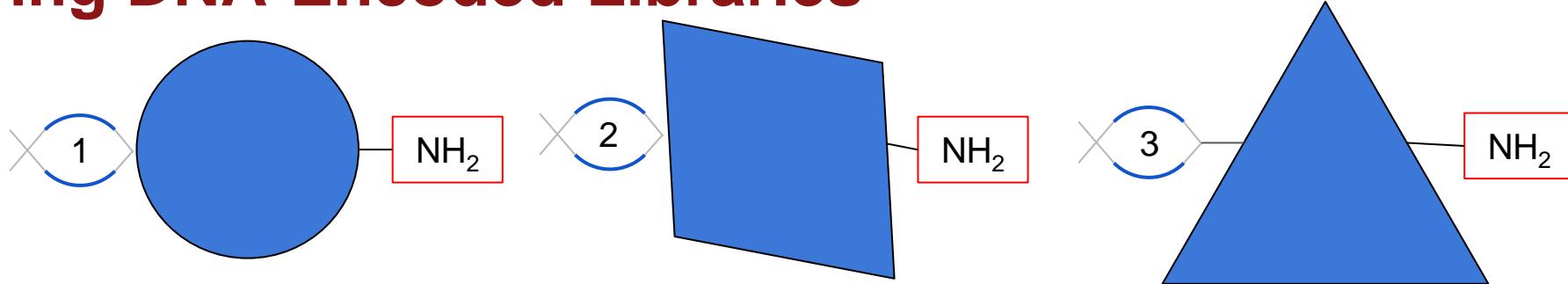
- Generate a library of compounds made from building blocks with DNA barcodes
- DNA can be used to decode structure
- Libraries can contain billions of compounds



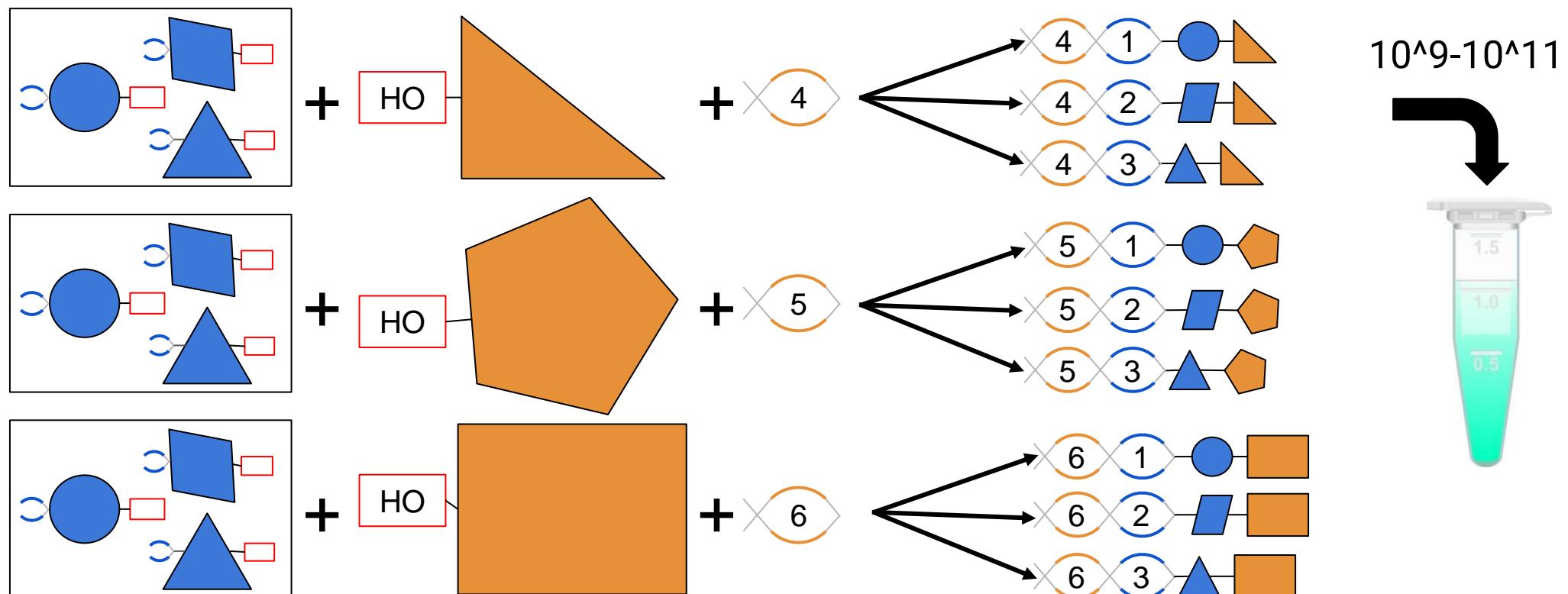
^a Nat. Rev. Drug Disc., 2023 22, 699

Preparing DNA-Encoded Libraries

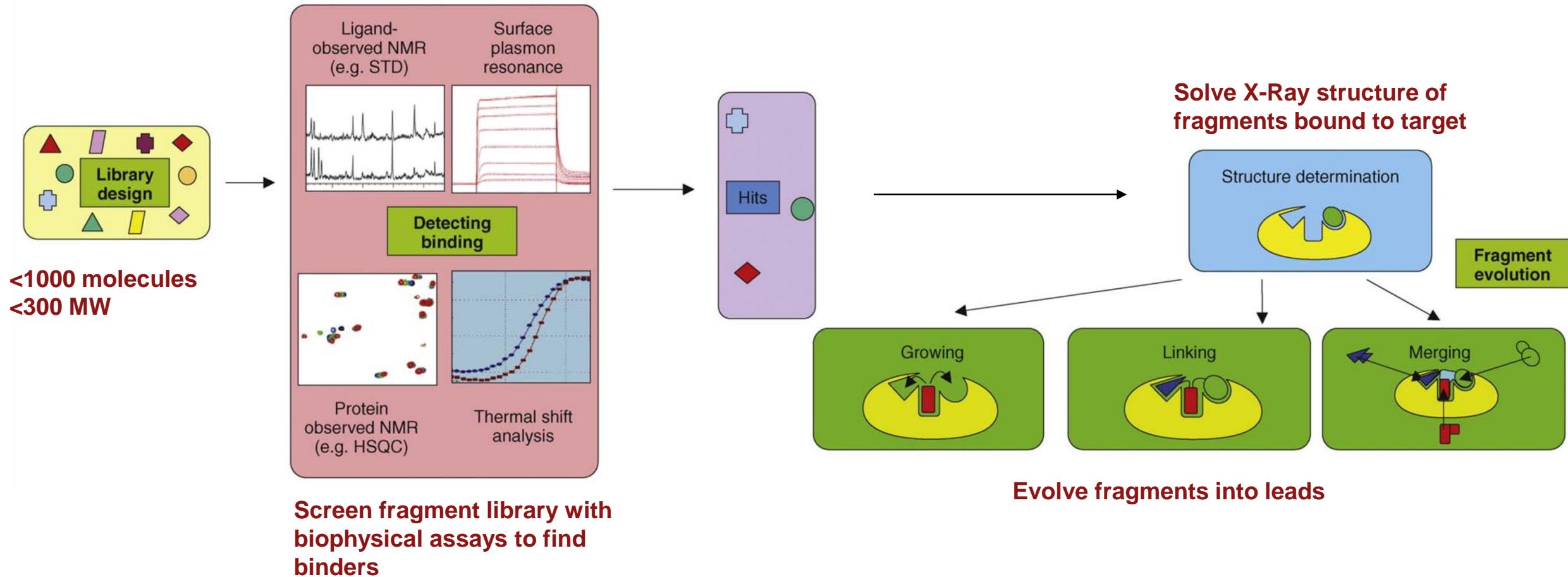
Step 1



Step 2



Fragment-Based Screening



^a *Front. in Molec. Med.* **2020**, 7, 180; ^b *Curr. Opin. Pharmacol.* **2009**, 9, 615

Hit Generation Using a Follow-On Strategy



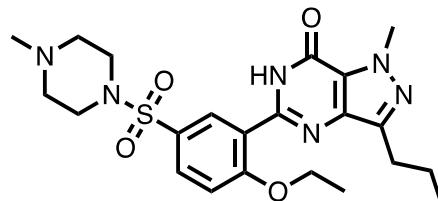
- A great way to find a drug is by starting with another drug
- Natural products can serve as viable starting points

^a Drug Disc. Today, 2009, 14, 516. ^b CRIPS, 2022 94.

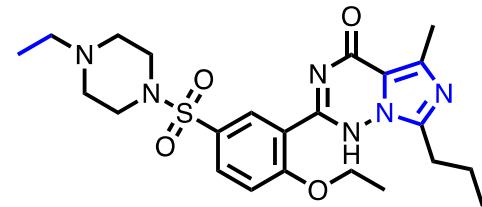
Hit Generation Using a Follow-On Strategy: PDE5 Inhibitors



- Originally designed for cardiovascular disorders → failed
- Approved in 1998 for erectile disorder → drug repositioning
- Huge market → blockbuster drug



Sildenafil



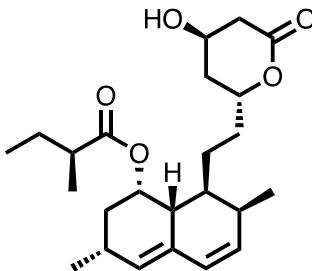
Vardenafil



- Bayer saw opportunity to tap a huge market
- Used Sildenafil as a starting point and designed compounds outside their IP space
- Identified Vardenafil → one carbon longer and one N-atom moved
- Reduced PDE6 activity → eliminates vision side effects

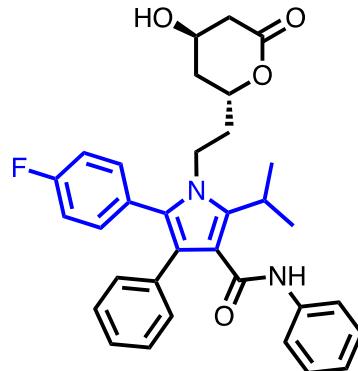
^a Drug Disc. Today, 2009, 14, 516. ^b CRIPS, 2022 94.

Hit Generation Using a Follow-On Strategy: HMGR



Lovastatin

- Fungal metabolite
- Statin medication approved in 1987 for reducing risk of cardiovascular disease
- Complicated chemistry needed to manufacture API



Atorvastatin



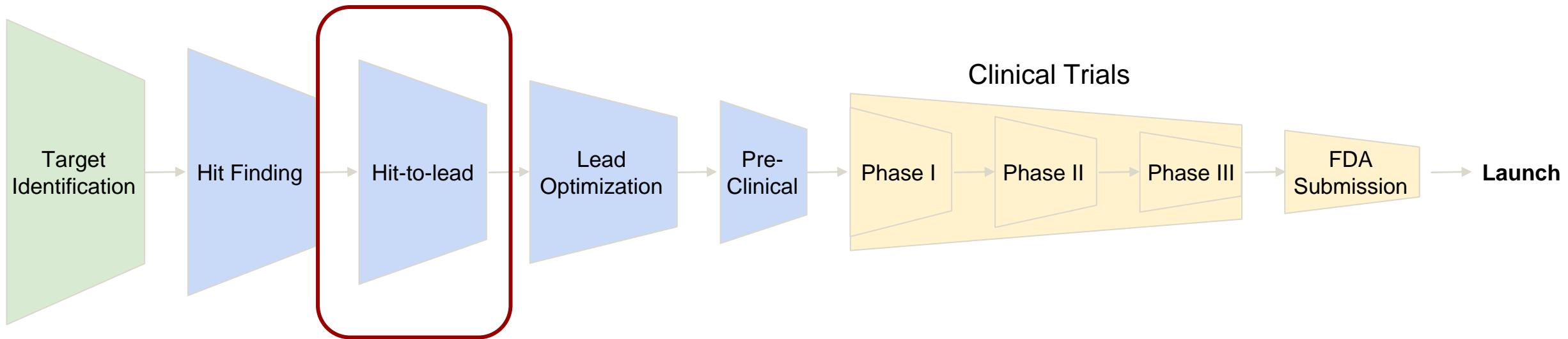
- Identified during a surge in Statin research
- Research on Lovastatin informed that **pyrrole core** could mimic the complicated core of Lovastatin
- Demonstrated superior efficacy to Lovastatin
- Significantly easier to manufacture

^a Drug Disc. Today, 2009, 14, 516. ^b CRIPS, 2022 94.



Hit-to-Lead

Hit-To-Lead: Build Confidence In Target and Chemical Matter



Key Question: how do I improve the potency and drug-like properties of my hit to gain confidence in a path forward towards a lead molecule

Objectives:

- Identify **lead molecules** (or series of molecules) with improved potency, metabolic stability, and other drug-like properties.
- Dose lead molecules in animal disease models to demonstrate in vitro or ideally, in vivo efficacy.

Strategy:

- Optimize multiple hits for target modulation, evaluate their potential on progression to lead series and quickly identify potential liabilities
- Identify 2-3 distinct lead chemical series and profile representative compounds to satisfy Drug Candidate Profile Criteria

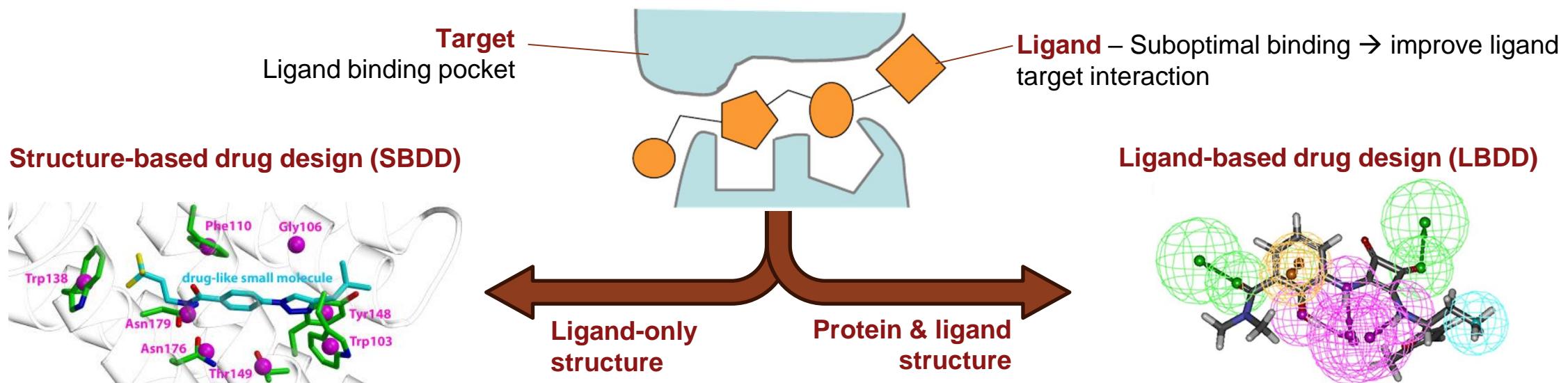
Hit to Lead, What is a Lead?

A **lead molecule** is a compound (or series) that meets specific criteria and possesses sufficient potential to be modified into a development candidate.

Specific criteria will vary between programs and organizations

Property	Criteria
Pharmacological potency	< x nM for 2+ chemical series, tractable SAR
Selectivity	> x-Fold selectivity over other targets of concern
Chemical diversity	2+ chemical series, no standard definition of a series
Target validation	Small molecules modulate pathway in cellular assays, and ideally in animal models
<i>In vitro</i> metabolic stability	Moderate stability or better
Rodent <i>in vivo</i> PK	Moderate stability or better, oral bioavailability >20%
Safety assessment	Safety panel (CEREP), drug-drug interactions (CYP450 inhibition, PXR activation), Kinase panel
<i>in vivo</i> efficacy model	Available or line-of-sight
Intellectual property	FTO search on chemical matter and draft patents ready to file

Initial Goal Of Hit-To-Lead Is To Increase Compound Potency



Basic approach: Use structure of protein or protein + hit to optimize activity systematically

Examples:

- Homology modeling
- Molecular docking
- Molecular dynamics simulations
- Virtual screening
- Receptor-based pharmacophore modeling

Basic approach: Use ligands' structure, 3D conformation to optimize activity systematically

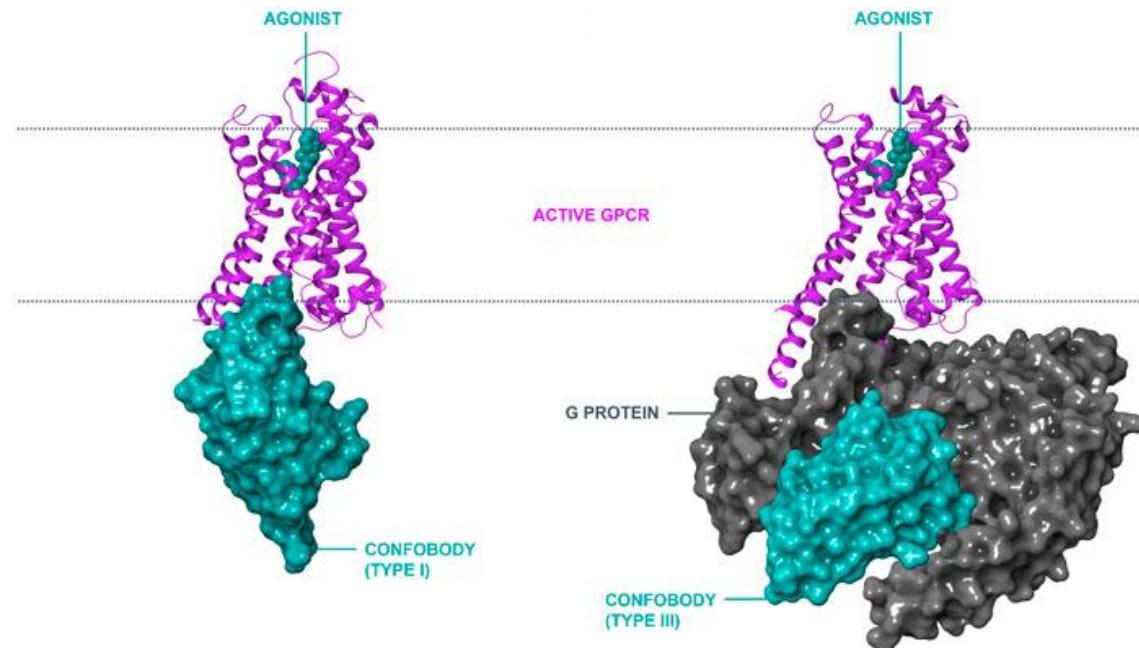
Examples:

- Molecular modeling
- Pharmacophore modeling
- QSAR
- Scaffold hopping

^a *Chemoinformatics and Bioinformatics in the Pharmaceutical Sciences* 2021, Chapter 2, pages 27-53

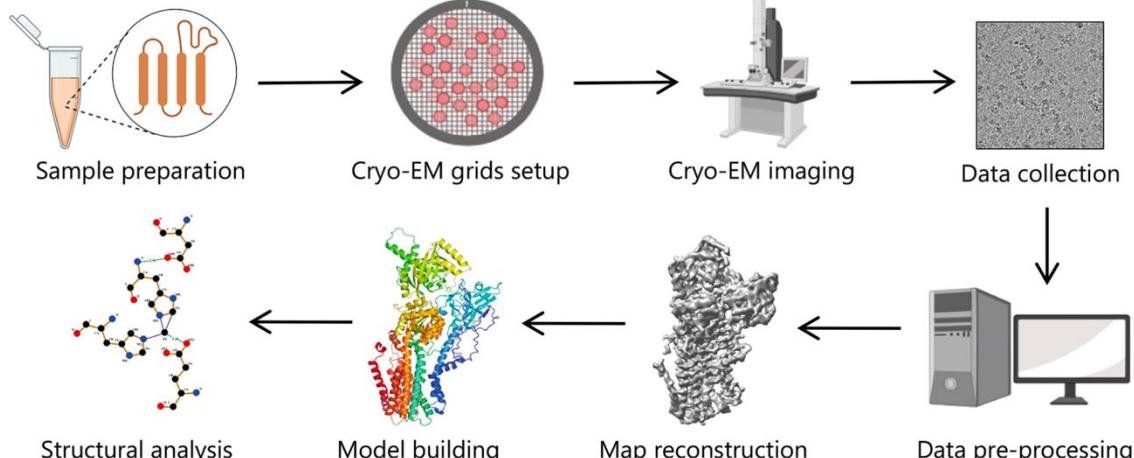
Poll 3: How Do You Use Structure In Your Work

- I look at structures of biomolecules
- I look at structures of small molecules
- I look at structures of biomolecules and small molecules
- I don't use structural biology in my work



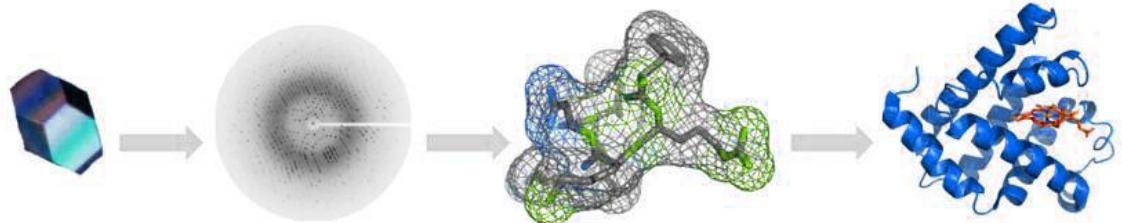
Structural Biology Techniques To Improve Potency

Cryo-Electron Microscopy Cryo-EM



- Newer method: Use has increased dramatically over last decade
- Uses electron beams to collect scatter patterns of frozen amorphous protein samples
- **Pros:** does not require protein crystallization | Can capture native states of protein.
- **Cons:** Lower resolution (for now) | applicable to higher MW samples

X-Ray Crystallography



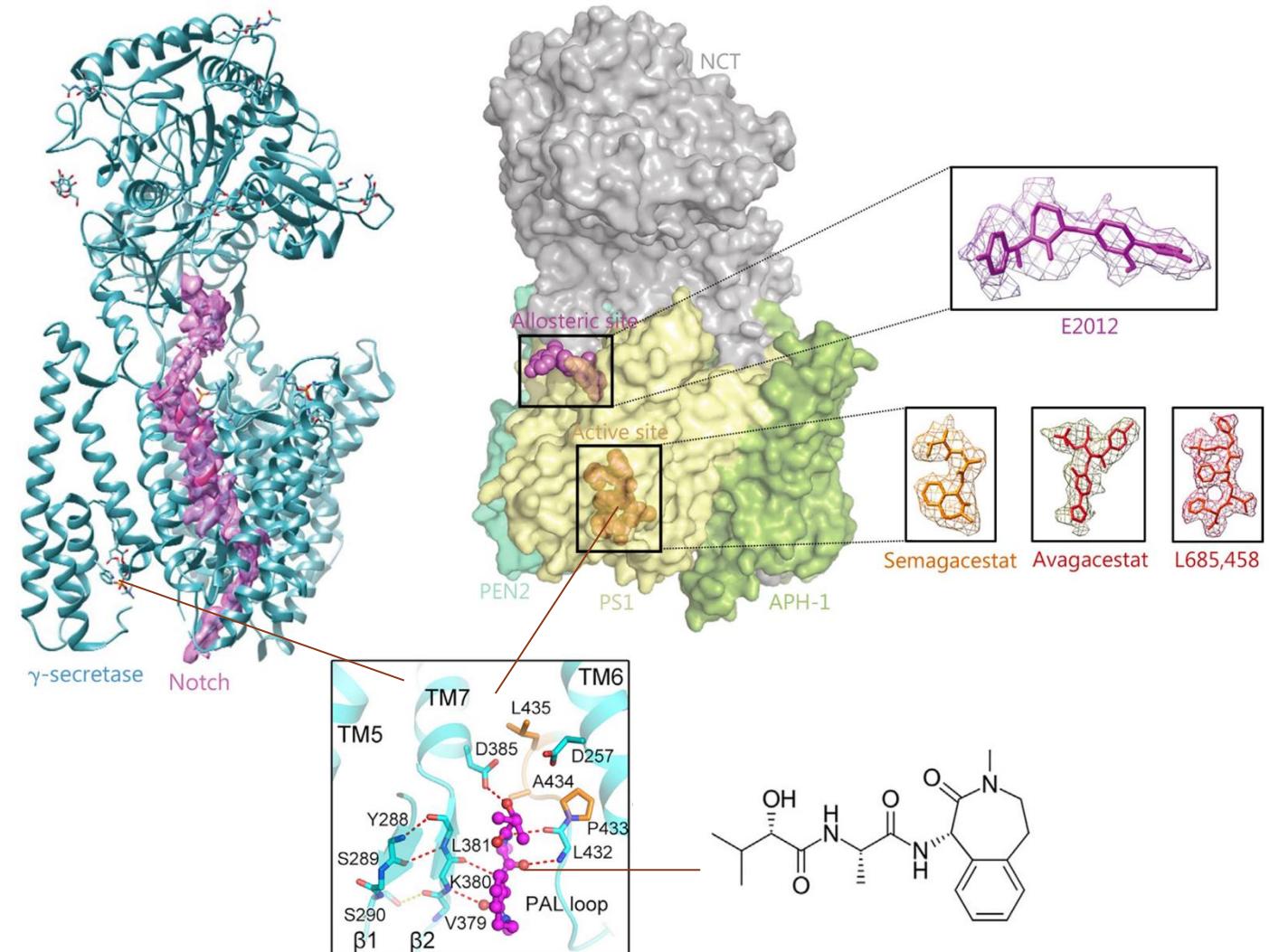
- Oldest method: Myoglobin structure solved in 1958
- Uses X-ray on crystallized protein samples to determine the position of atoms in a crystal
- Provides an electron diffraction map that can be processed to solve structure
- **Pros:** higher resolution | more versatile and established method: can solve structures across MWs and protein environments
- **Cons:** requires crystallization of target. Shows static solid-phase state of the structure

^a *Protein Sci.*, 2017, 26, 32. ^b *Nature* 2020, 578, 201 ^c *Acta Cryst.*, 2017, 174 d *Ann. Rev. of Pharmacol. and Tox.* 2020, 60, 51

Structural Biology Enables Structure-Based Drug Design

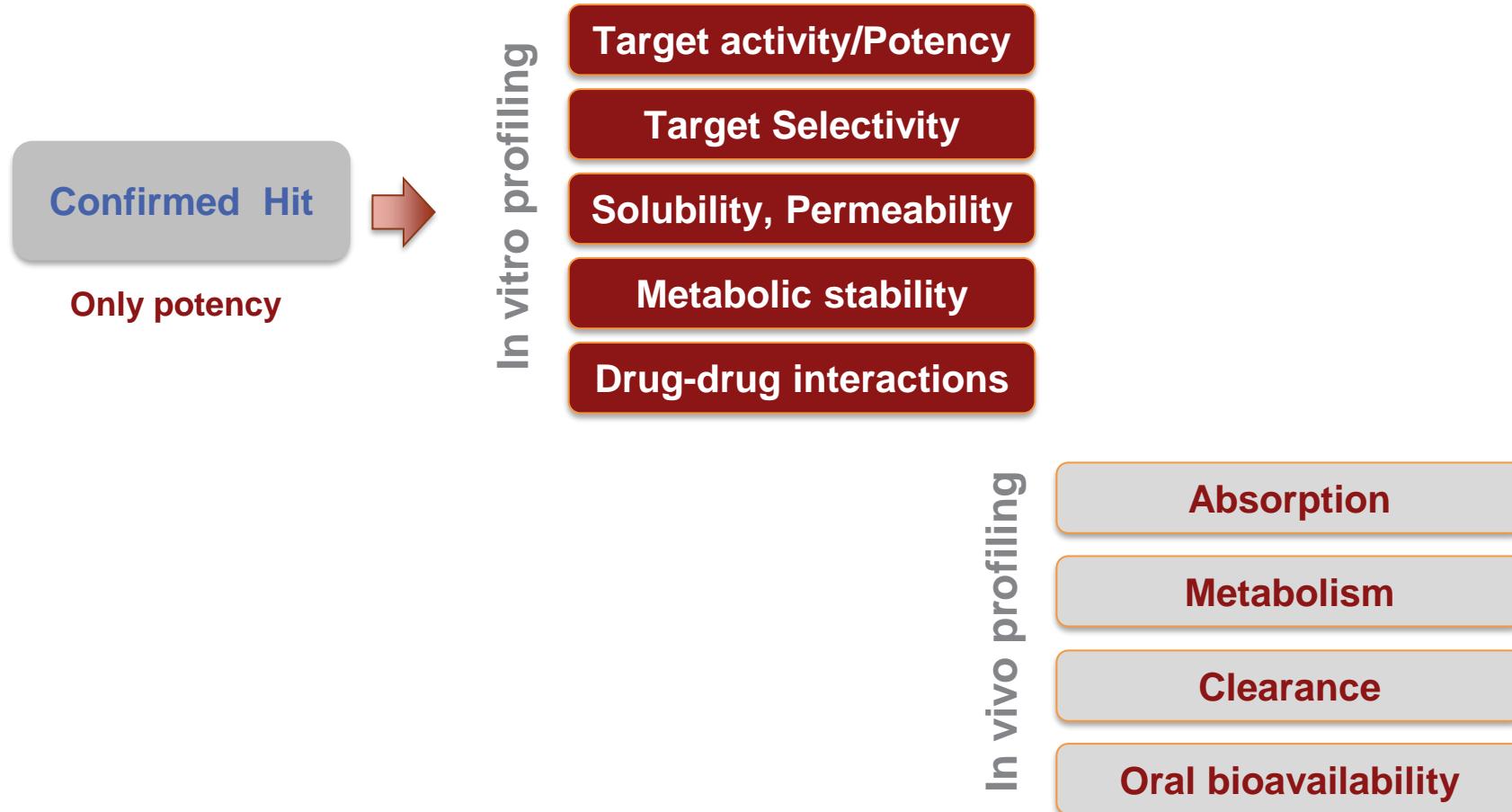
You have a structure of your protein. What do you do with it?

- Solved structures provide a 3D picture of the protein +/- ligand
- Used to enable structure-based drug design strategies to improve compound activity by optimizing interactions
 - Binding site identification
 - Key protein-ligand interactions
 - Conformation of ligand in bound state
 - Structural analog docking
 - Homology modeling for related targets
 - Molecular dynamics
 - Virtual screening



^a Int J Mol Sci. 2019 20, 2783. ^b Cell 2021, 184, 521

Multiple Parameters Typically Optimized To Identify A Lead



Molecular Properties Influence Drug-Like Properties

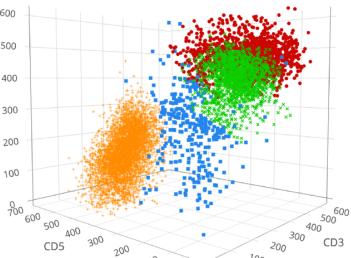
Many molecular properties are of interest in small molecule drug discovery

Molecular Properties	Drug-Like Properties
Hydrogen bond donors/acceptors	Metabolic stability
Polar surface area	Absorption
Lipophilicity	Oral bioavailability
Molecular weight	Clearance
Solubility	Volume of distribution
Partition coefficient	Half-life
Dissociation constant	

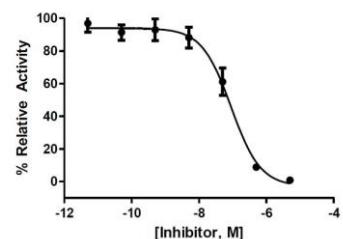
Lipinski's Rule of Five

- Set of rules that most successful oral drugs possess
- **More of a suggestion – many approved oral drugs do not meet many of these rules**
- Seminal paper published in 1997 by Lipinski described commonly found features of orally-active approved drugs
- Features
 - ≤ 10 hydrogen bond acceptors
 - ≤ 5 hydrogen bond donors
 - Molecular weight < 500
 - $\text{LogP} < 5$
 - LogP is a measurement of the hydrophobicity / hydrophilicity of a given compound
- Widely extended to include many other properties to help identify “drug-like” molecules
- **Most drug companies have their own modifications to these, based on experience**

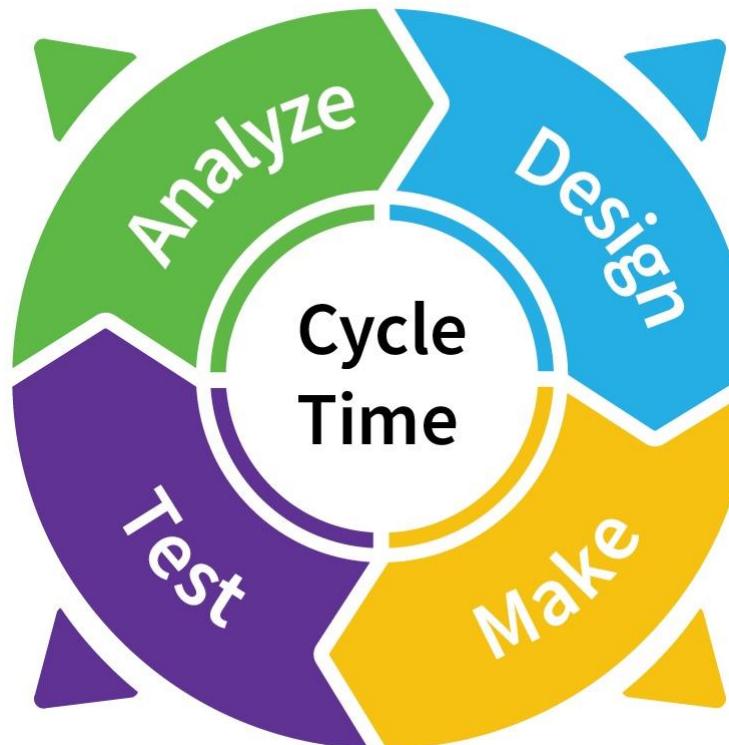
Hit-To-Lead Optimization is An Iterative Process



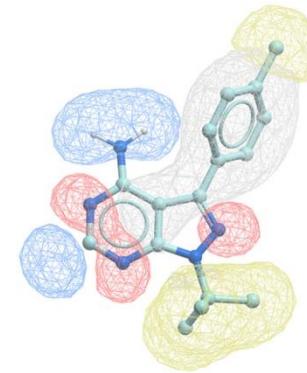
Interplay of data from multiple assays to inform on next cycle



Test compounds in sequential assays to meet lead criteria



Tools and strategies to optimize chemical matter



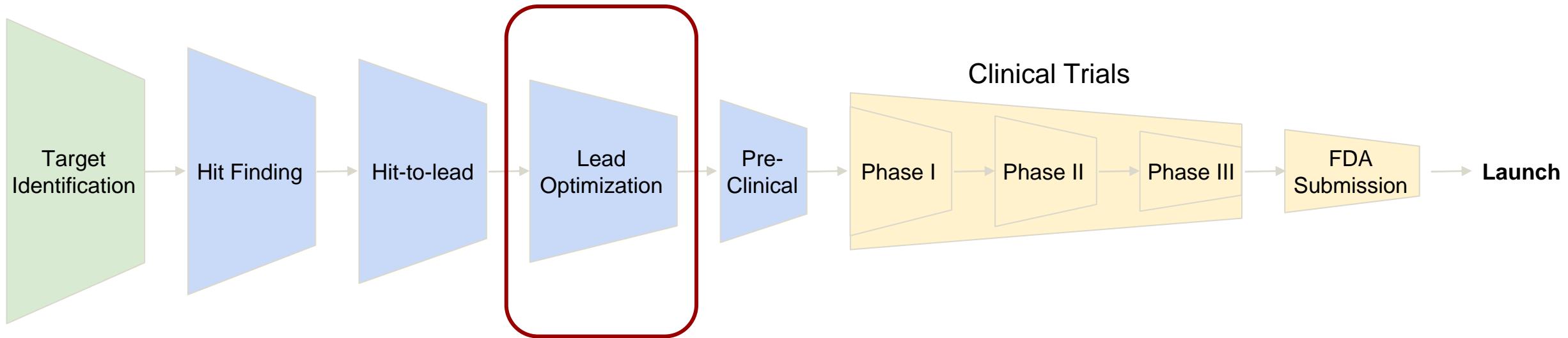
Synthesize compounds in the lab using organic chemistry





Lead Optimization

Lead Optimization: Identify Candidate Compounds



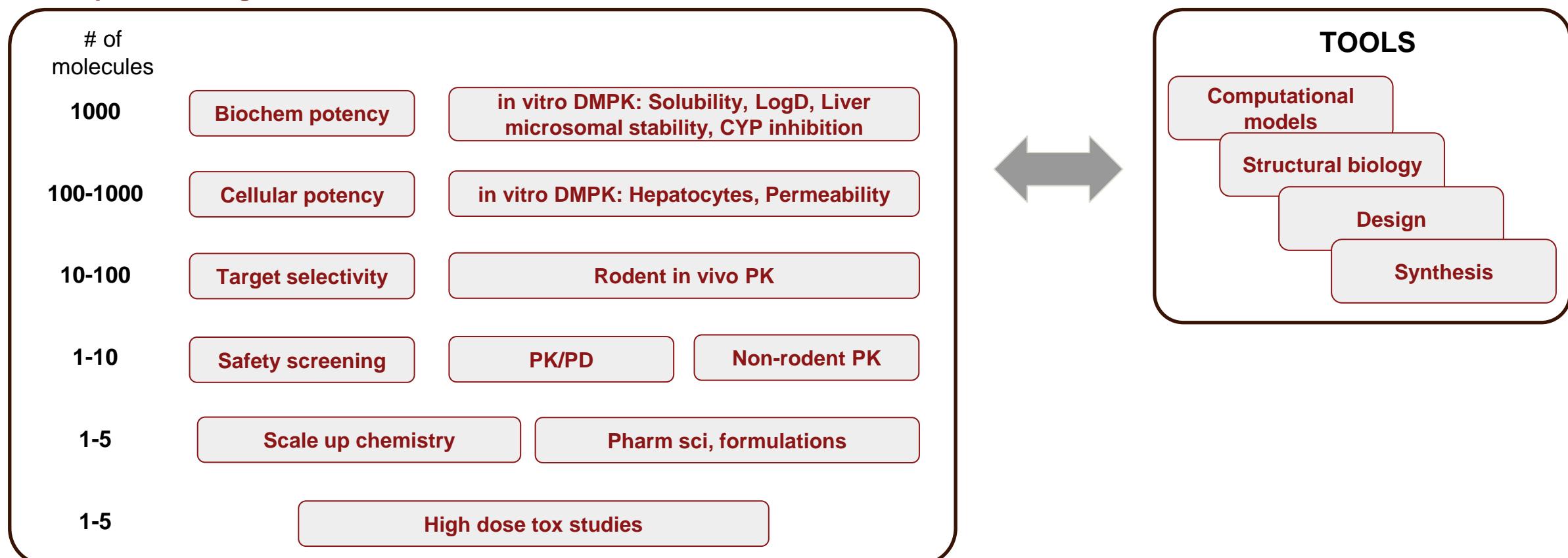
Key Question: How do I optimize my chemical matter to deliver a candidate molecule to progress into the clinic?

- Stage at which your lead series of compounds is optimized to deliver **candidate molecules** for toxicology studies and pre-clinical development
- Candidate molecule(s) needs to posses a combination of potency, selectivity, DMPK properties and safety
- A lot of overlap in activities between Hit-to-Lead and Lead Optimization

Lead Optimization Screening Cascade For Multi-Parameter Optimization

Lead Optimization requires optimization of multiple parameters in parallel. A screening cascade is used to gate compounds from low complexity assays to high complexity assays

Example screening cascade



Lead Optimization Screening Cascade For Multi-Parameter Optimization

Lead Optimization requires optimization of multiple parameters in parallel. A screening cascade is used to gate compounds from low complexity assays to high complexity assays

Multiparameter data is usually visualized and sorted in software like Vortex or Spotfire to identify compounds of interest and inform on next design cycle



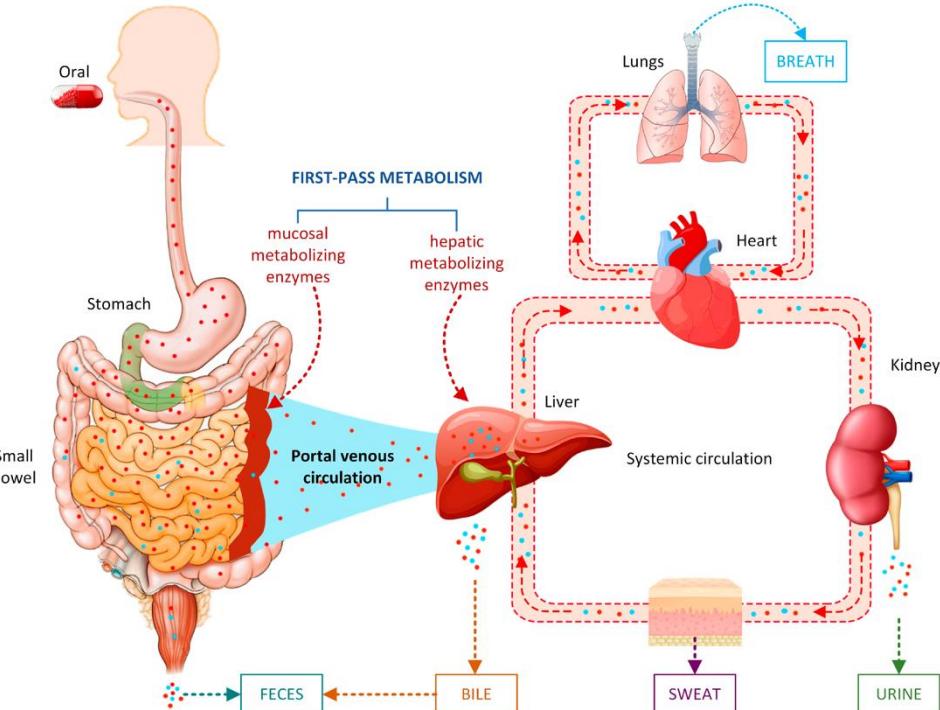
Drug Metabolism & Pharmacokinetics (DMPK) Optimization

Drug metabolism: conversion of a drug molecule into other compounds by metabolizing enzymes. Typically occurs by oxidation in the liver

Pharmacokinetics: measure of drug concentration throughout the body over time. Informs on how the body affects the drug after administration.

A drug must pass many hurdles before reaching its target of interest

- Withstand the pH ranges of the gut
- Cross multiple membranes
- Withstand metabolism in the liver
- Avoid active transport into the bile
- Avoid filtration and excretion in the kidneys
- Many others

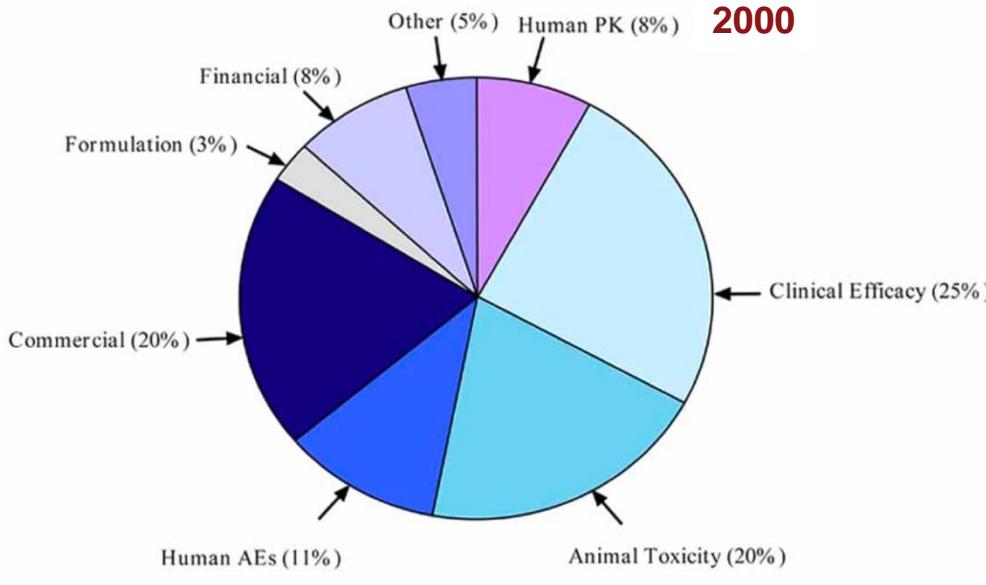
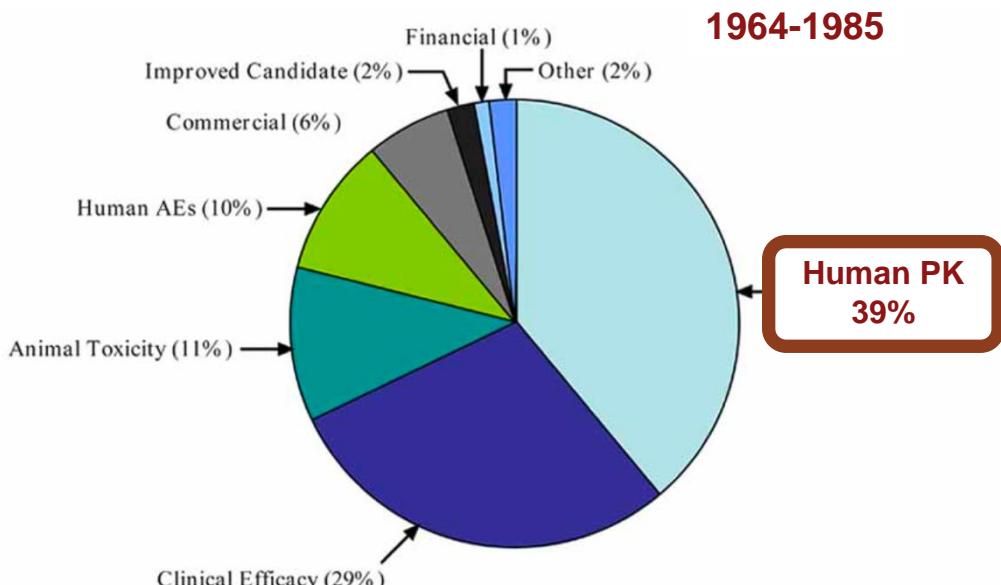


How do you predict DMPK properties in human at the discovery stage?

^a Acta Pharmaceutica Sinica B, 2019, 9, 1113e1144 ^b Acta Pharmaceutica Sinica B, 2022, 12, 2751e2777

Drug Metabolism & Pharmacokinetics (DMPK) Optimization: In Vitro

- In vitro assays are used to predict in vivo outcomes → assays and technologies to predict in vivo outcomes have improved over time
- Many drug failures occurred historically due to DMPK properties



^a *Nat. Rev. Drug Disc.* 2015, 14, 475. ^b *Comb. Chem. & HTS*, 2010, 13, 188.

Drug Metabolism & Pharmacokinetics (DMPK) Optimization: In Vitro

- In vitro assays are used to predict in vivo outcomes → assays and technologies to predict in vivo outcomes have improved over time
- Many drug failures occurred historically due to DMPK properties

Example in vitro assays used and what they predict

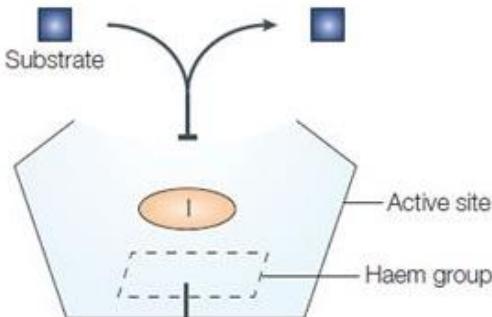
Absorption	Metabolism	Distribution
<ul style="list-style-type: none">• Physicochemical properties• Solubility assays• Artificial membrane permeability• Cellular monolayer permeability	<ul style="list-style-type: none">• Recombinant enzyme assays• Simulated biofluid stability• Microsomal stability• Liver fraction assays• Hepatocyte stability• CYP inhibition• CYP induction	<ul style="list-style-type: none">• Plasma and tissue protein binding

^a *Nat. Rev. Drug Disc.* **2015**, *14*, 475. ^b *Comb. Chem. & HTS*, **2010**, *13*, 188.

Lead Optimization: Drug-Drug Interaction Screening

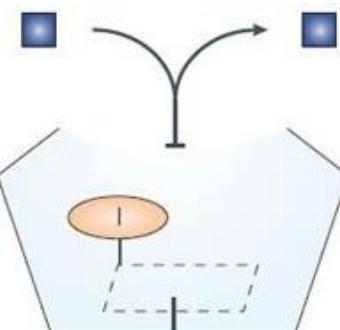
Drug-drug interactions (DDIs) occur when your compound activates or inhibits CYP enzymes to change the metabolism of other drugs in the body

Reversible CYP Inhibition



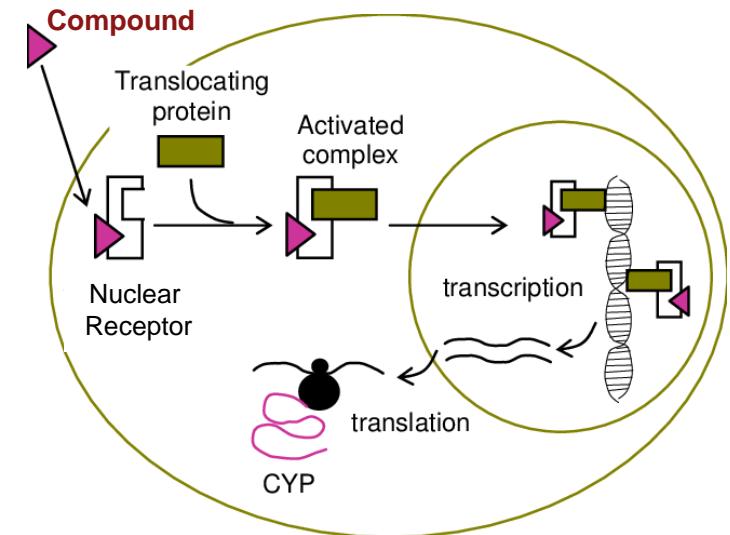
inhibitor blocks access of the drug to the active sites of the enzyme

Time-dependent CYP Inhibition



inhibitor is activated by the enzyme to form a reactive intermediate that covalently binds to the prosthetic haem group.

CYP Induction



Overall outcome: reduction in CYP activity and increased plasma concentrations of other drugs in the body.

Risk: Increased concentration of other drugs might be toxic

Overall outcome: increased transcription and translation of CYP enzymes – reduced plasma conc. of other drugs

Risk: increased biotransformation of drugs in plasma

^a Nat. Rev. Drug Disc. 2015, 14, 475. ^b Comb. Chem. & HTS, 2010, 13, 188.

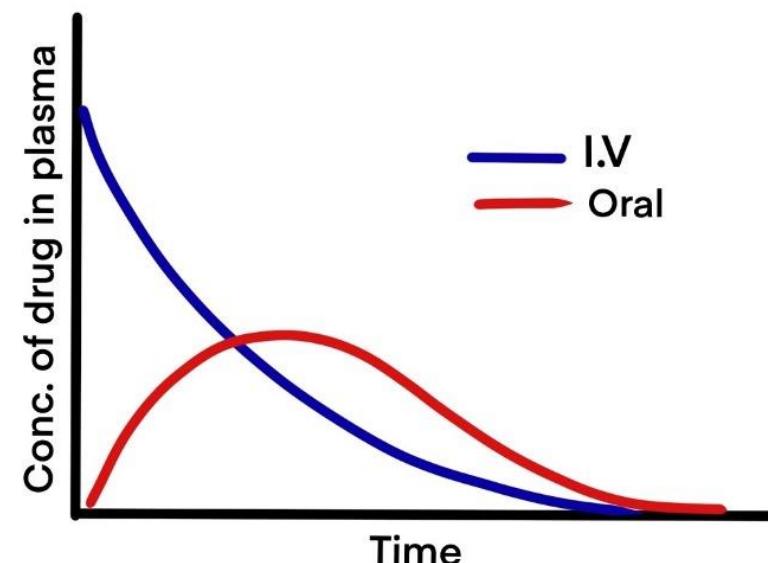
Drug Metabolism & Pharmacokinetics (DMPK) Optimization: In Vivo

Preclinical PK Species



- One rodent and one non-rodent species typically used
- Used to predict human PK

Generic IV and PO PK Profiles



- Examines the concentration of compound over time
- Profiles encompass **absorption, distribution, metabolism** and **excretion** (ADME)

Key PK Parameters

Clearance: the rate at which drug is removed

Volume of distribution: theoretical volume needed to account for the total amount of drug

Half-life: time needed for the drug concentration to reduce by half

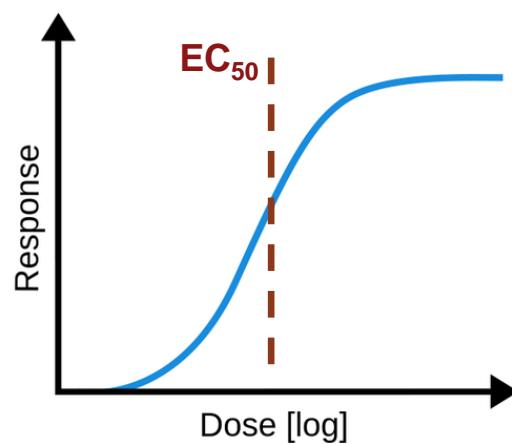
Oral Bioavailability: % of drug that reaches systemic circulation when orally administered

Area under the curve: area under the PK curve

Key takeaway: your compound should have ADME parameters that are predictable, permit plasma concentrations to reach therapeutic levels, avoid toxic levels, and allow for the drug to be metabolized and excreted from the body

In Vivo Efficacy Studies: Show An Effect In Animal Models

In Vitro Efficacy

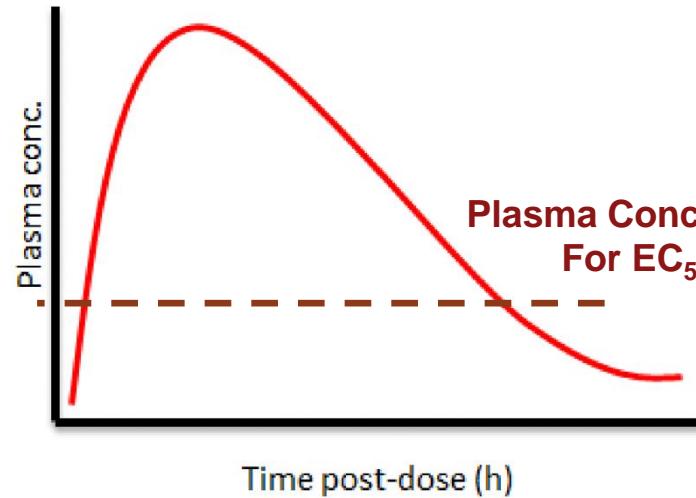


Determine compound potency from a relevant cellular assay – typically a pathway endpoint



Informs on the plasma conc. that needs to be covered in vivo

In Vivo PK

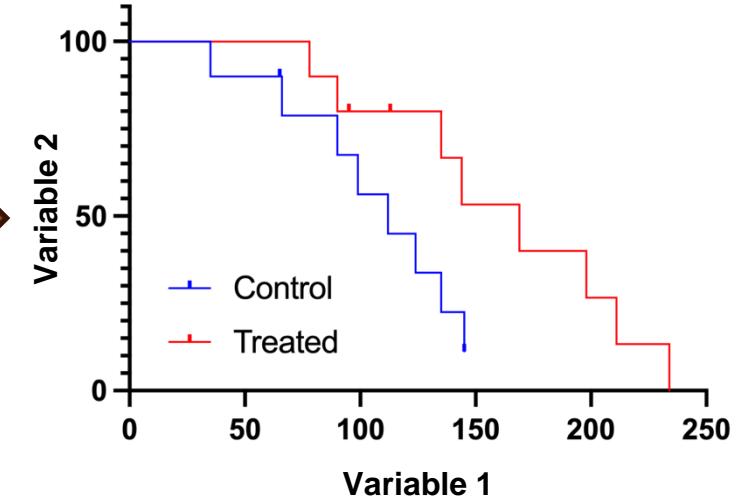


Obtain a compound PK profile in preclinical species model – typically mouse and dosed PO



Informs on dose that will need to be used in efficacy study

In Vivo Efficacy



Obtain in vivo response data that correlates with plasma concentration and supports progression of the compound

Lead Optimization Safety Screening: Show Compound Is Safe To Advance

On-Target Toxicity:

- Modulating your target of interest with a small molecule leads to toxic effects
- Might be missed in experiments where the target is modulated genetically (KO/KD)
- Path forward might still exist depending on safety margins between therapeutic and toxic effects
- Can be observed in vitro, in vivo, or in both

Off-Target Toxicity:

- Compound modulates other undesired targets and leads to toxic effects → secondary pharmacology
- Metabolite formed from the compound has secondary pharmacology
- Can be observed in vitro, in vivo, or in both
- Can be challenging to find the “smoking gun” when off-target tox is observed



^a *Nat. Rev. Drug Disc.* **2012**, 11, 909. ^b *Chem. Res. Toxicol.* **2016**, 29, 473.

Lead Optimization In Vitro Safety Screening

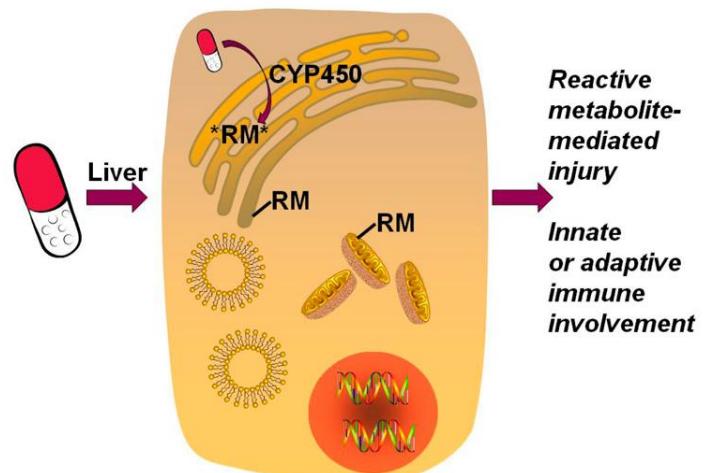
Off-Target Toxicity: Assessed using target panels – CEREP

- Most are high-throughput single point binding assays
- Hits are confirmed in functional or cellular assays

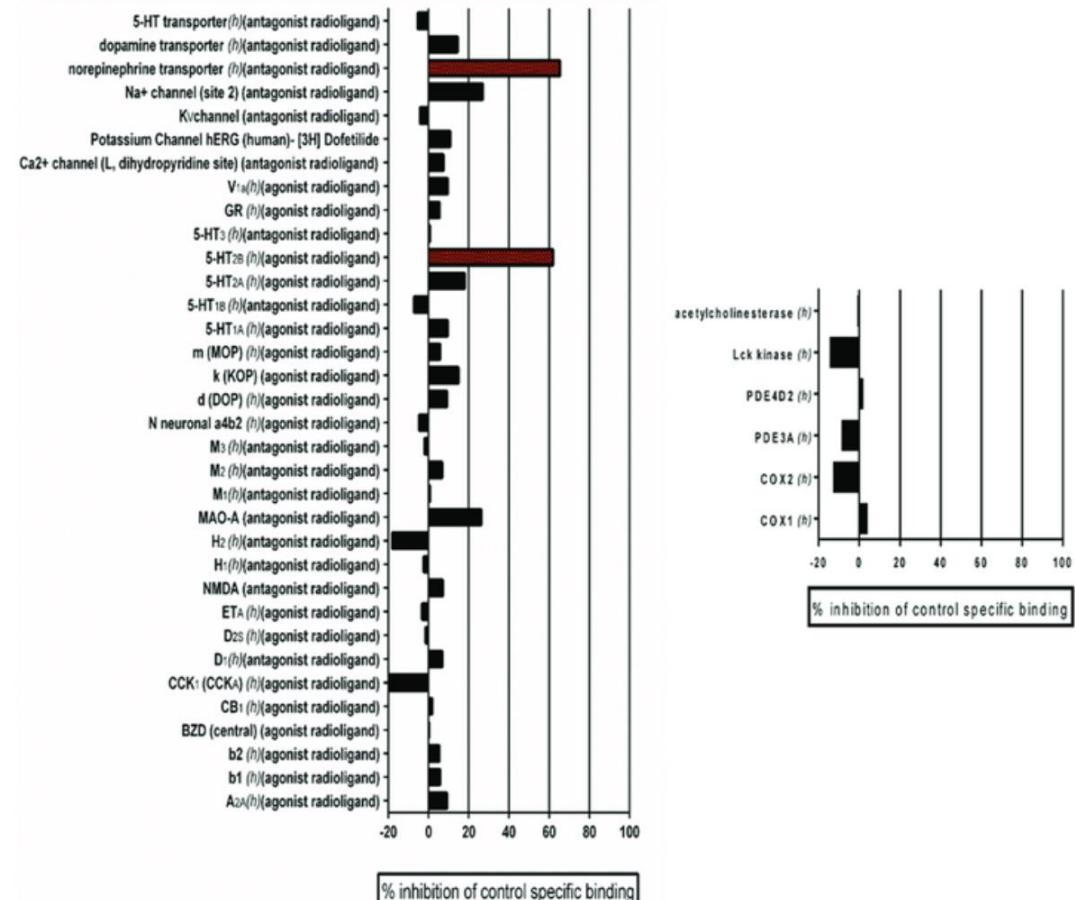
Reactive metabolites: Drug is metabolized to a metabolite that leads to immune response or liver injury

- Detected using glutathione trapping experiments

Reactive metabolites can lead to liver toxicity or immune response



Example Results From a CEREP Off-Target Panel



^a Nat. Rev. Drug Disc. 2012, 11, 909. ^b Chem. Res. Toxicol. 2016, 29, 473. ^c J. Pharmacol. Tox. Methods., 2019, 99, 106609.

Lead Optimization In Vivo Safety: Exploratory Toxicology

Exploratory toxicology

- Provide toxicological and toxicokinetic assessment candidate molecule
- Identify doses for GLP toxicology studies used to regulatory filing.



1 Rodent species



1 Non-rodent species



Maximum tolerated dose (MTD): administer increasing doses of compound until tox is observed



Dose-range finding: use dose information from MTD study to conduct repeat dosing study (around 14 days) at multiple dose levels to observe tox.



Readouts: Clinical observations | Hematology | Clinical Chemistry | Pathology/Histopathology | Toxicokinetics

^a *Regulatory Tox. Pharmacol.* **2008**, 51, 237. b *Proc. West. Pharmacol. Soc.* **2009**, 52, 94

Lead Optimization and Candidate Molecule Material Requirements

- Toxicology studies require larger amounts of material
- Chemistry challenges can arise with scale up
 - New route discovery and optimization



Hit-to-lead
Early lead optimization
25 – 100 mg



Lead optimization
100 – 1000 mg



nGLP toxicology studies
50 – 250 g



Candidate Molecule Drug Candidate Profile (DCP)

PROTOTYPE CRITERIA	
DISEASE INDICATIONS	Primary disease indication. Secondary disease indication. Specific patient population.
DIFFERENTIATION	First in class or best in class
DISEASE LINKS	Target of interest is functionally and or genetically linked to disease of interest with POC data
THERAPEUTIC HYPOTHESIS	Modulating the target of interest provides a specific therapeutic benefit.
THERAPEUTIC PoC	Administration of small molecules that meet DCP provide a response that support the therapeutic hypothesis
IN VITRO POTENCY	<10 nM potency in relevant biochemical and or cellular assays. <30 nM in pathway endpoint assay
SELECTIVITY	Selectivity: > 100-fold selectivity over target homologs, kinases, receptors, and ion channels
IN VIVO EFFICACY	Appropriate <i>in vivo</i> model shows movement of pathway or disease-relevant biomarkers
DMPK	Appropriate metabolic stability for a projected human BID or QD oral dosing. BCS I or II. Anticipated dose 500 mg/day. Low risk of CYP based DDIs.
PHARMACEUTICS	
SAFETY	Appropriate <i>in vitro</i> selectivity against targets in safety panel. No genotoxicity. Acceptable safety window in rodent/non-rodent repeat dosing study

10 Minute Q&A, Break



Large Molecule Drug Discovery



Immersion Course: Session 2 - Drug Discovery

(e.g., Target Identification, Small Molecule and Protein-based Therapeutics Discovery)

Rob Wells Ph.D.

Denali Therapeutics

My path to a career in biotech



UC San Diego



Undergraduate
in San Diego

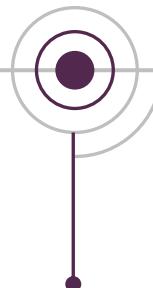
Biochemistry and cell
biology

Minor: Psychology

Graduate school in
Baltimore, Maryland

Biology, PhD

Studied mitochondrial
dynamics



JOHNS HOPKINS
UNIVERSITY



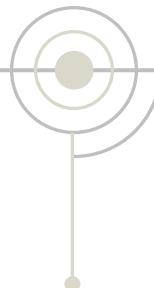
Post-doc in protein
engineering of non-
antibody scaffolds

University of Chicago,
Illinois

Shohei Koide lab

South San Francisco
Antibody & Protein
engineering

Designing biotherapeutics
for delivery into the brain
for neurodegenerative
disease



DENALI™
THERAPEUTICS

7+ years

Outline for large molecule session

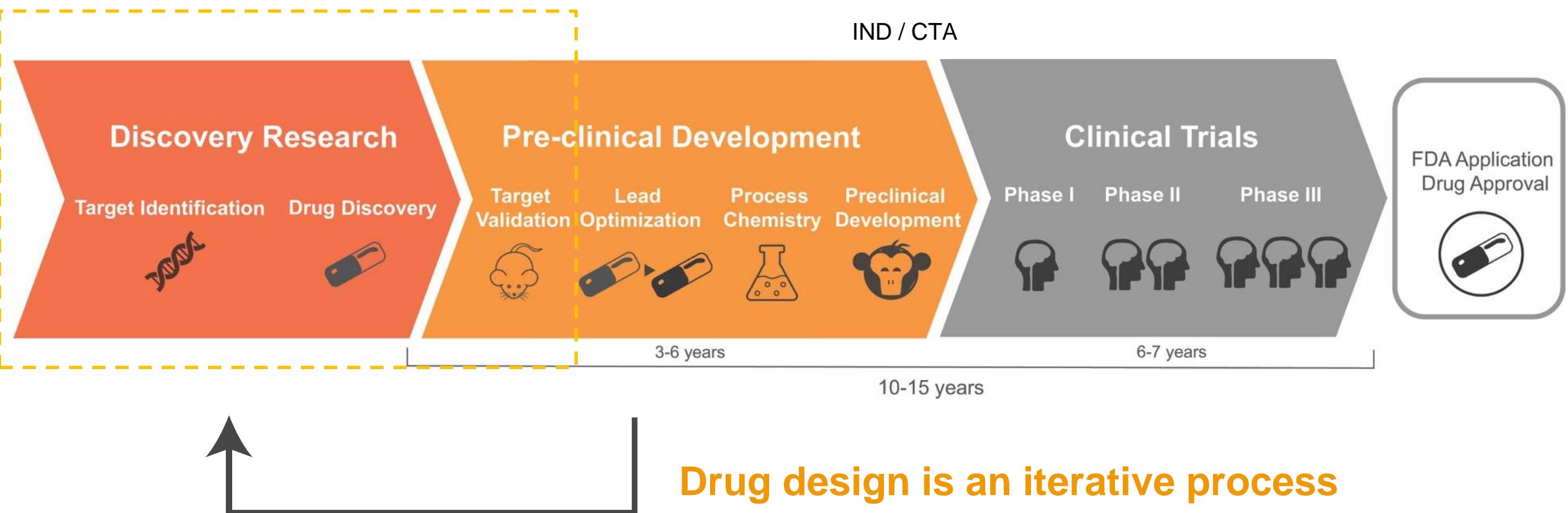
Drug discovery from a **Large Molecule** perspective

- Target identification
- Choosing a drug modality
- Antibody discovery
- Newer molecule modalities

Take aways:

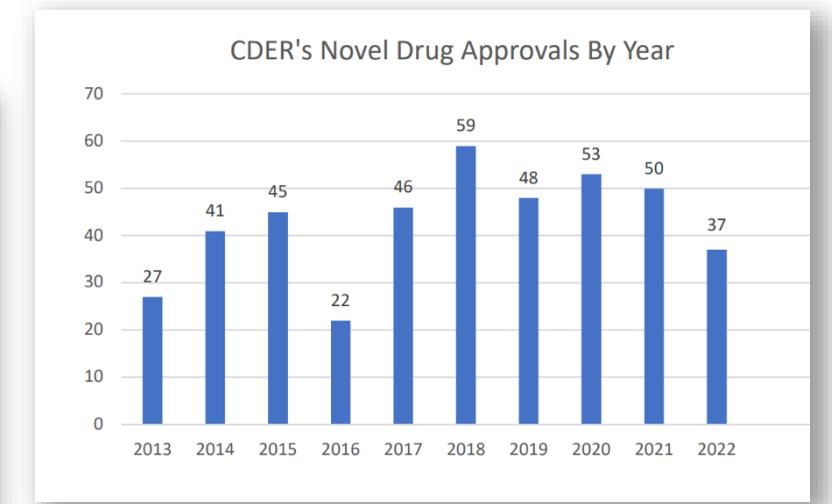
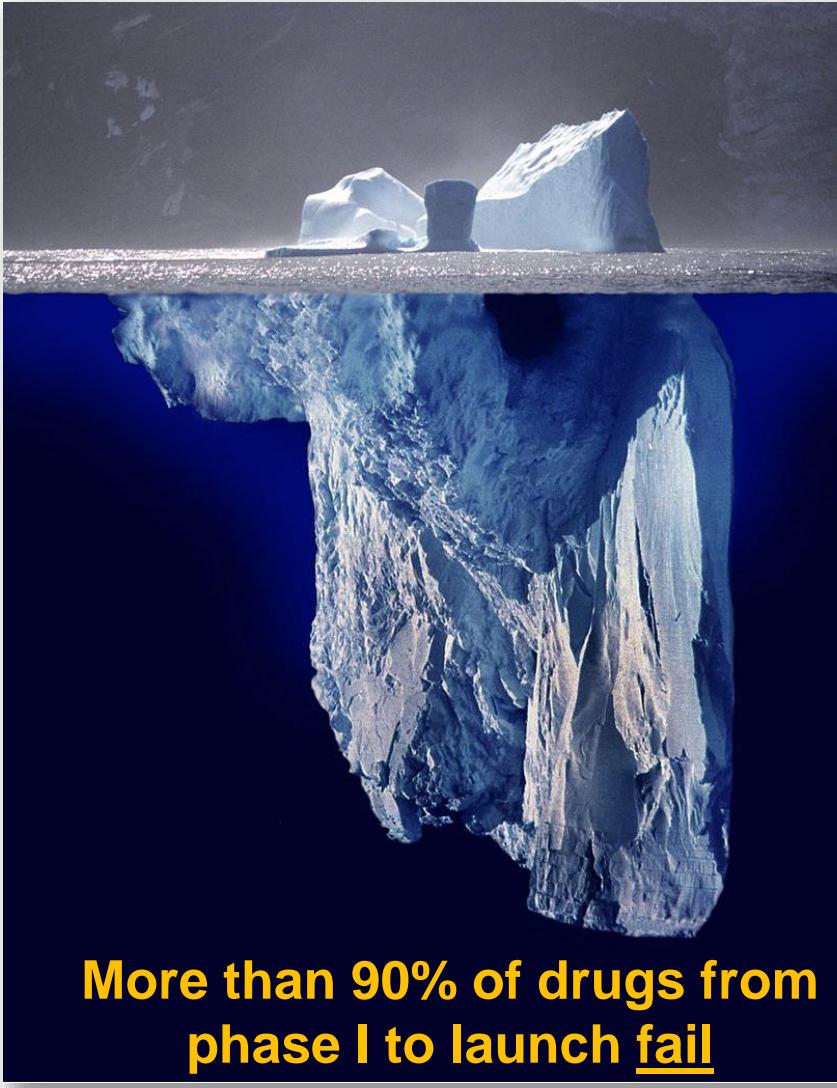
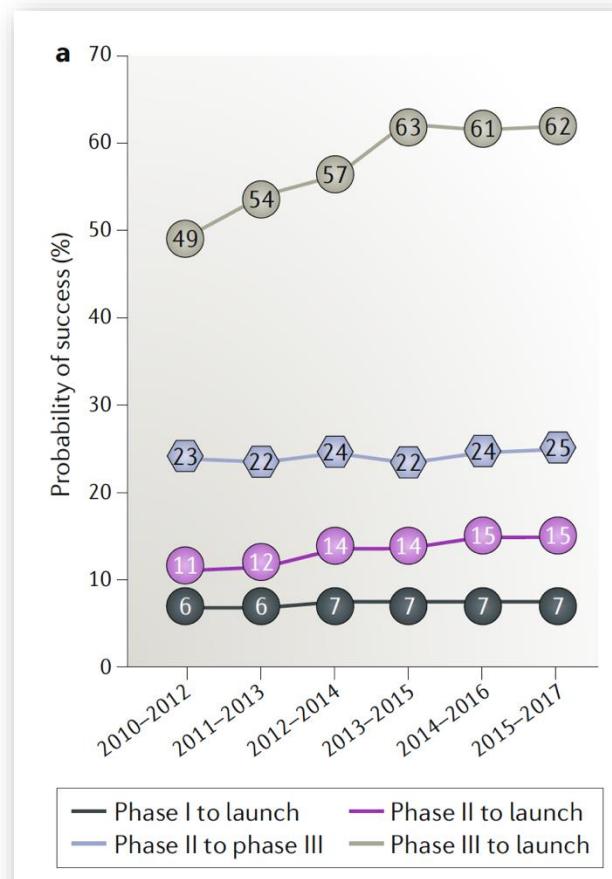
- Any jargon that someone new to the field (or old) may not know.
- Show examples of drugs that have been successful

THE DRUG DEVELOPMENT PROCESS – Large molecule



Discovery Research - What does the drug look like?

Most drugs fail (small + large)



CENTER FOR DRUG EVALUATION AND RESEARCH

Approved drugs are just the tip of the iceberg.

<https://doi.org/10.1038/d41573-019-00074-z>

<https://www.fda.gov/drugs/new-drugs-fda-cders-new-molecular-entities-and-new-therapeutic-biological-products/novel-drug-approvals-2022>

Discovery Research: How do we solve the low success rate for large molecules?

Identification of the right lead molecule(s) **before pre-clinical development.**

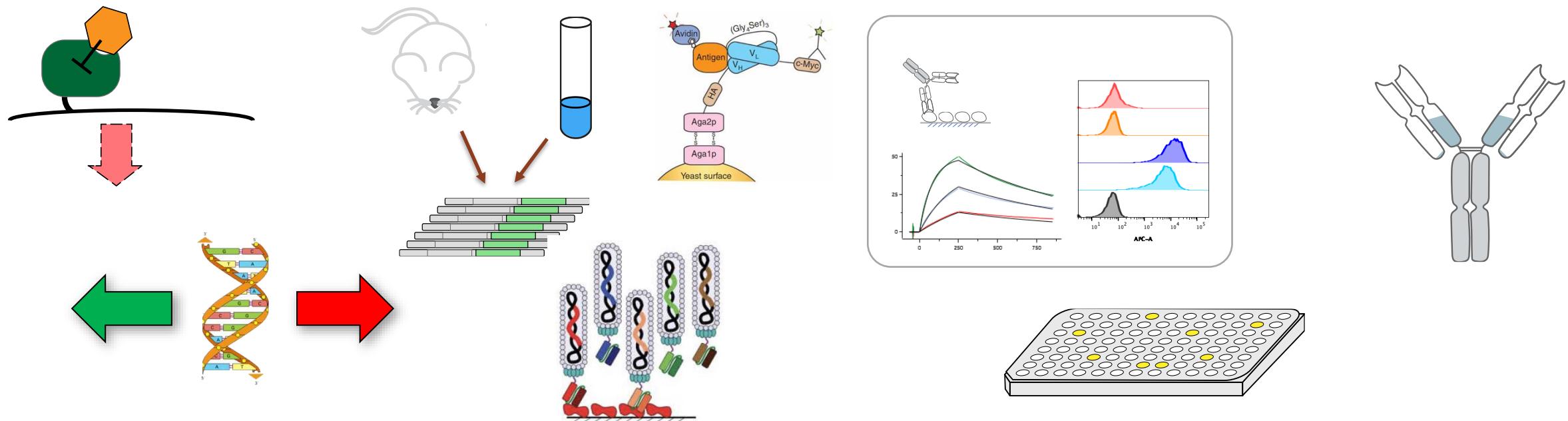
Target identification

Drug Discovery
(Hit finding)

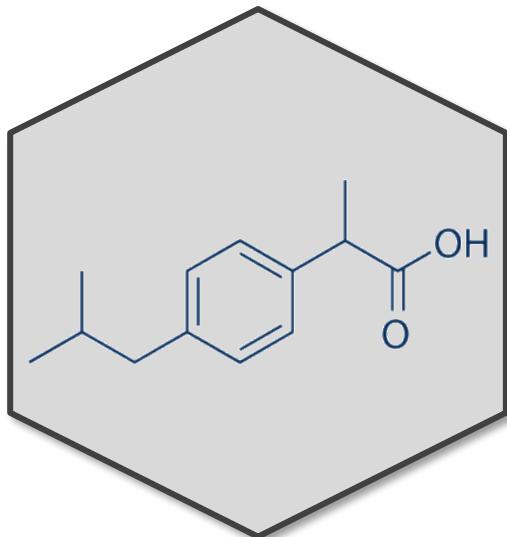
Target Validation

Lead optimization

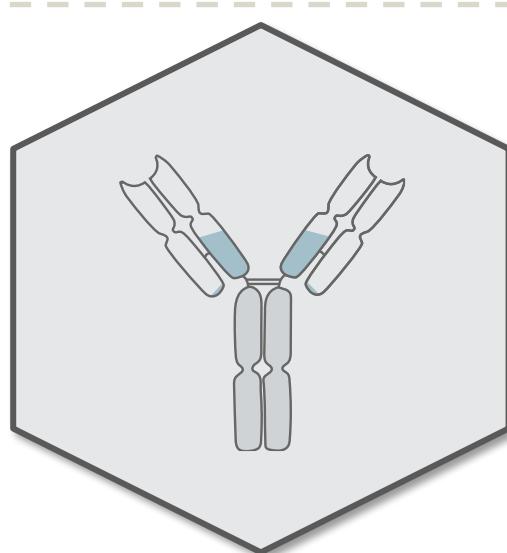
Lead ID



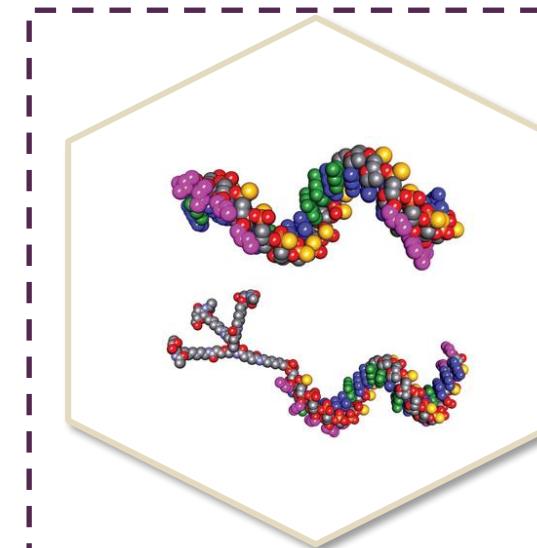
What drug modality are you delivering?



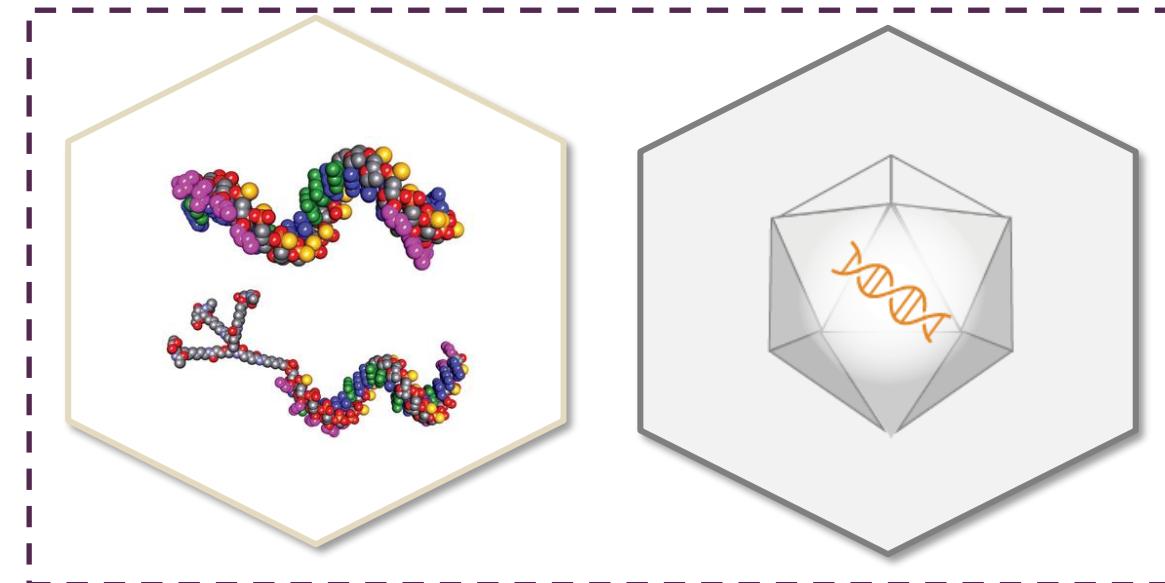
SMALL MOLECULES



ANTIBODIES, ENZYMES,
ANTIBODY DRUG
CONJUGATES



NUCLEOTIDES, VACCINES

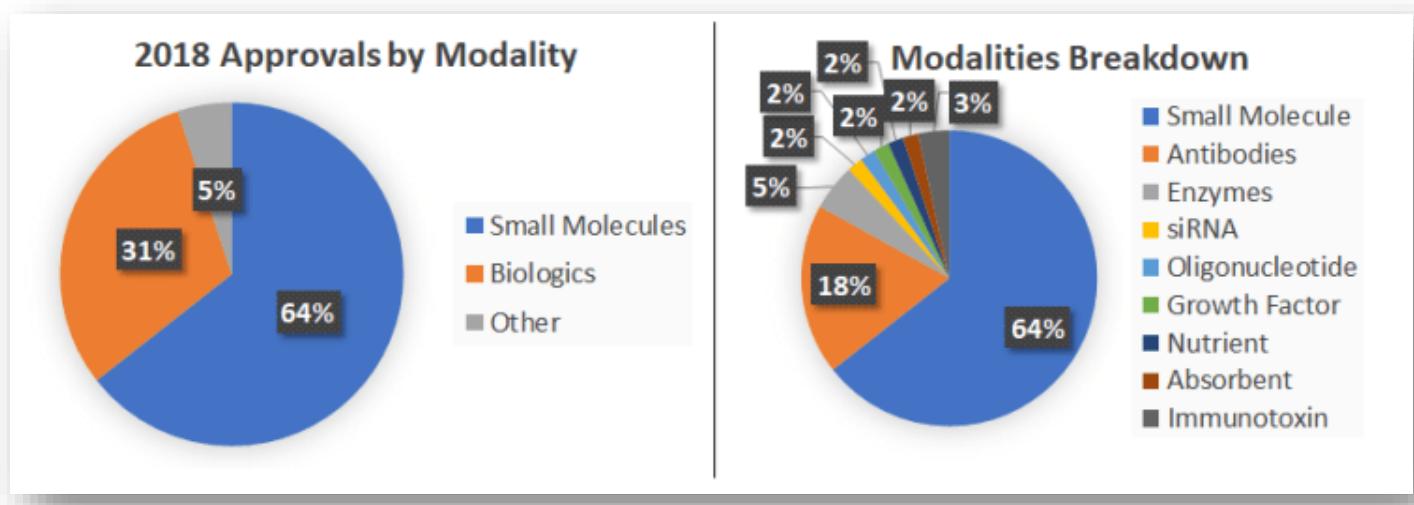


VIRAL DELIVERY/
GENE THERAPY/CAR-T

New formats are always being invented to **improve the potency, specificity and safety** of drugs to **new and old indications**.

Note: not drawn to scale

New Therapeutic Modalities are critical in modern Drug Discovery



Total 59 drug approvals in 2018

- Pioneer new approaches to drug discovery and explore new biology
 - Typically not addressed by traditional small molecules and monoclonal antibodies.
- Increase likelihood of finding the best tool for the best target
 - Design therapeutics for disease mechanisms previously considered difficult, precision medicine
- Require more creative approaches to clinical development

<https://www.fda.gov/drugs/new-drugs-fda-cders-new-molecular-entities-and-new-therapeutic-biological-products/novel-drug-approvals-2018>

Poll 4: What field are you most closely associated with...

- 1) Small molecule: chemist, chemical engineer, etc
- 2) Large molecule: biochemist, antibody/protein engineer, etc
- 3) Both small and large or a combination
- 4) None of the above



Target Identification Large Molecule Perspective

Target identification can be difficult

Existing drug targets: "Best-in-class" or "Biosimilar"

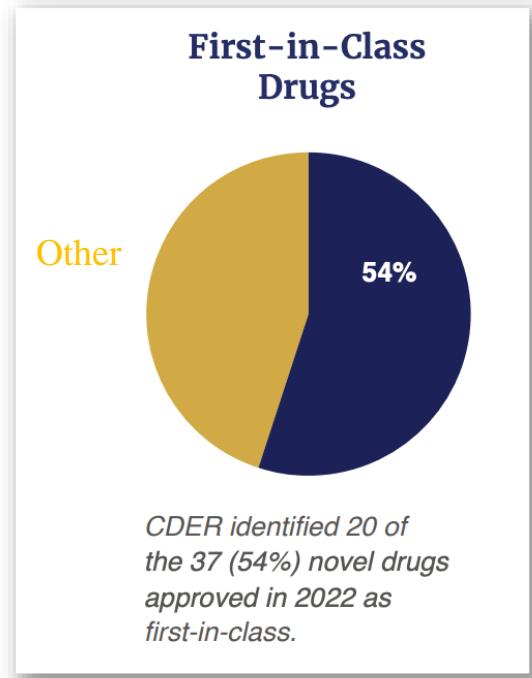
Novel indications: "First-in-class"

Publications/literature: Always repeat in house

- Biology – identification of disease associated pathways
- Genetics/proteomics –

Proof-of-concept in disease models:

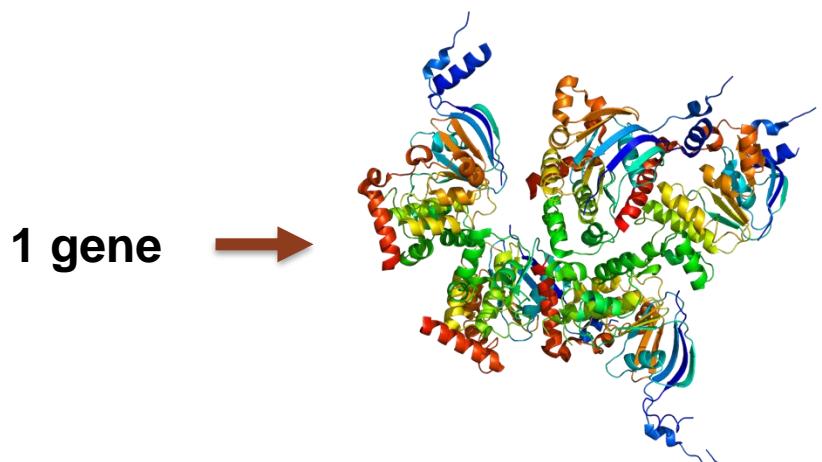
- Is there a "tool" molecule that can be tested to prove your therapeutic hypothesis in animals before the resources, time and effort is put into design?



Tool molecule : a molecule with undesirable properties of a drug but will have a similar effect on the biology to demonstrate a pathway can lead to beneficial outcomes. More easily and quickly available without the need for drug discovery.

Diseases have different complexities underlying their mechanisms

Cystic fibrosis



Alzheimer's Disease:

Many genes
+
Environmental
factors



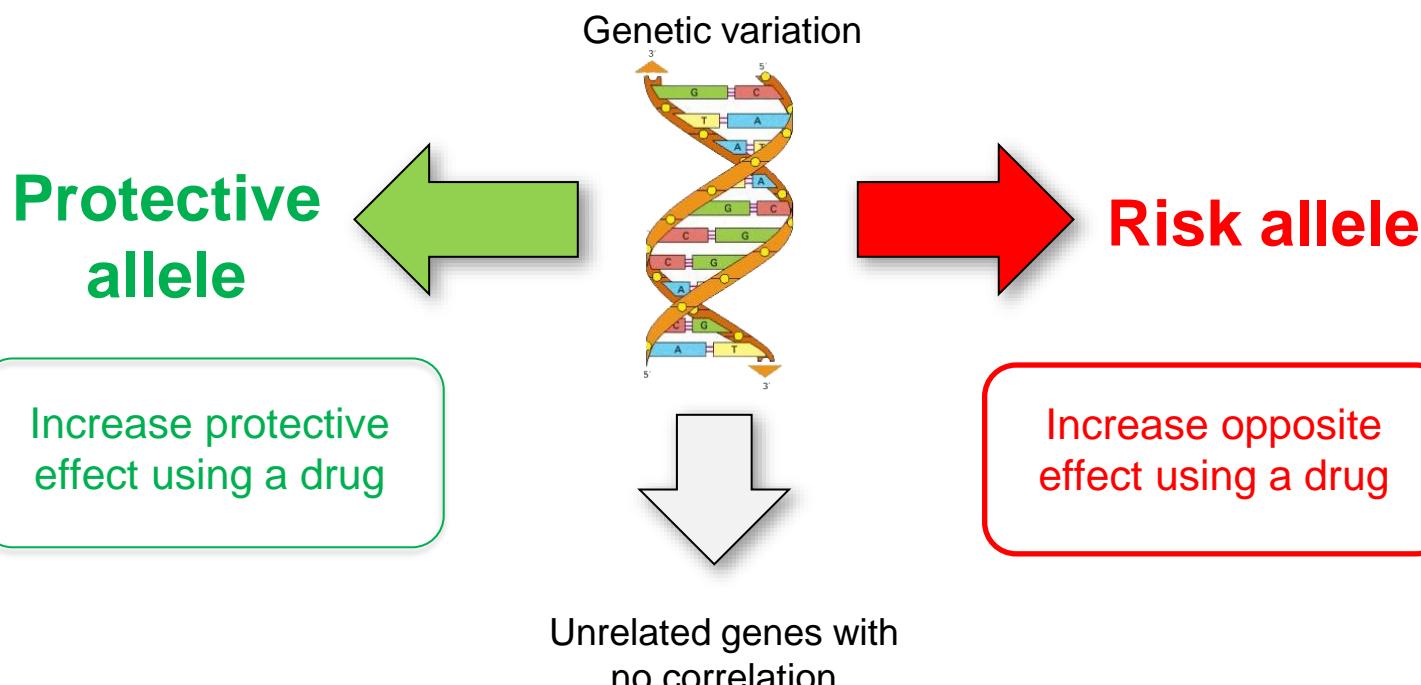
Alzheimer's assoc. Logo

“Drugability” – how easily the activity of your therapeutic target can be modulated by a drug.

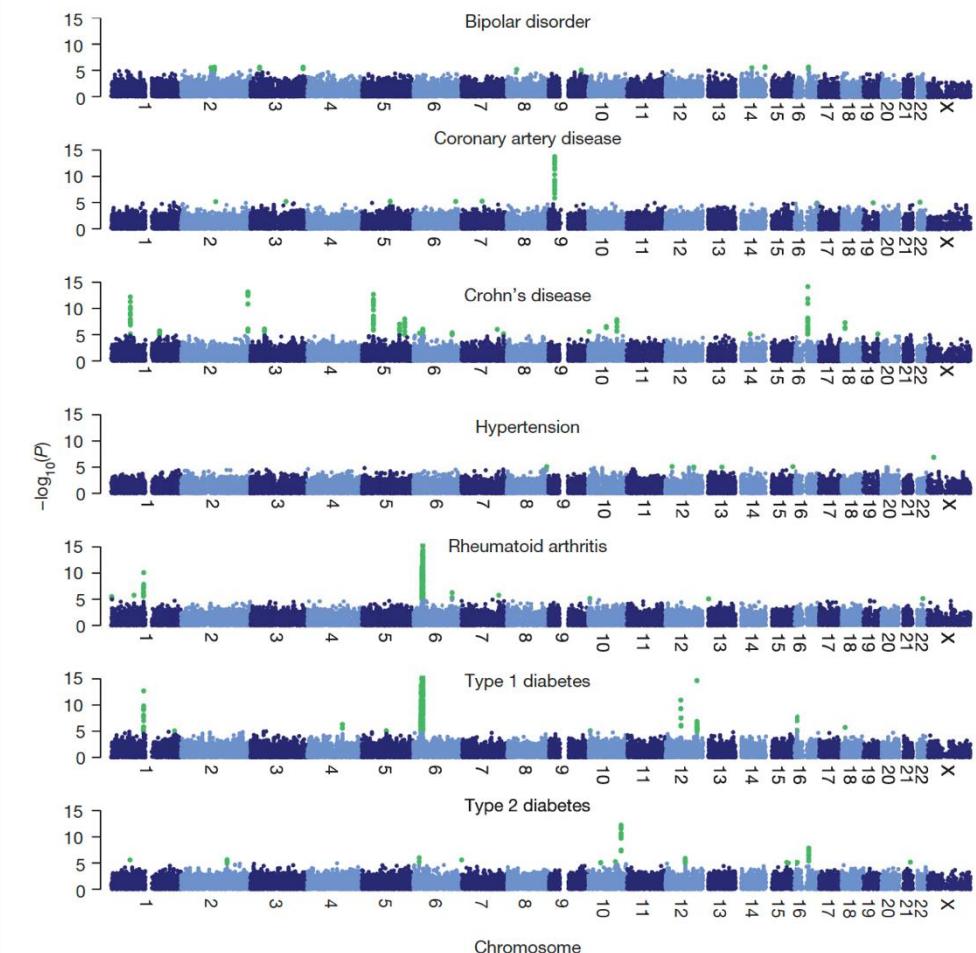
Finding disease causes: genome wide association studies (GWAS)

Correlate human genetic variation with disease

- **Single nucleotide polymorphisms – (SNPs)** : A genetic variation that exists in a high enough population (1% or more)
- Identify nearby genes to SNPs as disease correlated.
- GWAS studies can implicate **whole genetic pathways**
- Correlation doesn't always mean causation



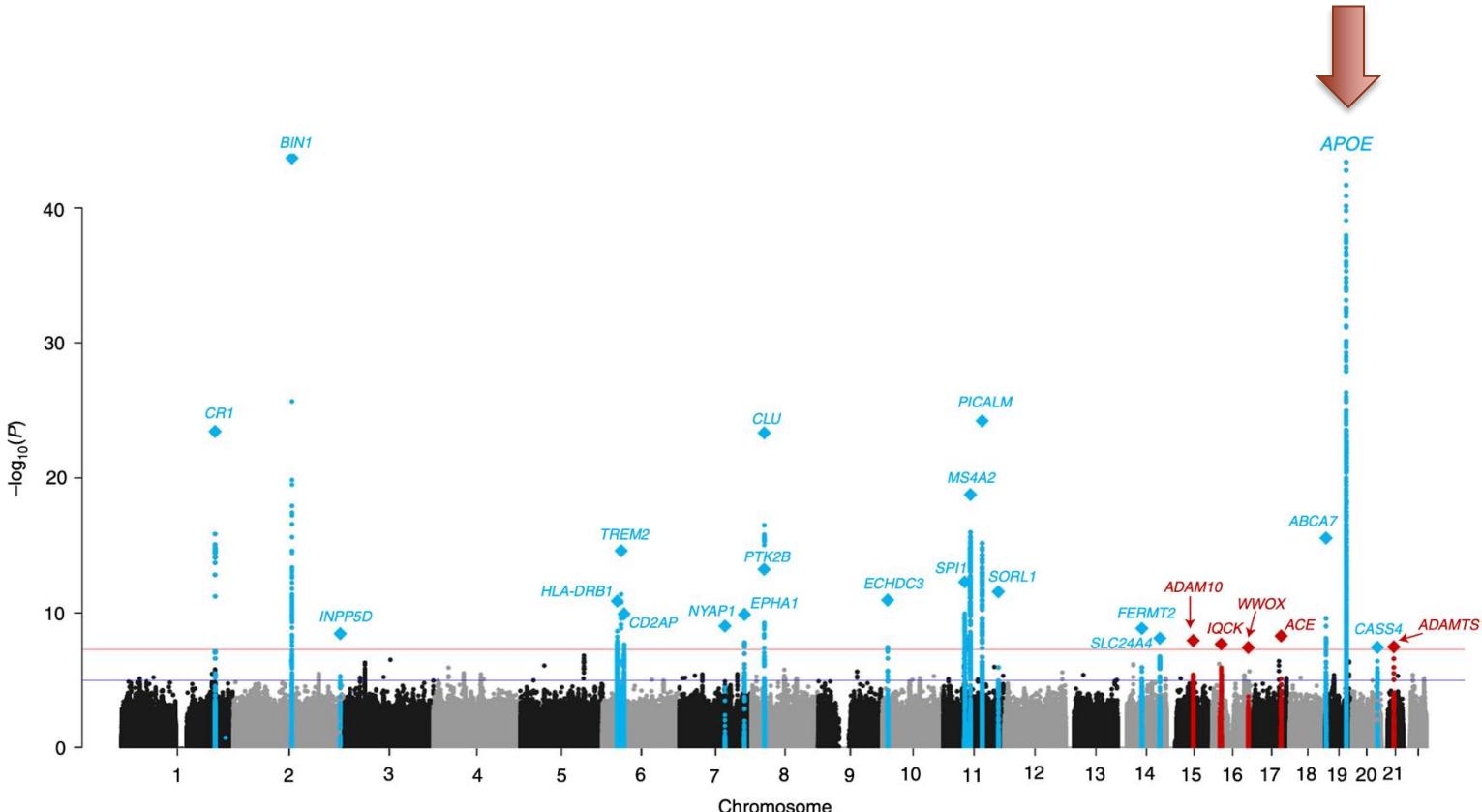
Plot of probability of disease versus genotype



Welcome Trust Case control Consortium Nature 2007 Jun 7;447(7145):661-78. doi: 10.1038/nature05911.

Example: GWAS studies for Alzheimer's disease

GWAS study for Alzheimer's disease

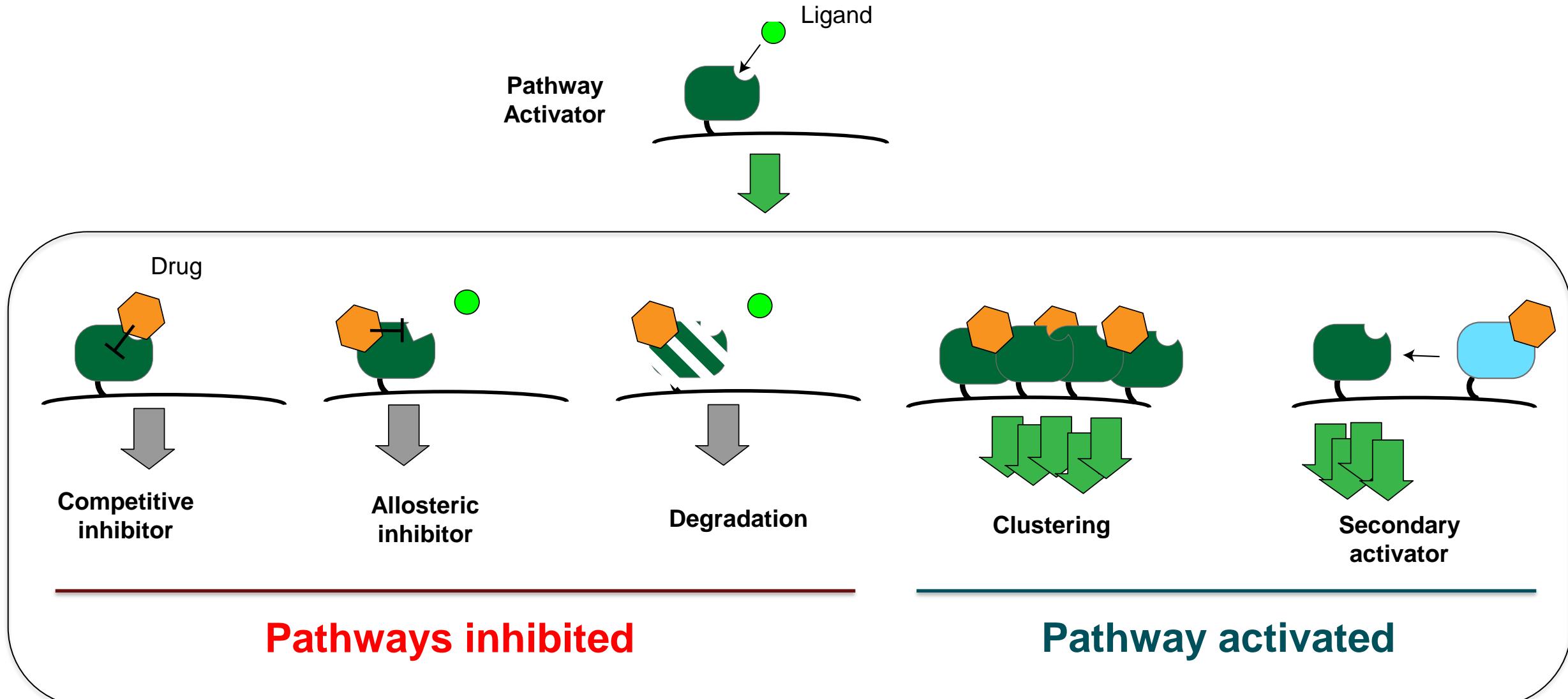


APOE: Apolipoprotein E – involved in lipid and cholesterol transport

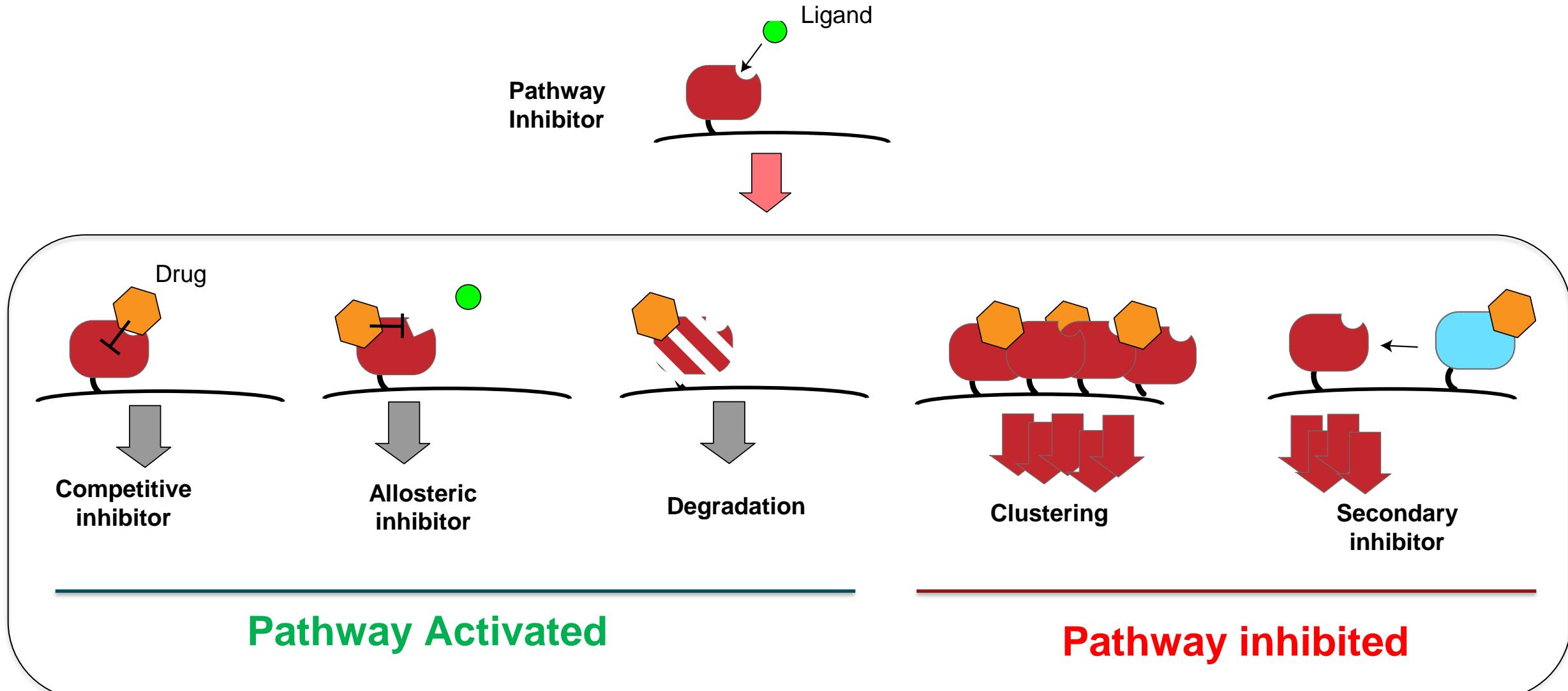
Common name	Genoset	Magnitude	rs429358	rs7412	Comment
Apo-ε1/ε1	gs267	6	(C;C)	(T;T)	the rare missing allele
Apo-ε1/ε2	gs271	2.5	(C;T)	(T;T)	
Apo-ε1/ε3	gs270	2.6	(C;T)	(C;T)	ambiguous ε2/ε4 or ε1/ε3
Apo-ε2/ε4	gs270	2.6	(C;T)	(C;T)	ambiguous ε2/ε4 or ε1/ε3
Apo-ε1/ε4	gs272	2.5	(C;C)	(C;T)	
Apo-ε2/ε2	gs268	4	(T;T)	(T;T)	good; lowest risk
Apo-ε2/ε3	gs269	2	(T;T)	(C;T)	
Apo-ε3/ε3	gs246	2	(T;T)	(C;C)	the most common
Apo-ε3/ε4	gs141	3	(C;T)	(C;C)	
Apo-ε4/ε4	gs216	6	(C;C)	(C;C)	~11x increased Alzheimer's risk

- APOE4 variants **increases risk**
- APOE2 variants **are protective**

How can drugs modulate pathways?



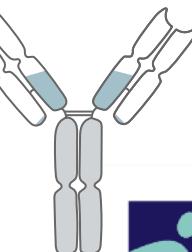
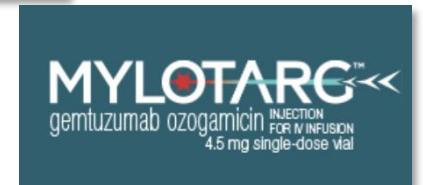
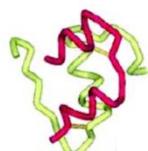
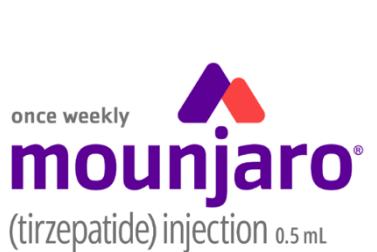
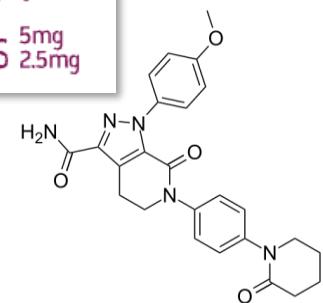
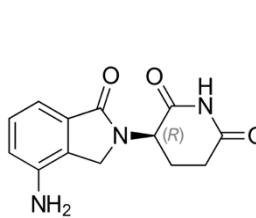
How can drugs modulate pathways?





Small and Large Molecules

Small molecules, peptides & large molecules



Molecular Weight (Daltons)

1000

10,000

100,000

300,000

Insulin structure: Adapted from Baker E et al. Phil Trans R Soc Lond 1988; B19:369-456.

Small versus Large molecule therapeutics

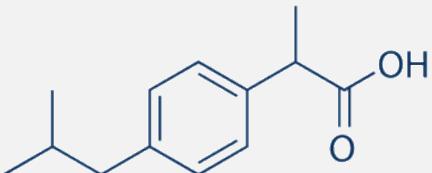
SMALL MOLECULE

Advantages:

- Orally bioavailable
- Cell/lipid permeable
- Low complexity/cost of manufacturing
- Precise homogeneous identity
- Fast on/off rates: tunable PD effect

Disadvantages:

- Specificity/off-target



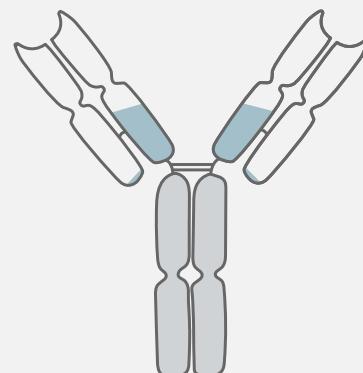
LARGE MOLECULES

Advantages:

- High specificity
- Long half-lives (monthly or bimonthly dosing)
- Modularity/functionality (Immune cell engagement, bispecific, others)
- Chemical conjugates allow novel modalities
- Enzymatic (Enzymes, nucleotides)

Disadvantages:

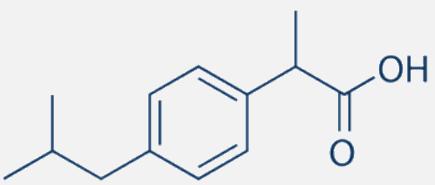
- Dosed IV or injection
- Immunogenicity
- Manufacturing



Small versus Large molecule Therapeutics

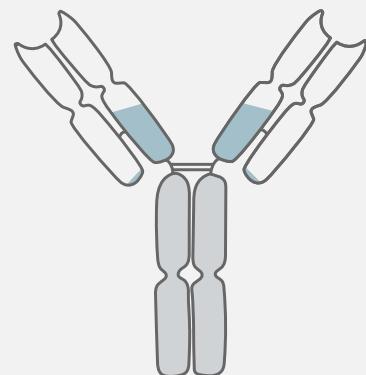
SMALL MOLECULE

- Target affinity < 50 nM
- Concentration-related activity in relevant functional assay
- Clear Structure-Activity Relationships
- Assessment of patentability
- Defined route of administration
- Void of highly promiscuous and reactive functional groups



LARGE MOLECULE

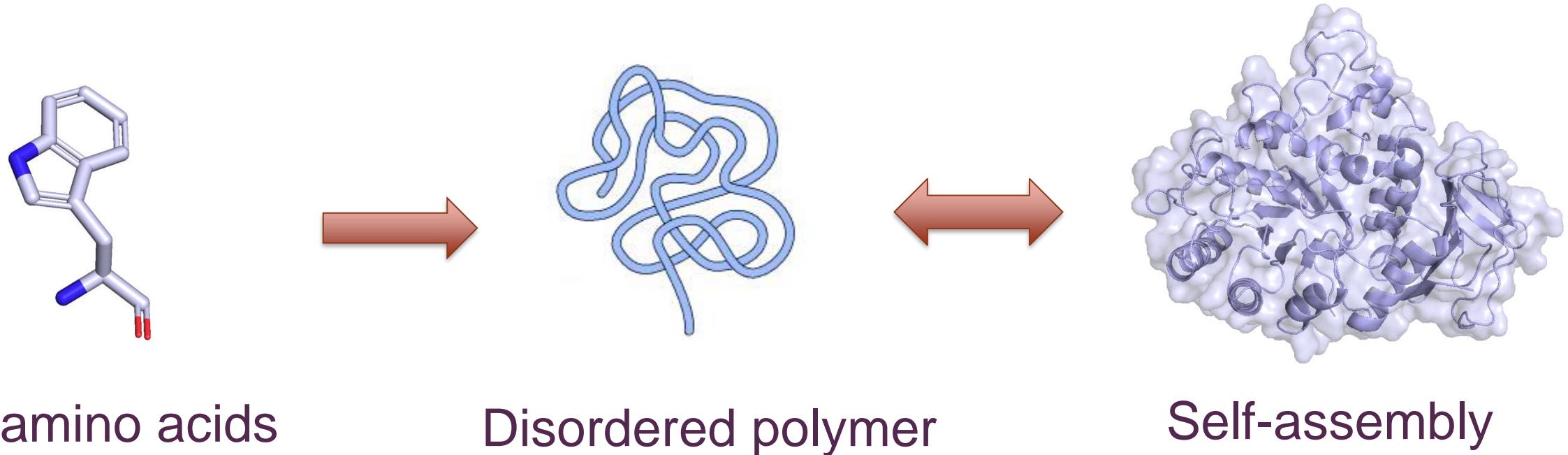
- Target affinity < 10 nM
- Concentration-related activity in relevant binding and functional assays
- Desired Cyno cross-reactivity
- Normal PK in mouse
- Unique sequences





Large Molecule Drug Discovery

Proteins are versatile molecules that drive evolution



- Evolved to **self-assemble** to form stable structures underlying all of life!
- **Proteins are meta-stable:** one mutation can unfold and aggregate.
- **How do we screen through this diversity to one stable drug?**

Poll 5:

How many **atoms** would it take to make one of every combination of a small
60 amino acid protein?

- A) All atoms in your lunch today
- B) All the atoms in the biomass of the earth
- C) All the atoms in the solar system
- D) All the atoms in the galaxy
- E) More than all the atoms in the universe

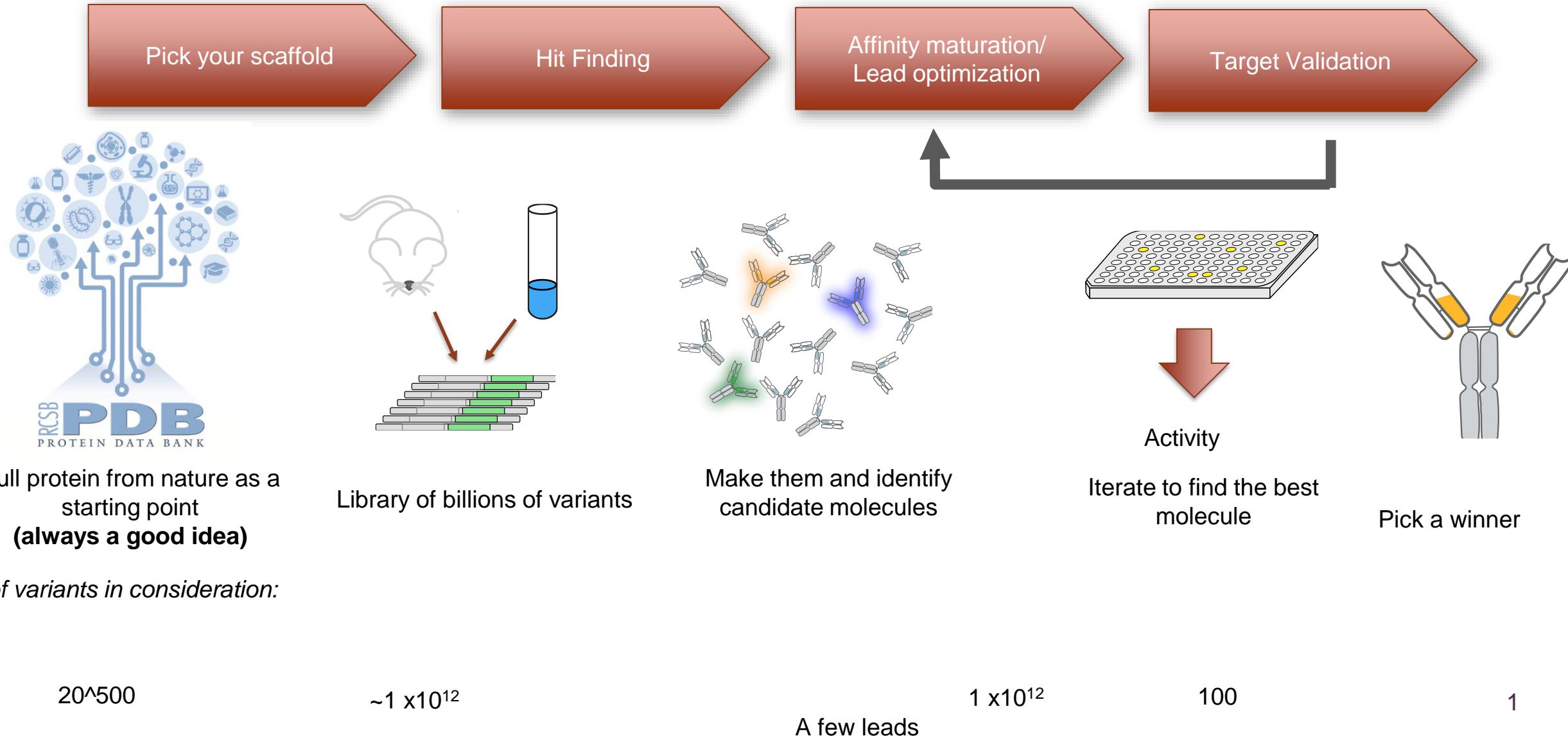
Answer

Total number of variants of a 60 amino acid protein = $(20 \text{ amino acids})^{60 \text{ positions}} \times \sim 1000 \text{ atoms} = 1.15 \times 10^{81}$

Total number atoms in the universe = $\sim 1 \times 10^{80}$

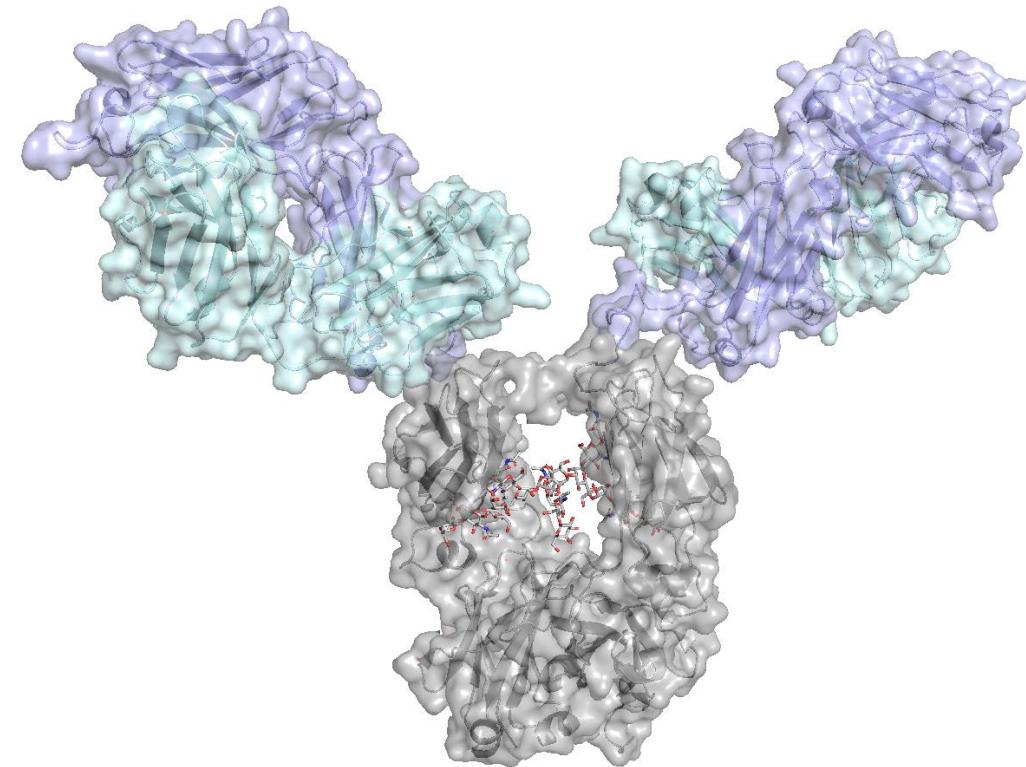
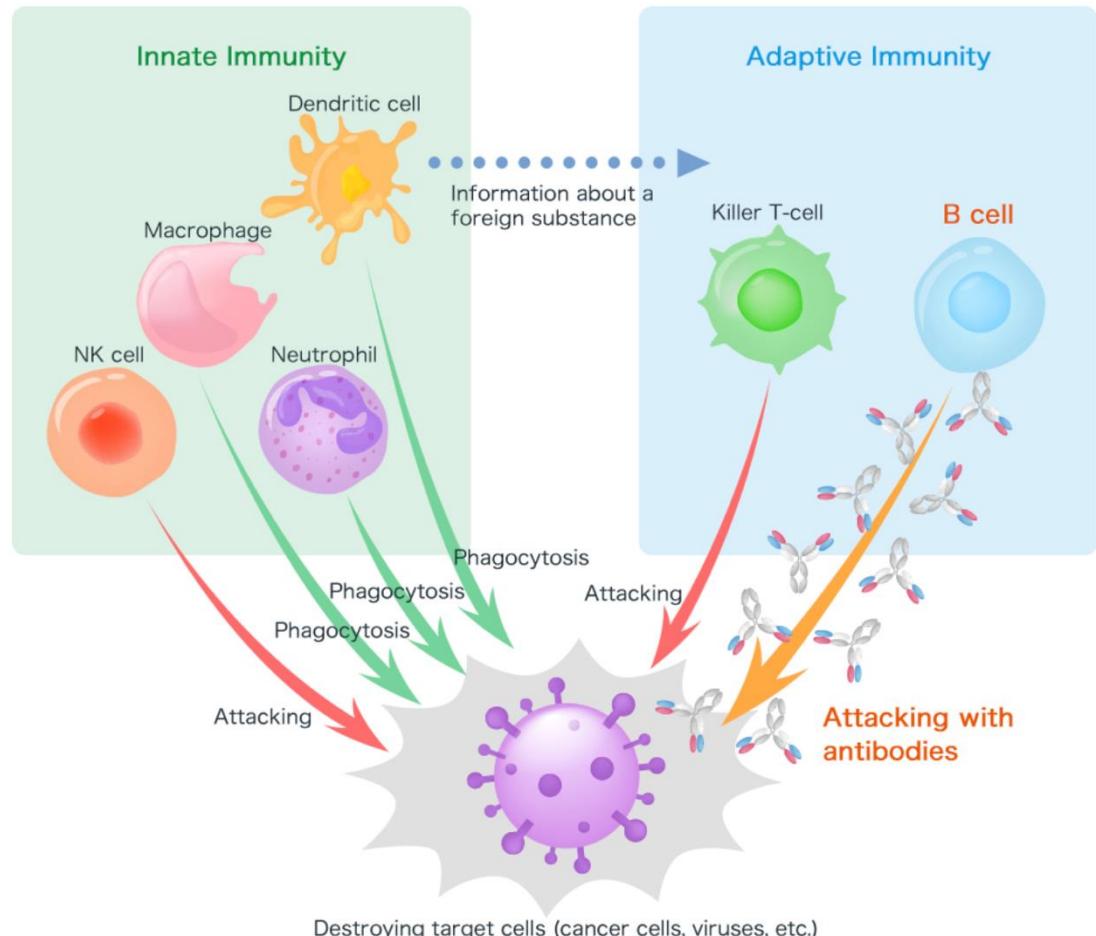
Answer E.) $>100,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000$ variants

Screening cascade for large molecule discovery



Antibodies evolved to bind with high affinity and specificity to their targets

Pick your scaffold



IgG1 Antibody

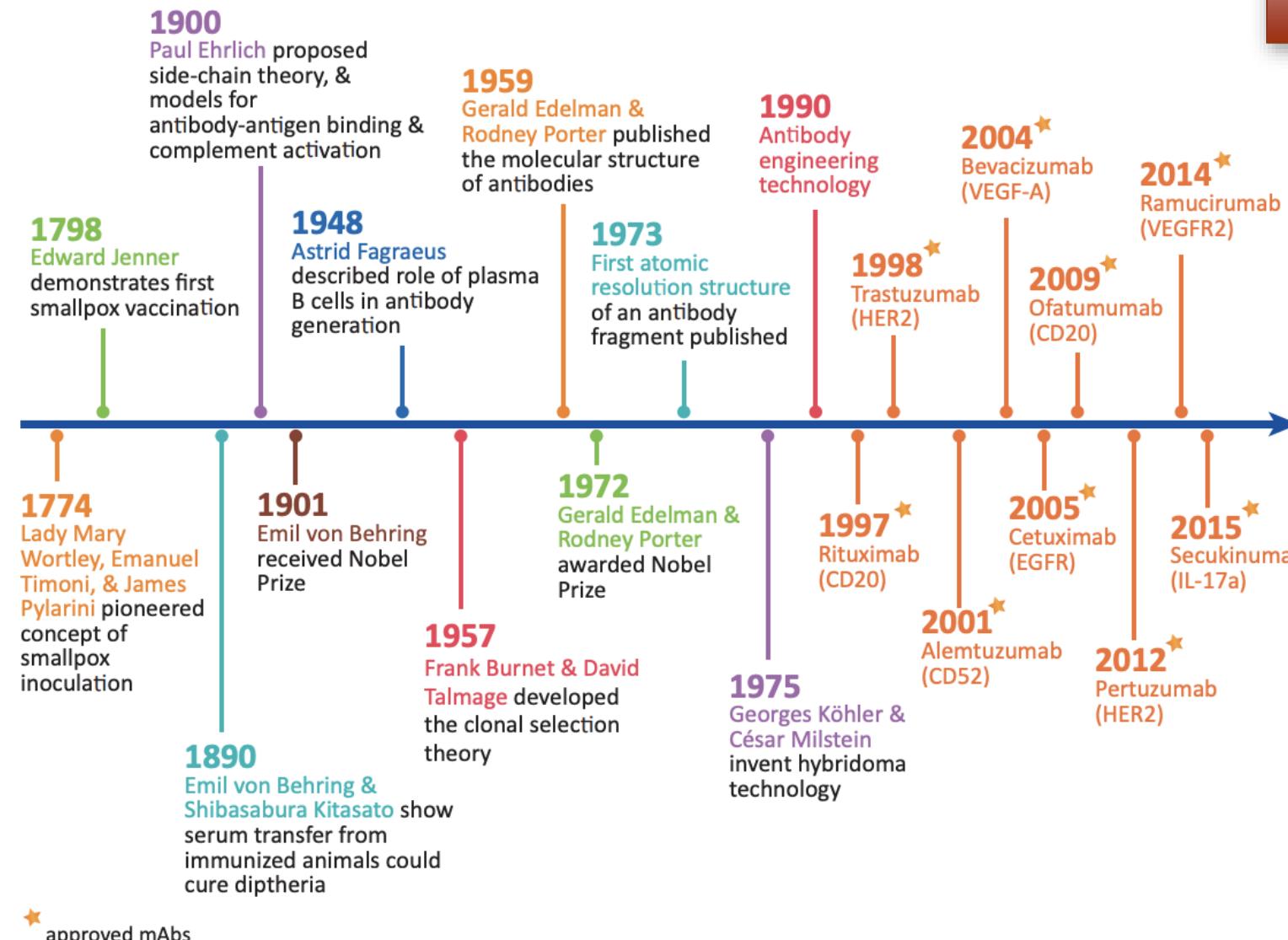
Antibodies in circulation recognize foreign material, while avoiding self-targets.

Sender R, Milo R. The distribution of cellular turnover in the human body. Nat Med. 2021 Jan;27(1):45-48. doi: 10.1038/s41591-020-01182-9. Epub 2021 Jan 11.

1hzh.pdb

History of antibody discoveries

Pick your scaffold



Genscript antibody handbook

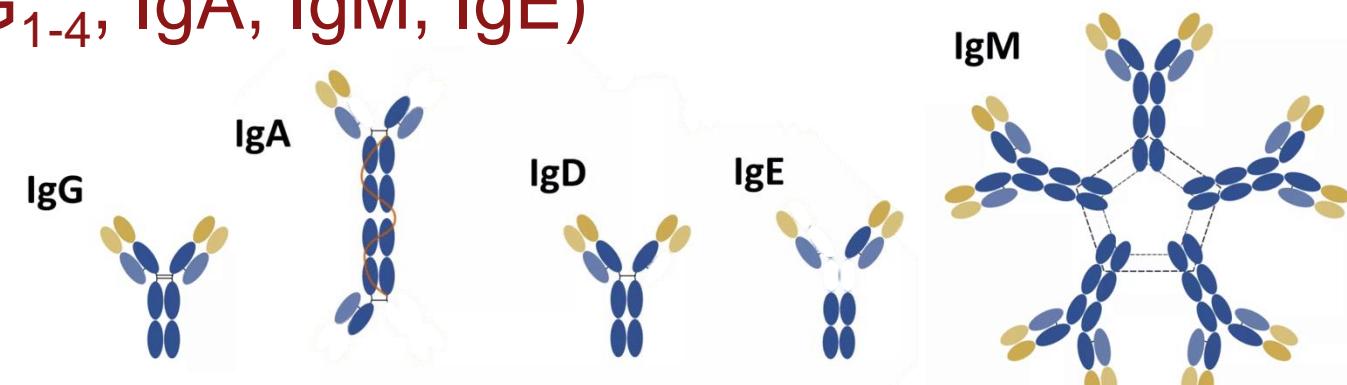
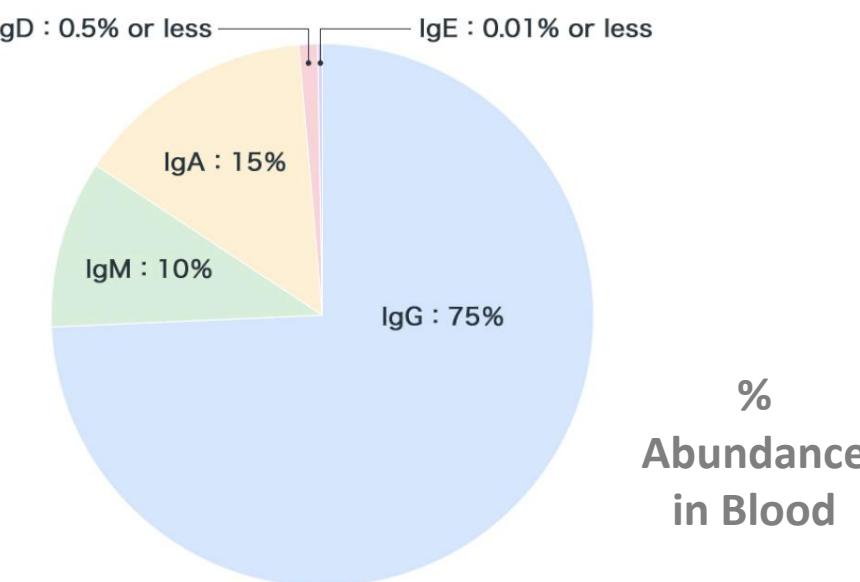
Antibody Isotypes and Roles (IgG₁₋₄, IgA, IgM, IgE)

IgG

- most abundant antibody in blood (only isotype that can pass through the placenta)
- binds **bacteria and toxins**
- complement, opsonization, neutralization and antibody-dependent cellular cytotoxicity (ADCC)

IgM

- mainly distributed in blood
- key role in the **initial immune system defense**
- antigen neutralization, complement, weak ADCC and opsonization.



IgA

- mainly present in blood as monomers
- forms dimers in **secretions** (bowel fluid, nasal discharge, and saliva) to prevent bacterial invasion
- also present in breast milk to protect the gastrointestinal tract of newborns from bacterial and viral infection
- antigen trapping and neutralization

IgD

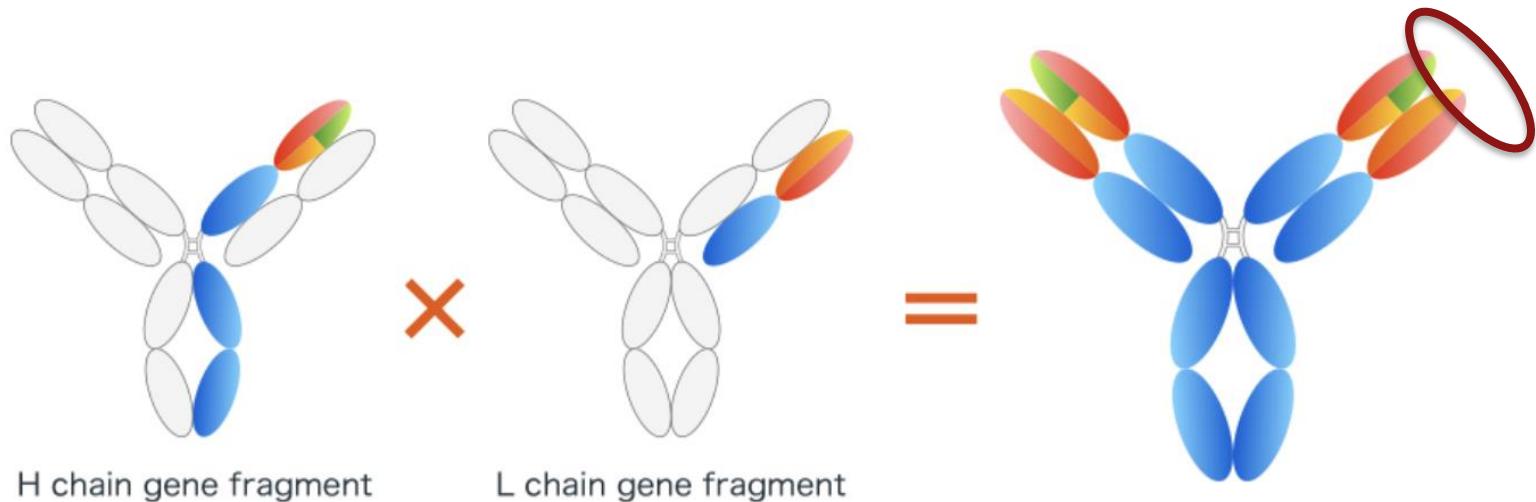
- present on the surface of B cells (poorly understood)
- plays a role in the induction of antibody production and the prevention of respiratory tract infections

IgE

- originally related to immune reactions to parasites
- involved in **allergies** when bound to mast cells
- bound to Fc receptors on mast cells, basophils and eosinophils

IgGs are the most popular drug form

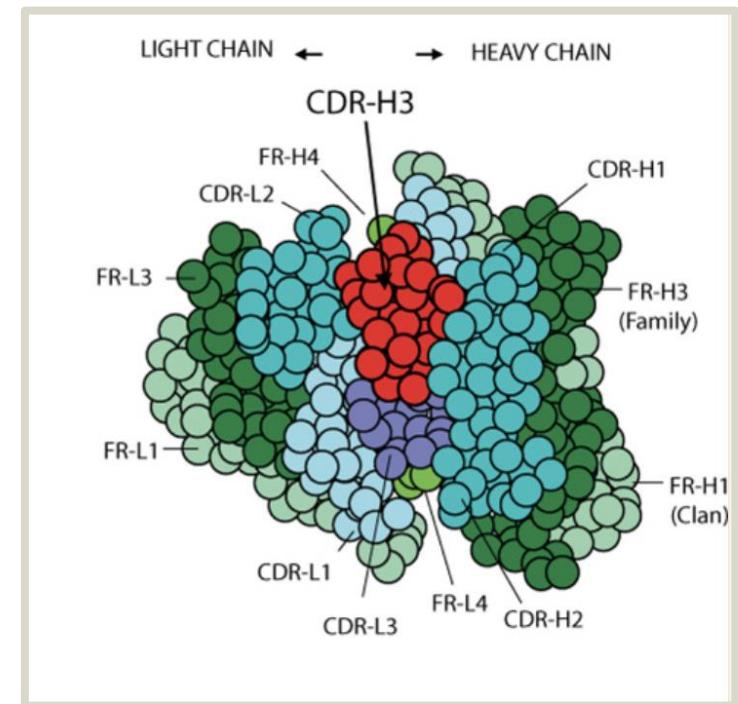
Antibody antigen recognition and diversity



3 Heavy chain CDRs

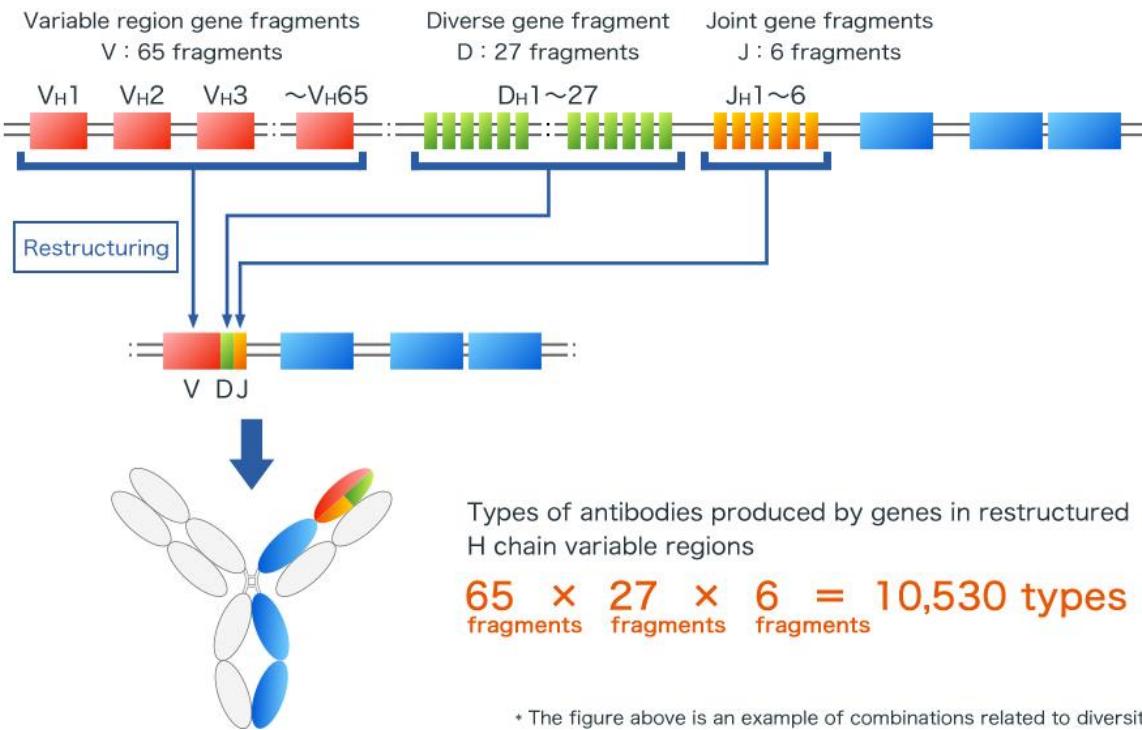
3 Light chain CDRs

9 total CDRs
x
2 identical arms

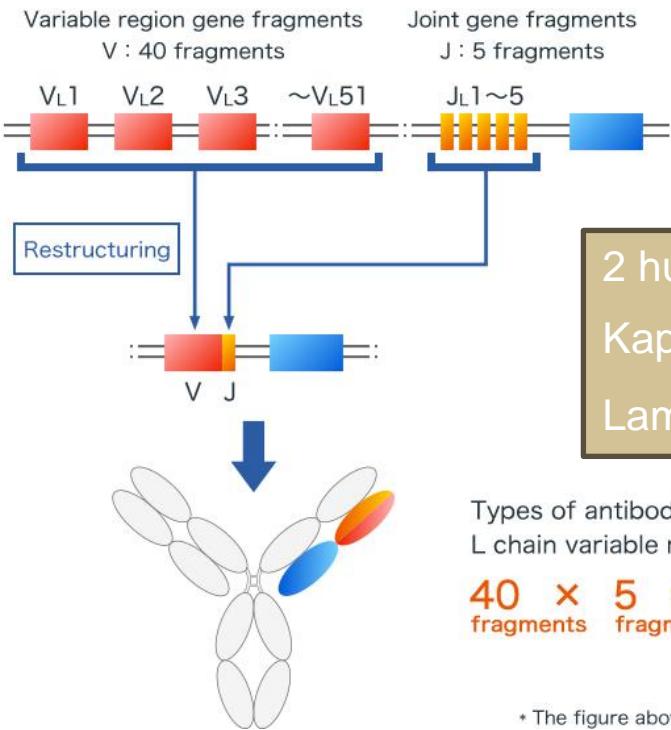


Complementarity Determining Regions (CDRs):
Highly variable loop regions in an antibody

Antibody diversity in the variable domains



* The figure above is an example of combinations related to diversity



2 human light chain loci:
Kappa: κ
Lamba: λ

* The figure above is an example of combinations related to diversity

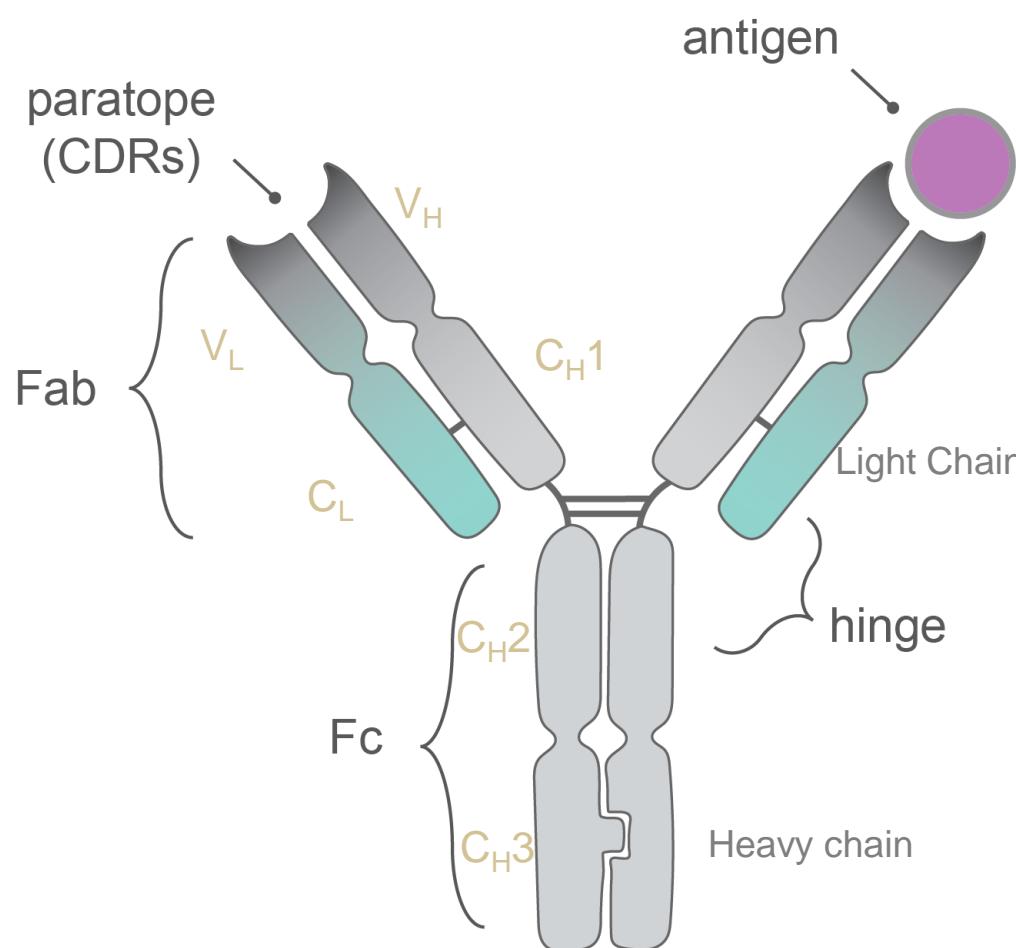
Naïve Diversity = 2×10^6 antibodies

Plus junctional diversity and somatic mutation

= Huge diversity

Synthetic libraries
can be even larger

Antibodies have defined structure and multiple functions



Two heavy chains and two light chains attached at the “hinge”: a dimer of dimers

Antigen: the target of an antibody

Epitope: the surface on the antigen bound by an antibody

Constant domains: Scaffold of the antibody, defines the isotype.

Heavy chain: (CH1, CH2, CH3)

Light chain: (CL)

Variable domains (VH and VL): Together, comprise the paratope that binds the antigen.

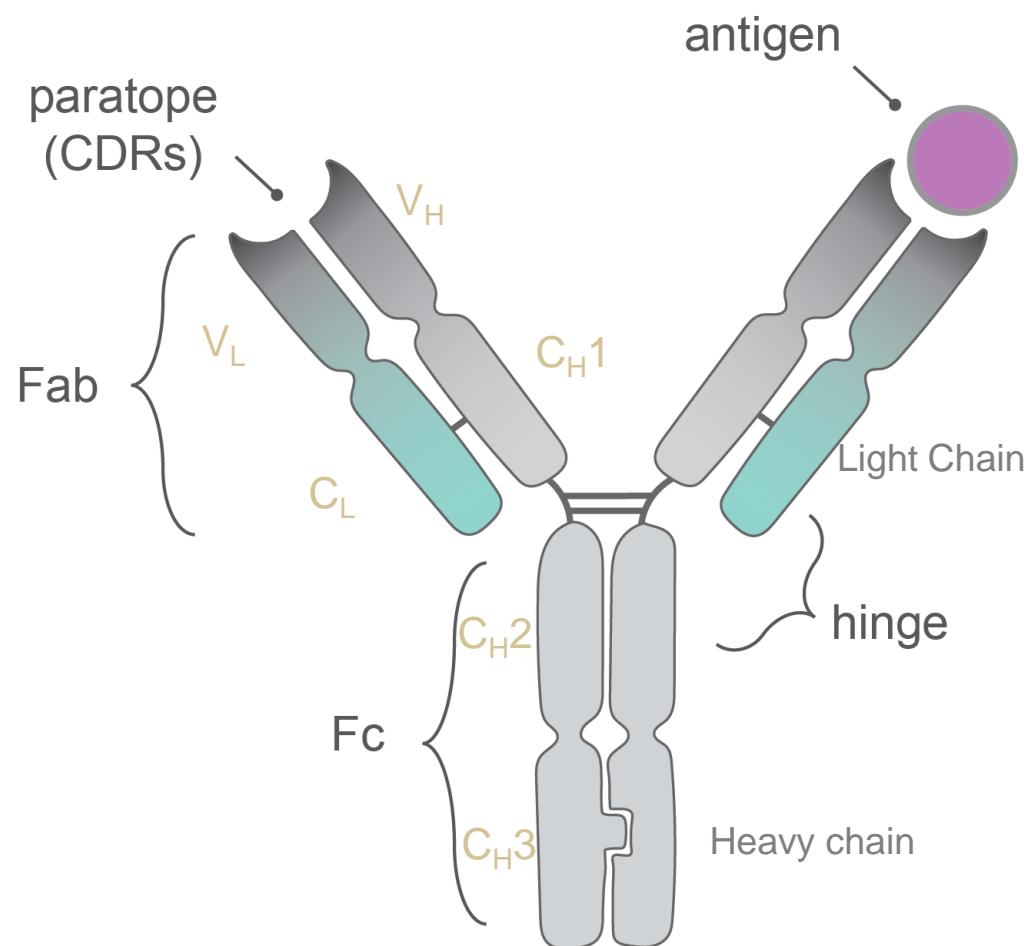
Complementarity-determining region (CDR): loops in the variable domains (three each in VH and VL) that dictate antigen binding

Fc: Part of the heavy chain constant region comprising the hinge, CH2, and CH3 domains

Effector function: triggered by interaction of cell receptors with Fc and leads to activation of immune cells and clearance of pathogenic species (can be enhanced or attenuated by mutations in the Fc)

FcRn: receptor found on many cell types responsible for recycling of antibodies, leading to their long retention *in vivo*

Antibodies have defined structure and multiple functions



Two heavy chains and two light chains attached at the “hinge”: a dimer of dimers

Species: The origin of the antibody sequence
(Human, rabbit, mouse, goat...)

Specificity: The target species
(anti-human, anti-mouse, cross-reactive...)

Polyclonal: A collection of heterogeneous antibodies to the target

Monoclonal:(mAb) A single homogeneous pool of antibodies to the target

Bispecific: An antibody that binds to more than one target

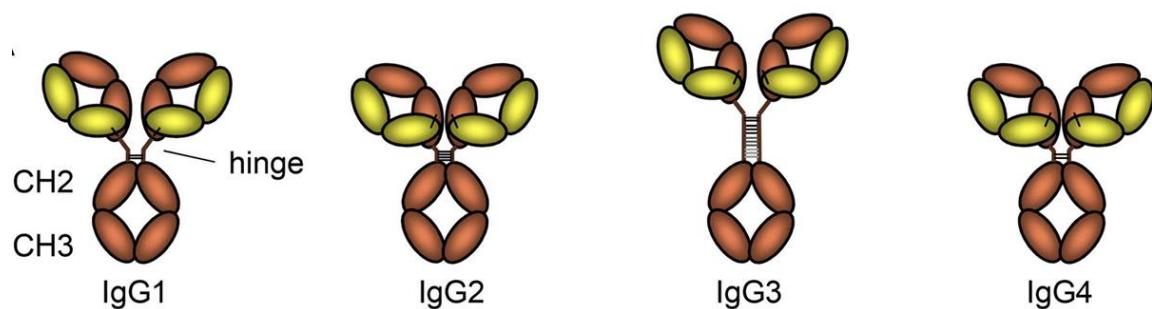
Isotype or antibody class: IgG, IgA, IgD, IgE, IgM
See class-switching.

Heavy Chain isotype/class: IgG1, IgG2, IgG3, IgG4

Light Chain isotype/class: Two in humans- Kappa and Lambda

Allotype: Genetic diversity inside isotypes in the human population that results in a small number of amino acid changes to the constant region.
- Varies depending on the human population

Antibody IgG Isotypes have different Fc domain functionality

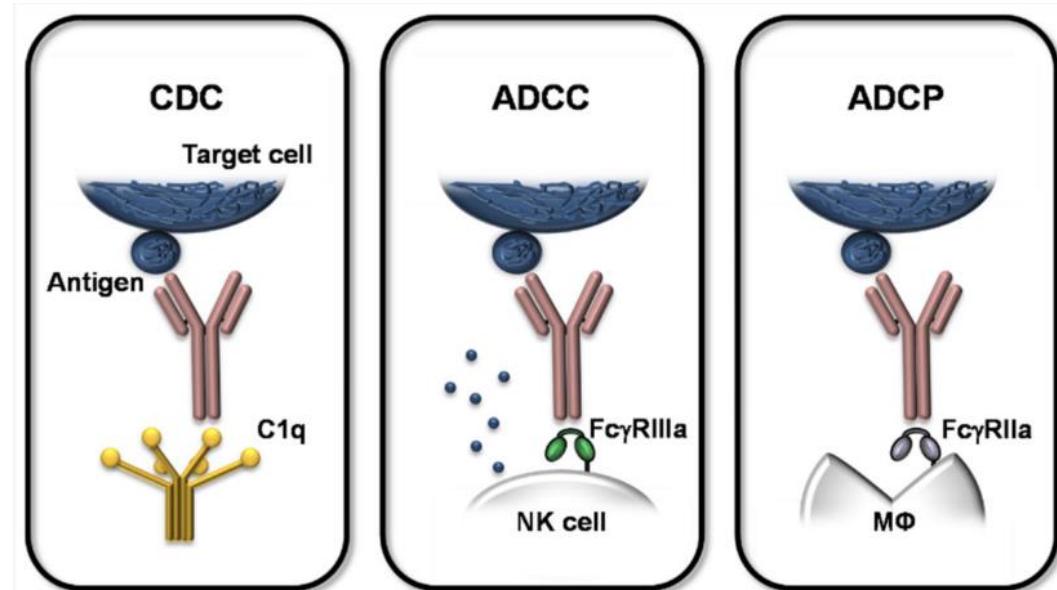


	IgG1	IgG2	IgG3	IgG4
Serum levels (mg/ml)	6.98	3.8	0.51	0.56
C1q (CMC)	++	+/-	+++	-
ADCC (Fc _y IIIa)	+++	+/-	+++	+/-
ADCP (Fc _y IIa)	+++	+	+++	++

** No standard for strength of responses.

Three ways to engage the immune system:

- CDC - Complement-dependent cytotoxicity
- ADCC - Antibody-dependent cellular cytotoxicity
- ADCP - Antibody-dependent cellular phagocytosis



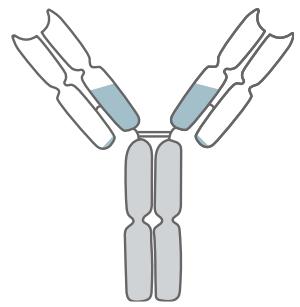
Ig1, Ig3, IgM, (IgG2)

Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol.* 2014 Oct 20;5:520. doi: 10.3389/fimmu.2014.00520. PMID: 25368619; PMCID: PMC4202688.

Gogesch P, Dudek S, van Zandbergen G, Waibler Z, Anzaghe M. The Role of Fc Receptors on the Effectiveness of Therapeutic Monoclonal Antibodies. *Int J Mol Sci.* 2021 Aug 19;22(16):8947. doi: 10.3390/ijms22168947.

Pick your Scaffolds: Antibodies and antibody-like molecules

Antibody and Antibody fragments



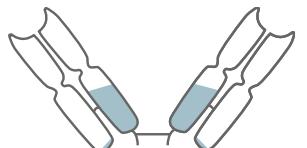
Standard Antibody



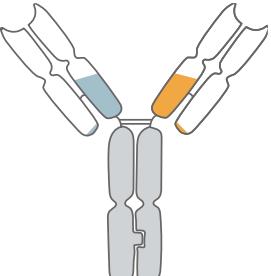
scFv



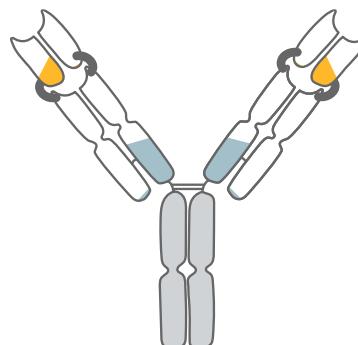
BiTE – bispecific T-cell engager



F(ab')2



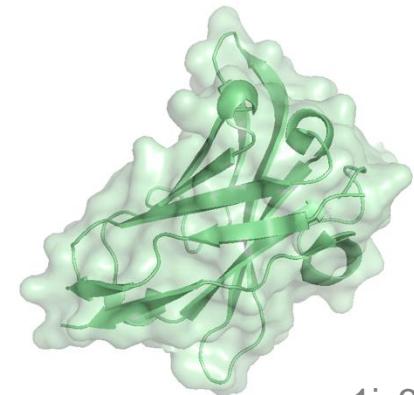
Bispecific antibody



DVD-Ig



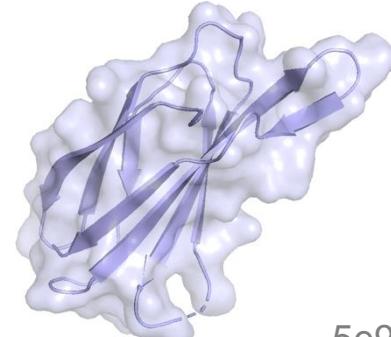
Camelid domain
VHH or Nanobody



1iv3.pdb

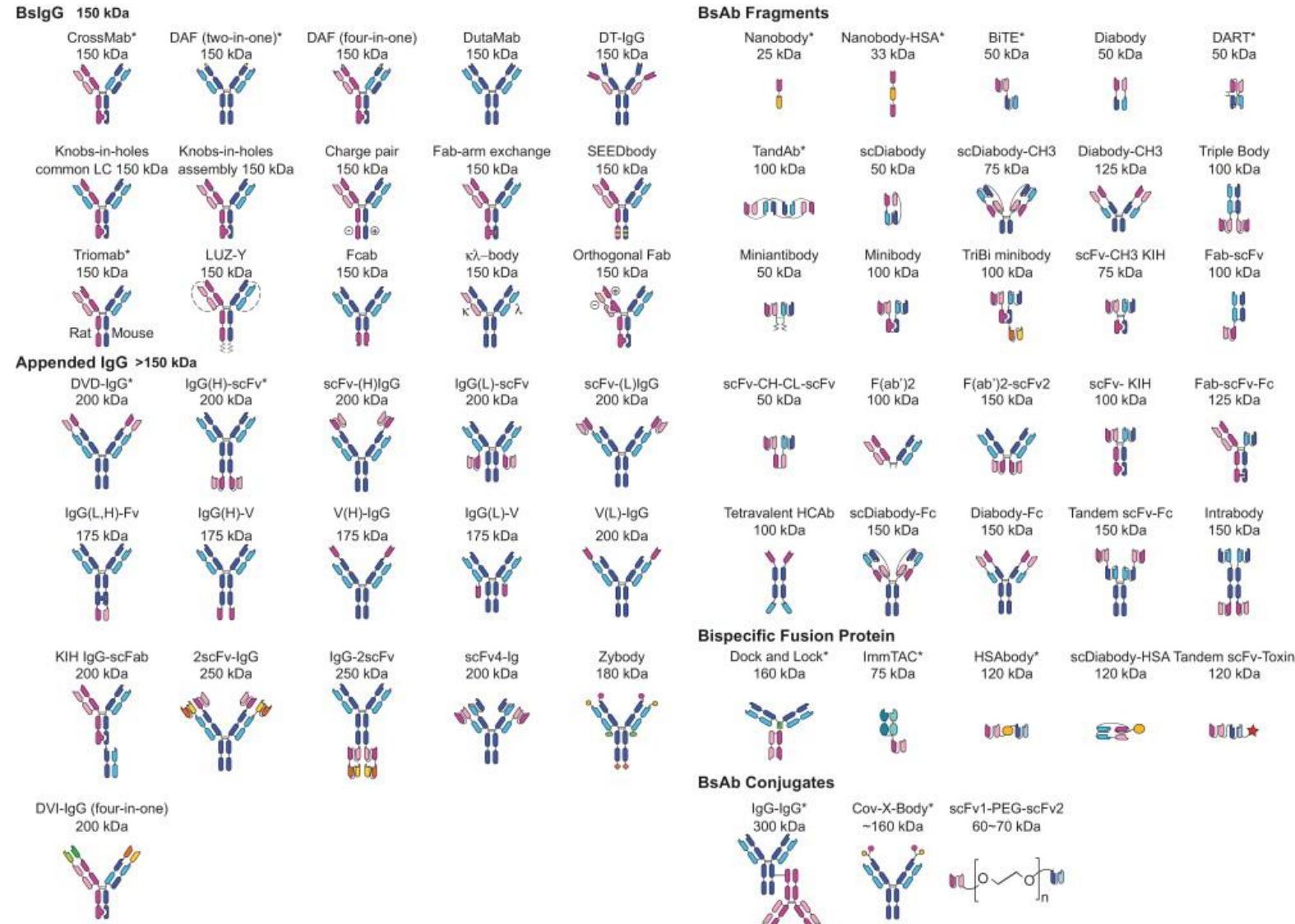


FNIII Monobody
adnexin



5e95.pdb

Many different antibody formats are possible

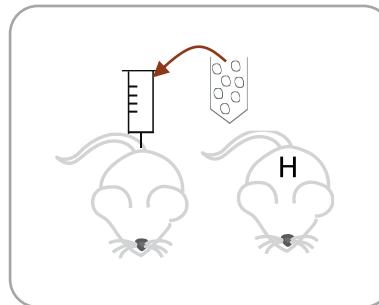


But may not be wise...

Why are antibodies the large molecules of choice?

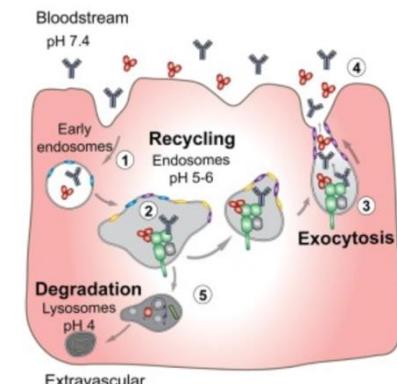
Antibody discovery can be done with **animal immunization**

- High specificity and affinity from natural diversity



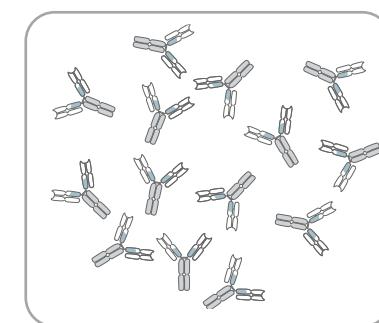
Fc domain binding to FcRn receptor **increases drug half-life**

- Protein A binding at the same site allows for purification



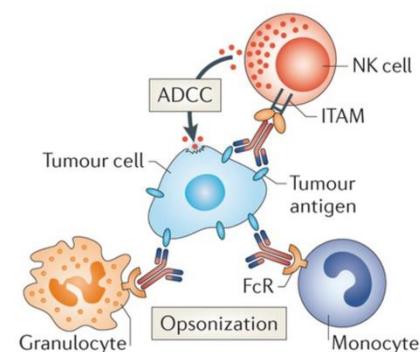
Humanized antibodies have **low immunogenicity**

- Blood concentrations of globulins are high (25 – 25 mg/ml)
- Antibodies naturally have variable CDRs



Engagement of **immune system** via Fcγ receptors and C1q

- C1q can increase Increased potency/mechanism of action.
- Mutations can “knock-out” these functions if desirable.





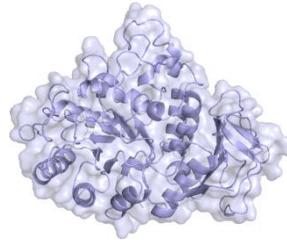
Hit Finding

What do you need for antibody discovery?

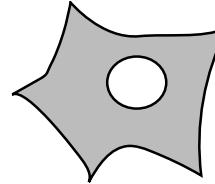
Hit Finding

Antigen:

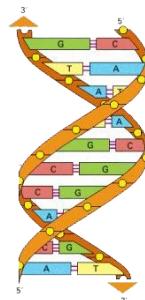
- Recombinant protein
- Cells with surface expression
- Target DNA



or



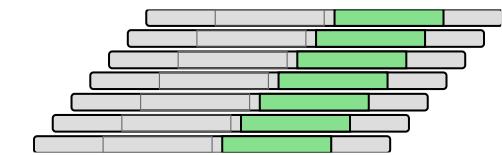
or



Crystal structures
are very helpful!

Library of proteins:

- Hybridoma/B-cell technology:
or
- Synthetic protein library:



Binding assays:

- Flow cytometry
- ELISA

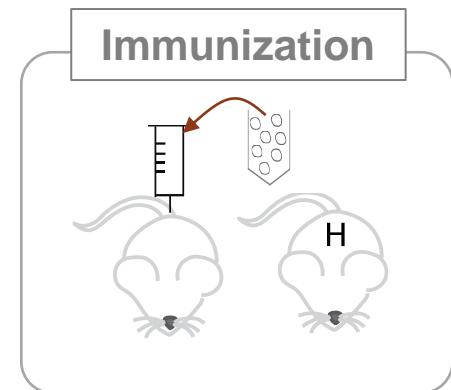


Functional readouts:

- Cell signaling
- Cell death
- Immune cell recruitment

Antibody hit generation by immunization

Hit Finding

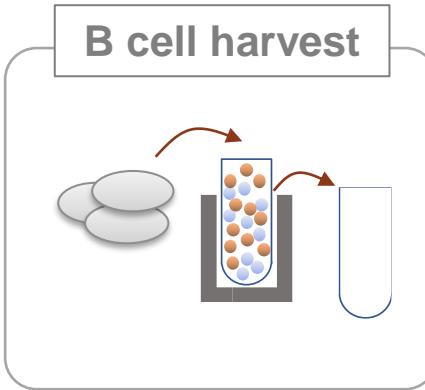


Antigen design

- Recombinant proteins
- Peptides, VLPs, Liposomes
- Whole cells
- Target DNA

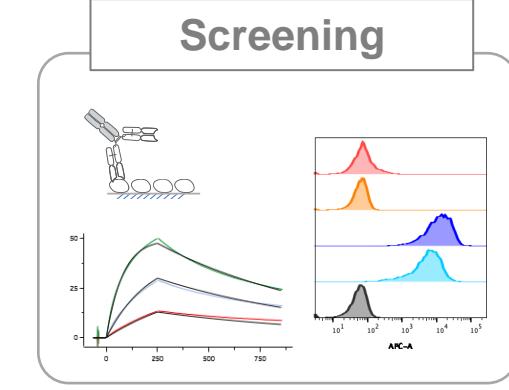
Strategy

- Animal strain
- Adjuvants
- Boost schedule



Screening platform

- Hybridoma
- B-cell cloning
- Immune library (phage/yeast)



Primary screen

- ELISA
- Flow-based B-cell selection (usually binding-based)

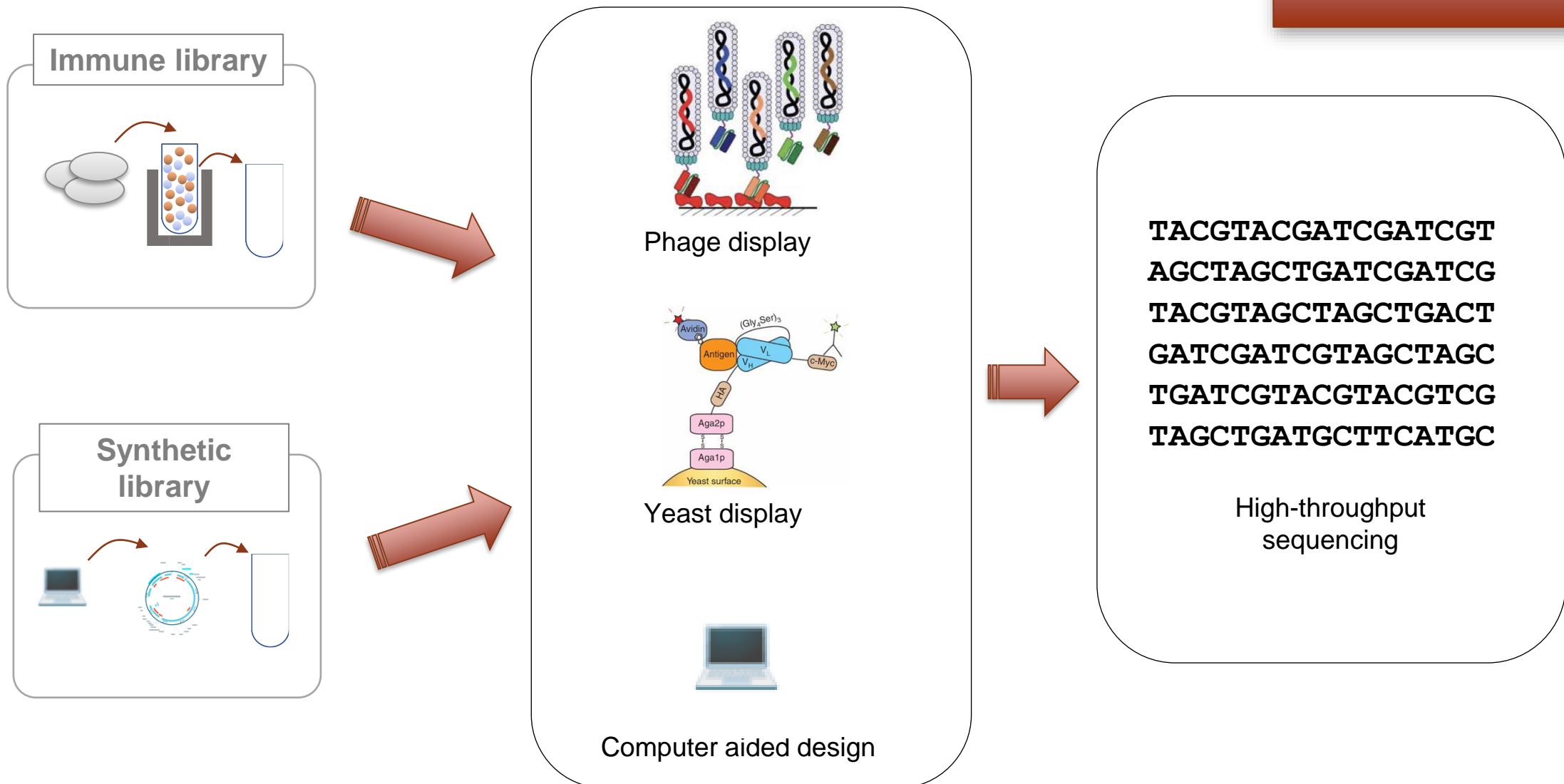
Secondary screen(s)

- Often functional
- Cell-based
- On recombinant mAbs

Teams work closely with Target Biology, Protein Sciences and Protein Engineering

Antibody libraries screened by surface display

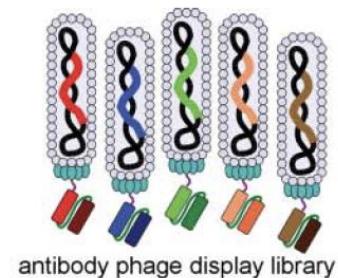
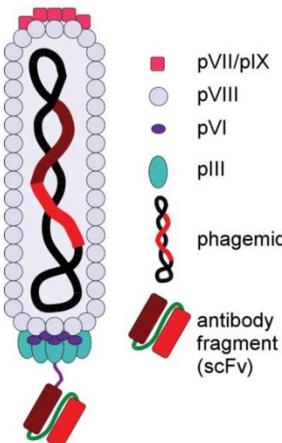
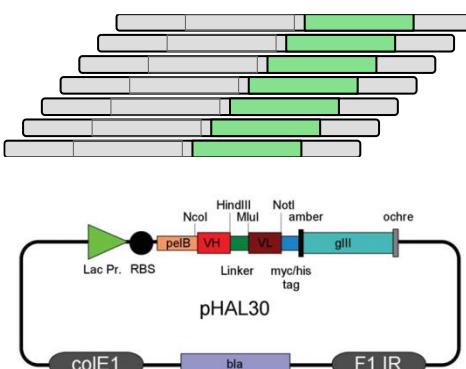
Hit Finding



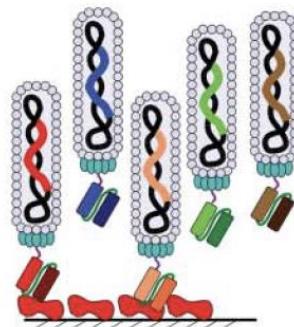
Adapted from Chao G, et al. Nat Protoc. 2006;1(2):755-68. doi: 10.1038/nprot.2006.94..

Phage display: library of your protein on a bacterial virus

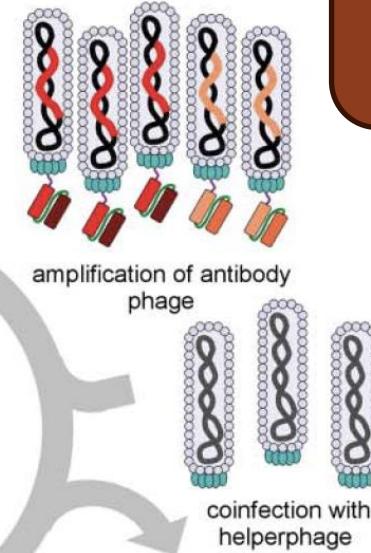
DNA library of
~ 1 trillion variants



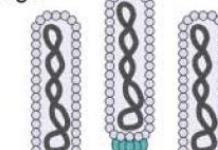
antibody phage display library



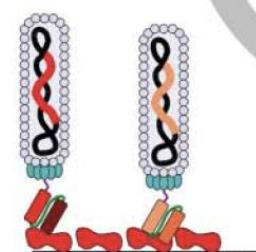
surface immobilized antigen.
During *in vitro* selection, the panning conditions (e.g. pH, temperature, competitors, co-factors...) can be controlled and adapted to the final antibody application



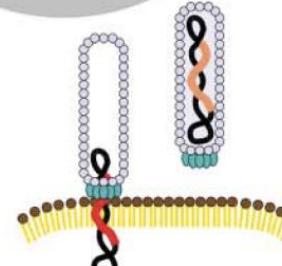
amplification of antibody phage



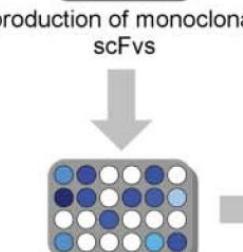
coinfection with helperphage



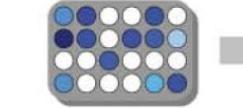
Removal of non and unspecific binders by washing under controlled conditions



elution of antibody phage and reinfection of *E. coli*



production of monoclonal scFvs



identification of monoclonal binders by ELISA

Humira was one of the first phage display derived antibodies

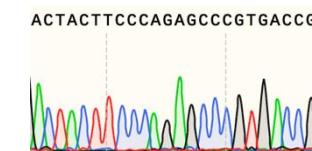
Advantages:

- Large library sizes 1×10^{11} - 1×10^{13} members
- Screen fully humanized synthetic libraries
- Total screening 1-2 weeks

Disadvantages:

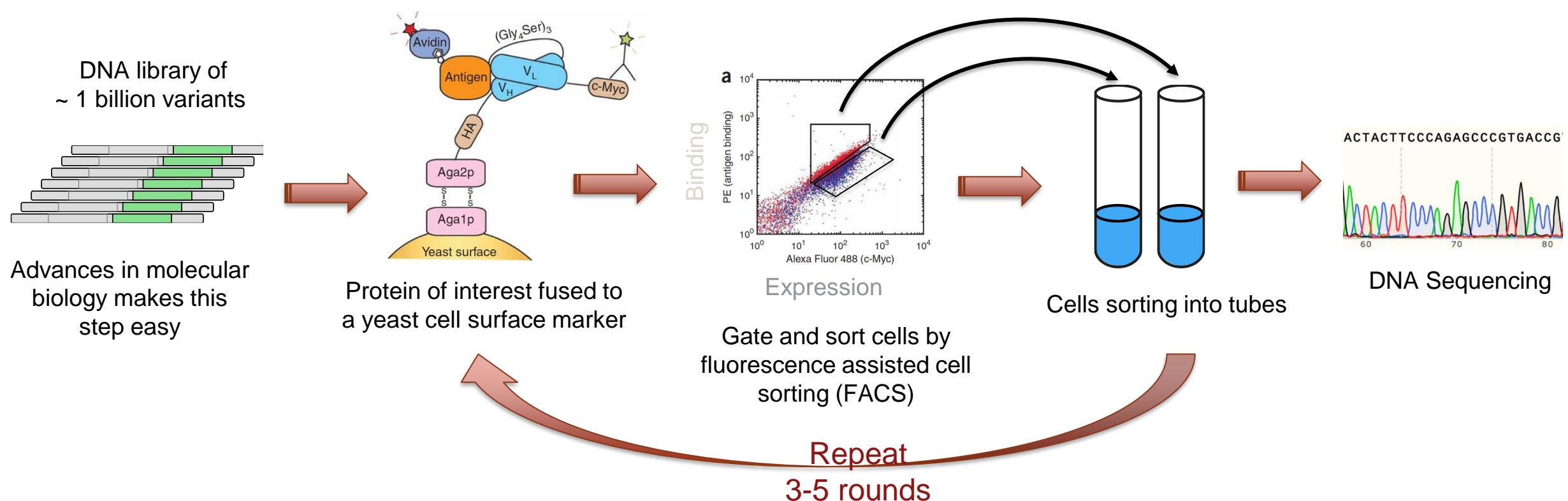
- Protein production in a prokaryote (*E. coli*)
- Phage bodies can affect activity/functional screens

Frenzel A, Schirrmann T, Hust M. Phage display-derived human antibodies in clinical development and therapy. MAbs. 2016 Oct;8(7):1177-1194.



DNA Sequencing

Yeast Display: Library of your protein on the yeast cell surface



Advantages:

- Yeast can produce proteins similar to mammalian cells
- Select for binding to more than one antigen simultaneously
- Can estimate binding affinity during selection

Disadvantages:

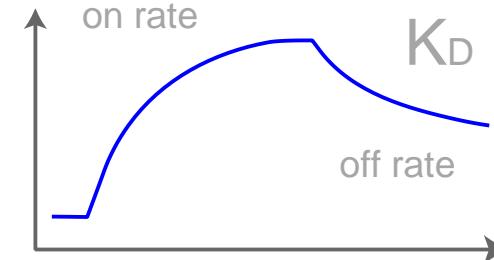
- Library sizes of only 1×10^9
- Screening total: ~2 weeks (3 days for each round.)

Adapted from Chao G, et al. Nat Protoc. 2006;1(2):755-68. doi: 10.1038/nprot.2006.94..

Understanding the properties of hits

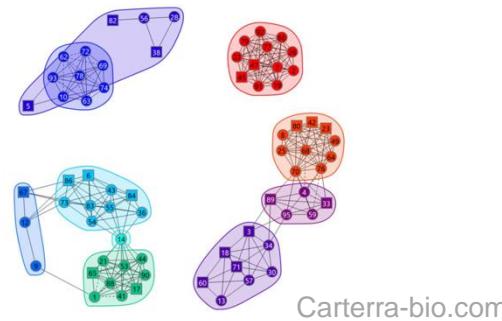
Affinity: How tightly does the purified drug bind to target?

- ELISA: (enzyme-linked immunosorbent assay)
- SPR: (surface plasmon resonance) / BLI (bio-layer interferometry)
- **Cross-reactivity** – species specificity (mouse, monkey, human)



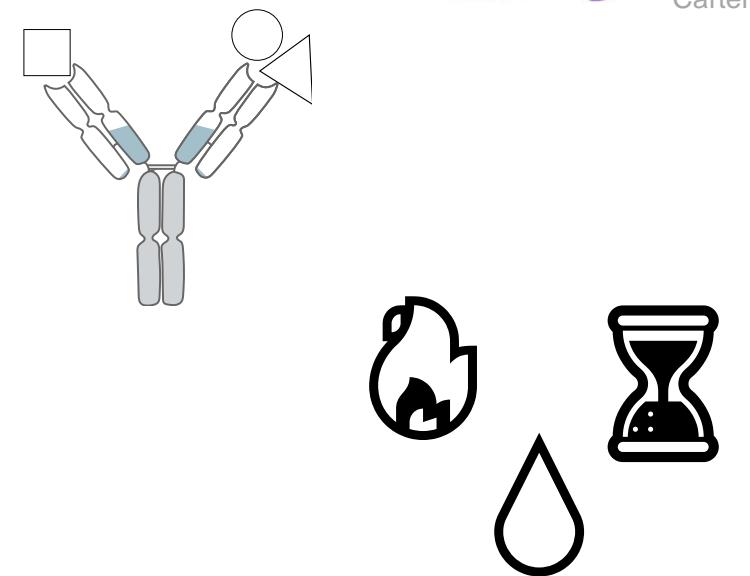
“Binning” - Map where the drug binds to the target

- Epitope matters and can narrow down lead finding efforts.
- High-resolution structure determination of co-complex



Off-target binding: What else does your drug binding to?

- BV ELISA (baculovirus), non-specific binding assays
- Wild-type mouse Pharmacokinetics



Developability: Can your drug be manufactured?

- Stability, aggregation, post-translational modifications
- Formulation: What solution keeps your drug stable?

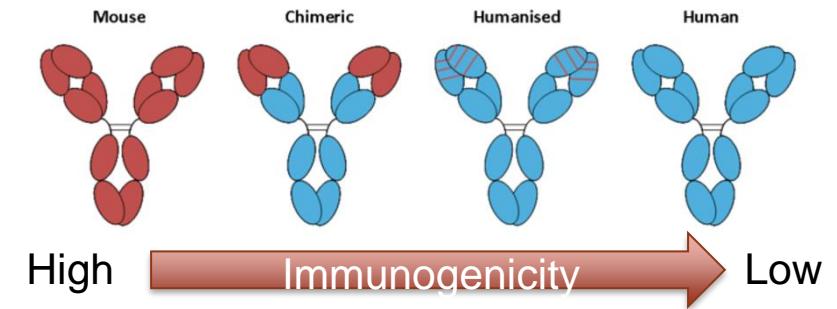


Lead Optimization

Iterate on the design if needed

Affinity maturation/
Lead optimization

Humanization: increases the number of residues found in the human antibody repertoire while maintaining the **same binding properties as the original molecule.**

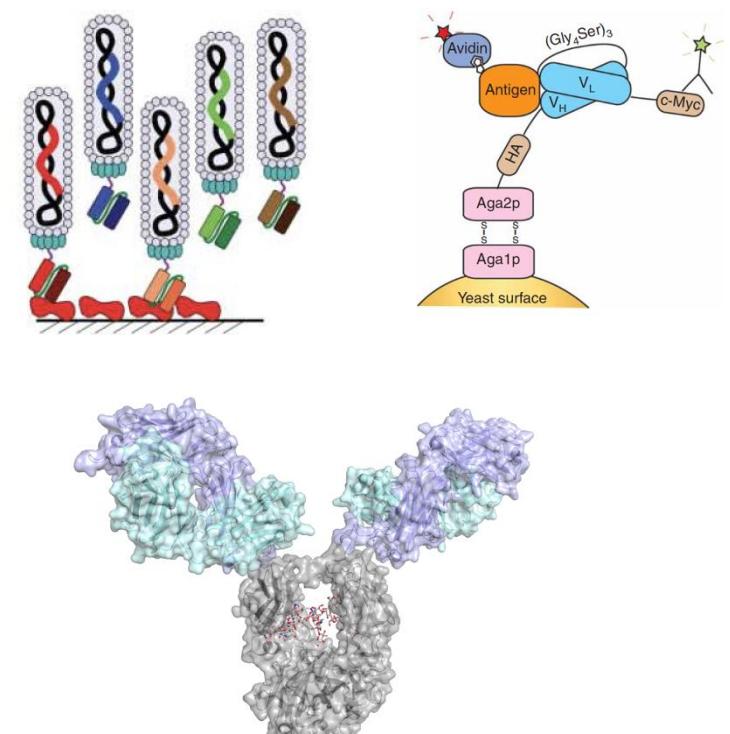


Affinity maturation: improving the affinity to the target

- **Cross-reactivity:** Mouse, Monkey, Human
- Yeast display
- Phage display
- Rational design – computational/AI

Format:

- Effector function/complement:
 - IgG1, IgG2, IgG3, IgG4
 - Non-natural blocking mutations – “LALA” “PG” “PS”
- Valency/bispecific
- Half-life modifying mutations – “YTE” “LS”



DCP: DRUG CANDIDATE PROFILE

A list of requirements for your therapeutic

- ✓ Disease indication
- ✓ Therapeutic hypothesis
- ✓ Therapeutic modality
- ✓ Therapeutic proof of concept study
- ✓ In vitro potency
- ✓ Selectivity
- ✓ In vivo efficacy
- ✓ DMPK
- ✓ Safety
- ✓ Biomarker strategy

EXAMPLE

Therapeutic hypothesis	Blocking this target will reduce the rate of disease progression
Therapeutic PoC	Administration of drug will reduce the rate of disease progression
Modality	Small molecule, large molecule, gene therapy
Stability	Melting temperature, purity, other analytical properties
In vitro activity	Assessment of ability to bind well-established target
Cellular activity	Rescue of known genetic pathway marker
In vivo distribution and efficacy	What correction / increase / decrease/ rescue would you like to see in which animal models?
Administration and clinical efficacy	Weekly infusion; clinical efficacy assessed by correction of increased brain level of disease marker
Safety profile	Assess tumorigenic potential (chronic)

Compounds fail in development for many reasons

Off-target toxicity: Toxicity can arise at any stage of preclinical or clinical development and can be difficult to predict with preclinical systems.

On-target toxicity: Drugging a given target is itself toxic, with limited or no therapeutic window

Poor Pharmacokinetics (PK): Human PK is different than what was predicted by projection of PK in preclinical species

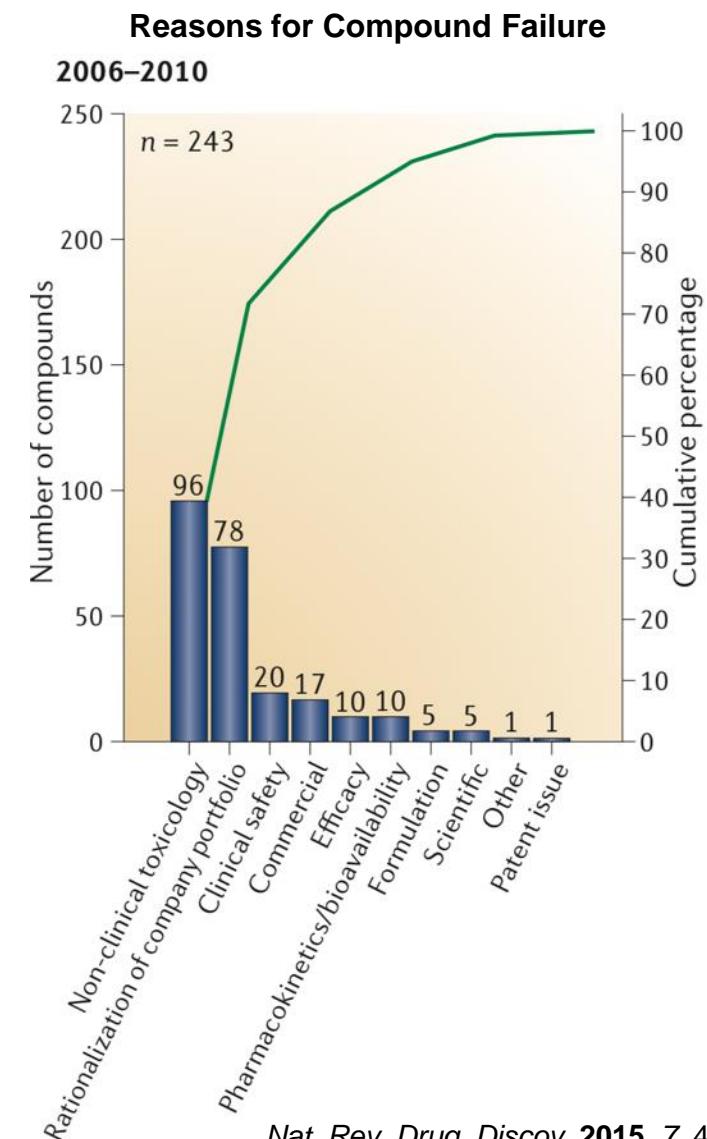
Efficacy: Drugging a given target does not modulate disease as expected

- Especially true in the neurodegeneration space

Formulation: A given compound cannot be formulated to deliver an efficacious dose

IP and other reasons: May not be able to own the compounds you develop or other undisclosed business reasons

Pharmacokinetics – the process of uptake, distribution, metabolism and elimination of a drug overtime in the body.



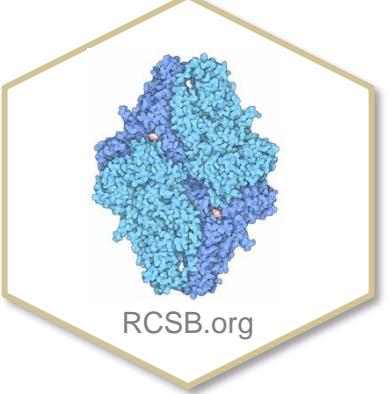
Nat. Rev. Drug. Discov. 2015, 7, 475

Pick your Modality: Large molecules

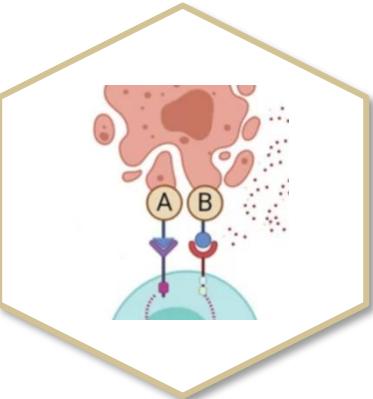
Other modalities



Vorob'yeva et al, 2019



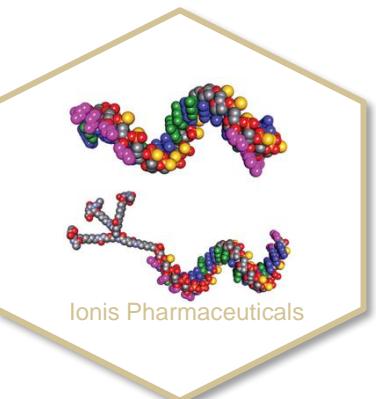
RCSB.org



Non-antibody
scaffolds

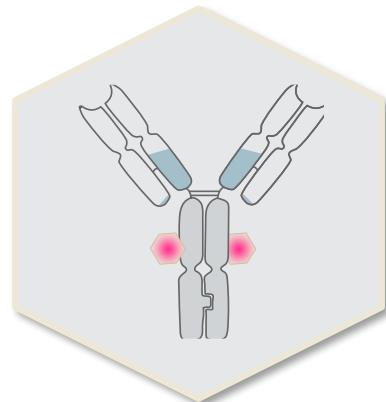
Protein/enzymes

CAR T /Cell
immunotherapy

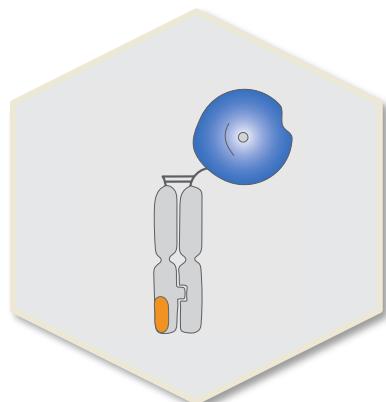


Nucleotides

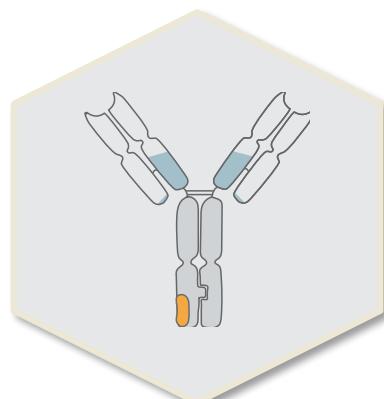
Large molecule fusions



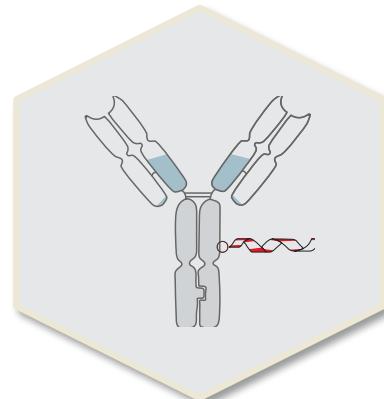
ADC



Protein Fusions



Modified Fc

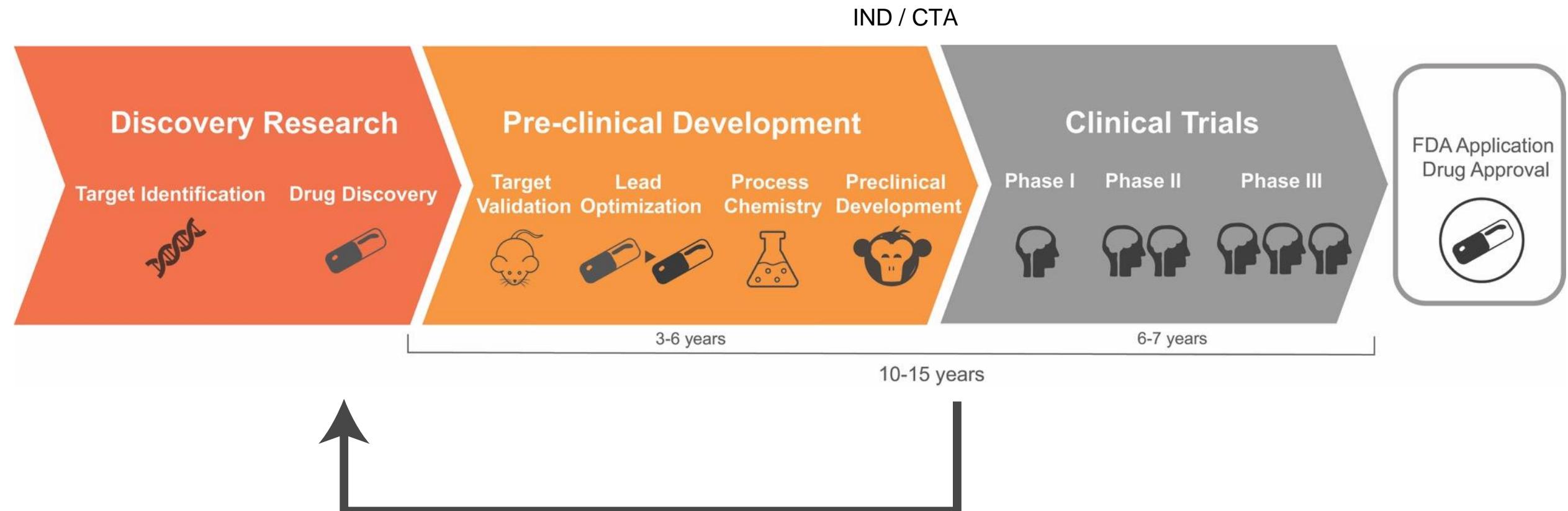


Nucleic acid
conjugates

Viral Vectors

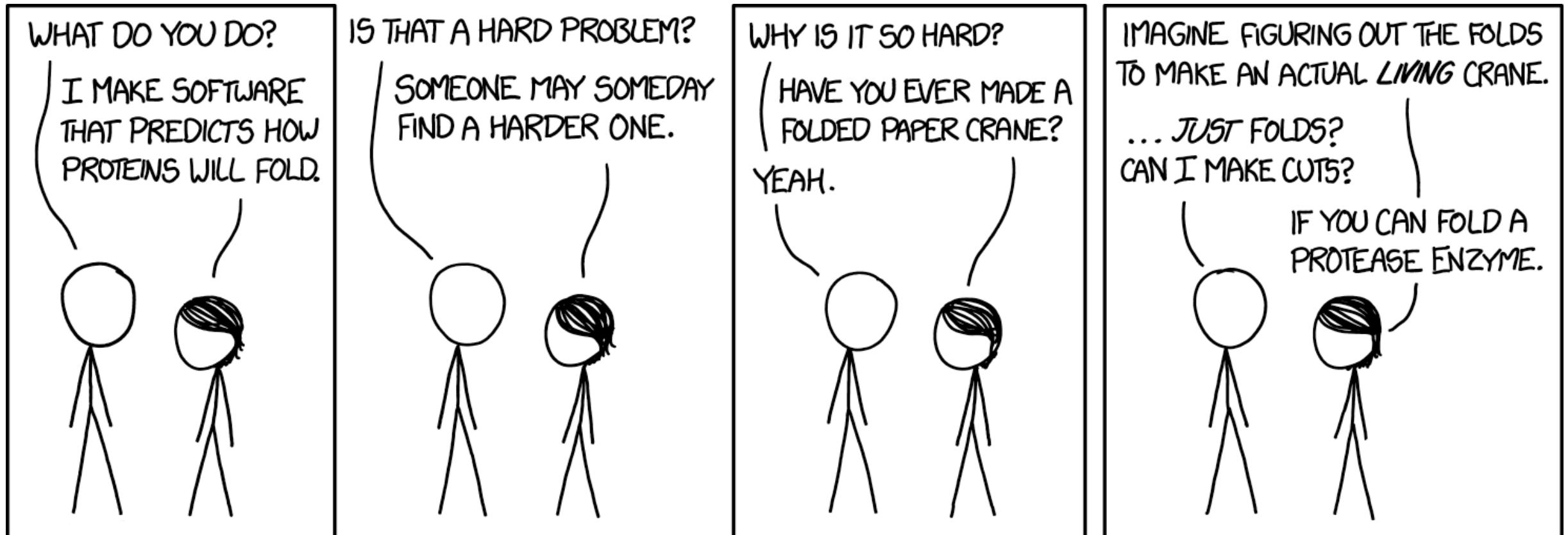
THE DRUG DEVELOPMENT PROCESS (simplified)

Lead ID



You can iterate until you have a design that works!

QUESTIONS FOR THIS SECTION...



xkcd.com

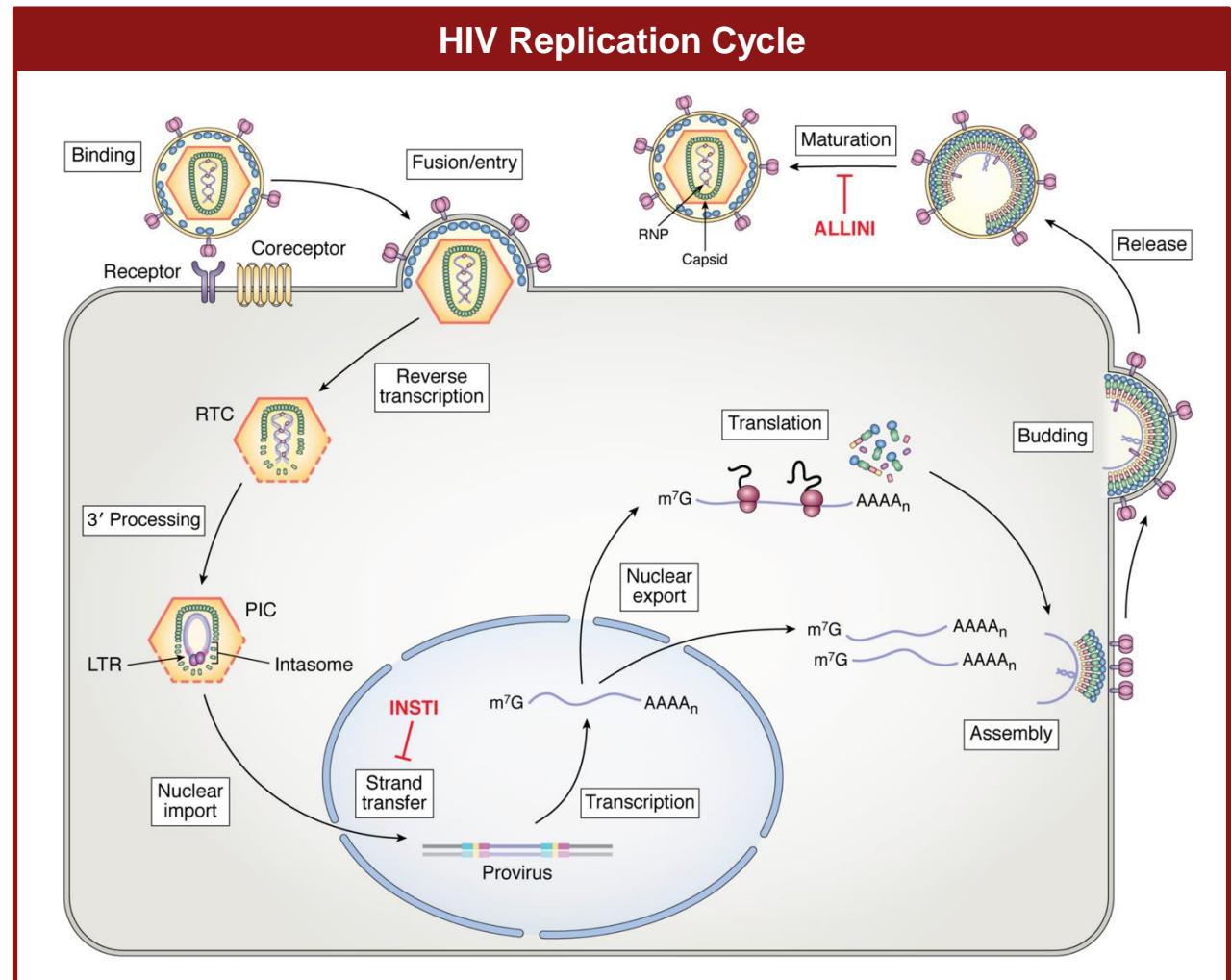


Case Studies: Small Molecules

Case Study 1: Raltegravir For The Treatment Of HIV

Target Identification: Inhibition of integrase can serve as an HIV therapeutic.

- Each step of the HIV replication cycle offers an opportunity for therapeutic intervention
- Typically, multiple mechanisms are targeted as part of HAART regimen
- HIV-1 integrase **catalyzes the insertion of the viral DNA into the cellular genome** of the host cell through a multistep process that includes two catalytic reactions: 3' endonucleolytic processing of the viral DNA ends and strand transfer, i.e., joining of the viral and cellular DNAs

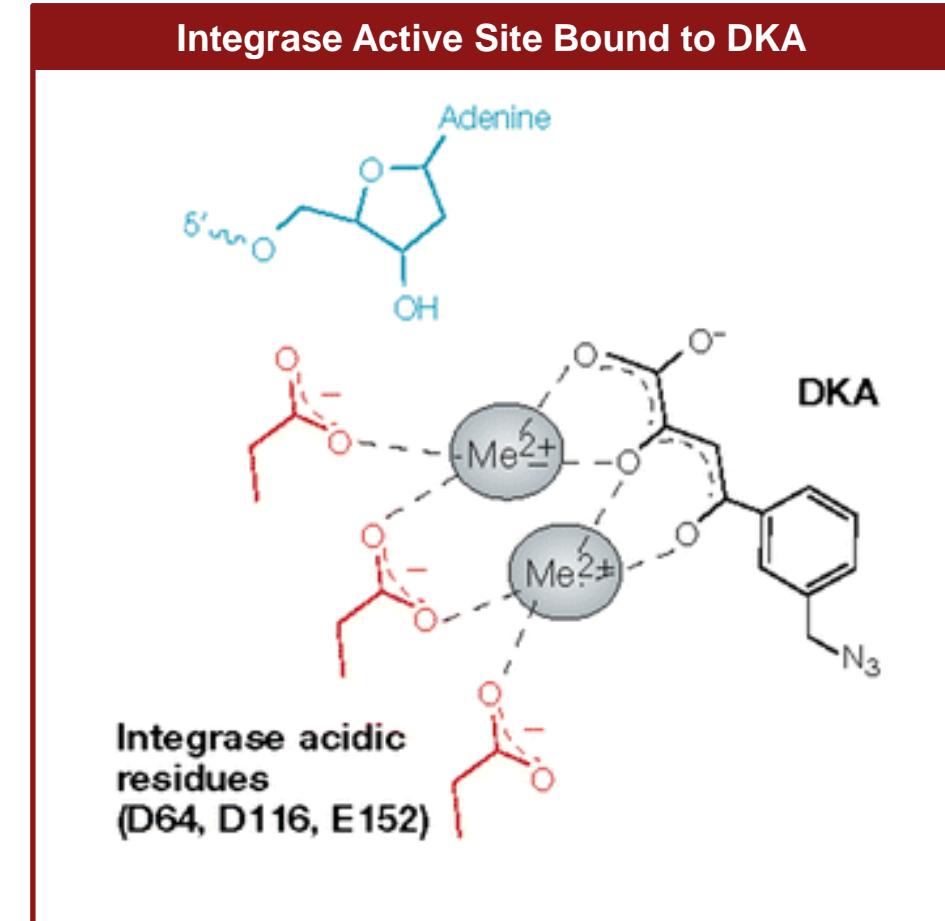
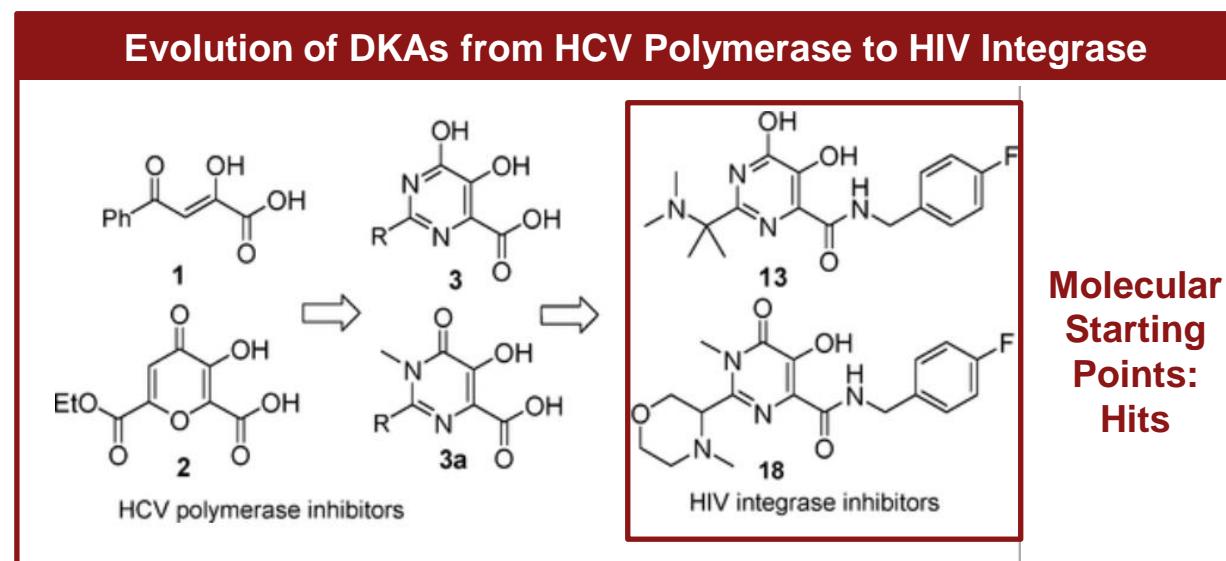


^a J. Biol. Chem. 2019, 294, 15137. ^b Nat. Rev. Drug Disc. 2005, 4, 236

Case Study 1: Raltegravir For The Treatment Of HIV

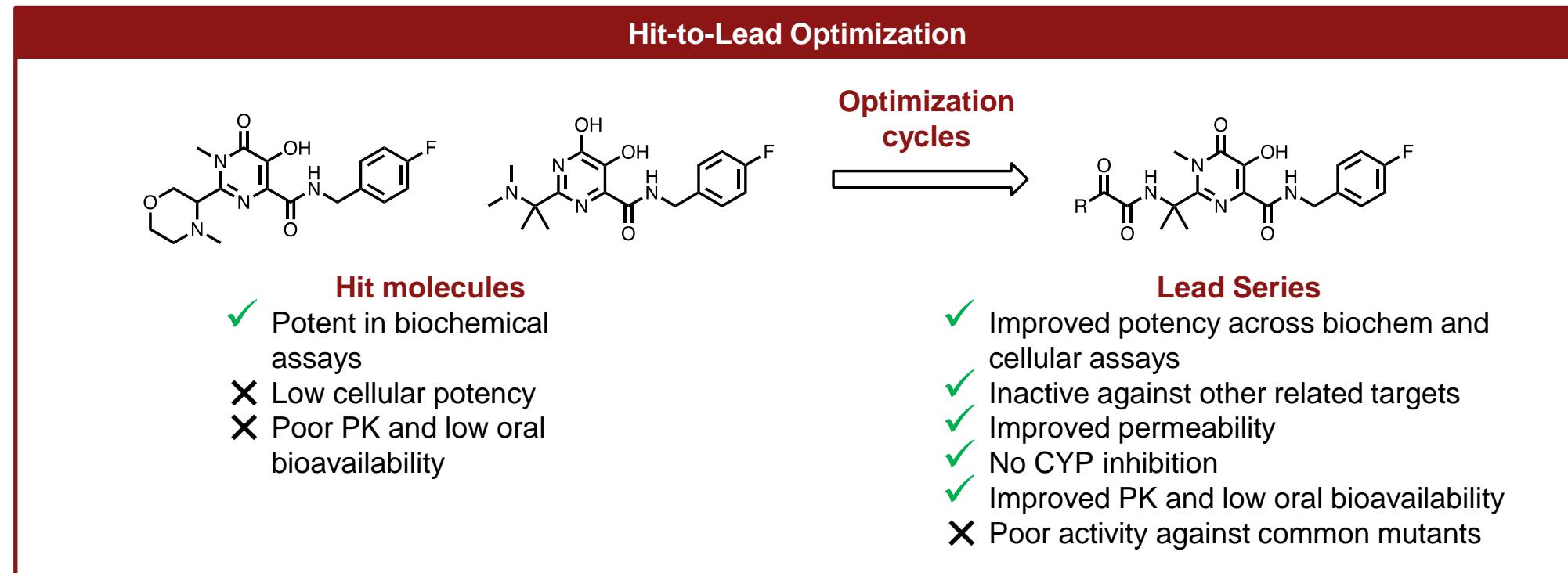
Hit Finding:

- 4-Aryl- 2,4-diketobutanoic acids (DKA) previously shown to be integrase inhibitors in cellular assays
- DKAs also NS5b RNA-dependent RNA polymerase inhibitors: Merck also had a program on this target: **use lead molecules from another program as a starting point**



^a *J. Biol. Chem.* **2019**, *294*, 15137. ^b *Nat. Rev. Drug Disc.* **2005**, *4*, 236 ^c *J. Med. Chem.* **2006**, *49*, 6646

Case Study 1: Raltegravir For The Treatment Of HIV



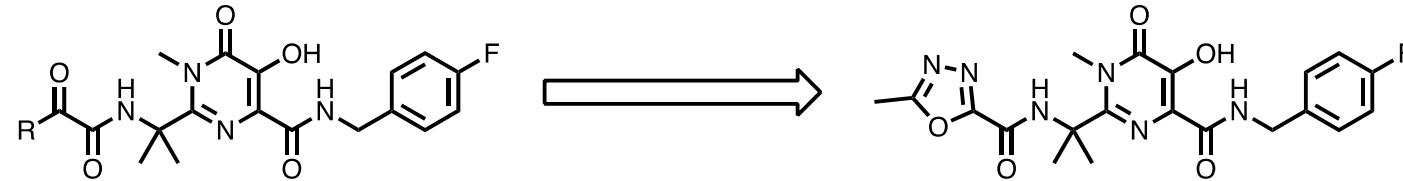
Combination of structure-based and ligand-based drug design used:

- HIV integrase structure known but without inhibitor in the active site
- Specifics not disclosed, but multiple ligand-based strategies appear to be used: pharmacophore modeling, scaffold hopping, library approaches

^a *J. Biol. Chem.* **2019**, 294, 15137. ^b *Nat. Rev. Drug Disc.* **2005**, 4, 236 ^c *J. Med. Chem.* **2006**, 49, 6646

Case Study 1: Raltegravir For The Treatment Of HIV

Lead Optimization



Lead Series

- ✓ Desirable overall profile for further optimization
- ✗ Moderate in vivo PK properties
- ✗ Poor activity against common HIV mutants

Candidate Molecule

- ✓ Further improved potency across biochemical and cellular assays
- ✓ No off-target activity risks
- ✓ No DDIs
- ✓ Excellent PK and oral bioavailability across preclinical species
- ✓ Excellent activity against common mutants

Raltegravir was the first integrase inhibitor approved for the treatment of HIV1



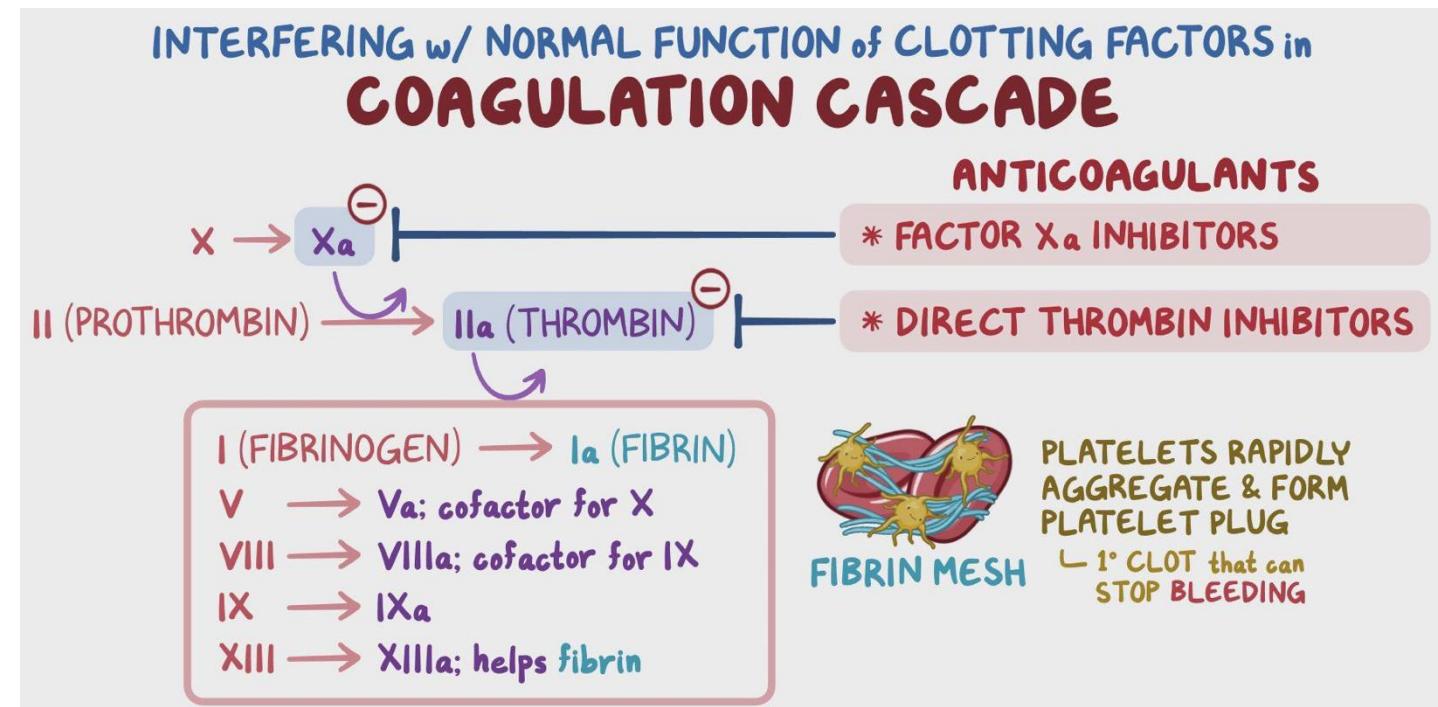
^a J. Biol. Chem. 2019, 294, 15137. ^b Nat. Rev. Drug Disc. 2005, 4, 236 ^c J. Med. Chem. 2006, 49, 6646

Case Study 2: Apixaban For The Treatment of Thrombosis

- Thrombosis is a leading cause of death worldwide
- Few safe antithrombotic agents exist due to bleeding risk

Target Identification: factor Xa (FXa) converts prothrombin to thrombin and is a viable strategy to treat thrombosis

- Reversible inhibition of thrombin formation carries low risk of bleeding relative to other approaches (Warfarin)



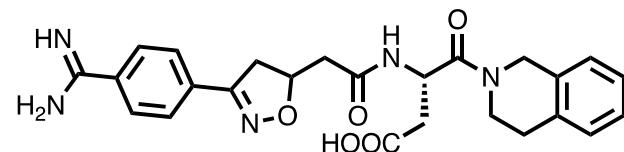
^a J. Med. Chem. 2007, 50, 5339 ^b J. Thromb Thrombolysis, 2011, 31, 478.

Case Study 2: Apixaban For The Treatment of Thrombosis

Hit Identification:

- GPIIb/IIIa receptor shares active site similarity with FXa
- BMS had an internal GPIIb/IIIa inhibitor program with a significant compound collection
- The internal collection of GPIIb/IIIa inhibitors was screened against FXa
- Identified a hit with weak inhibitory activity in biochemical assays

Internal library of GPIIb/IIIa inhibitors



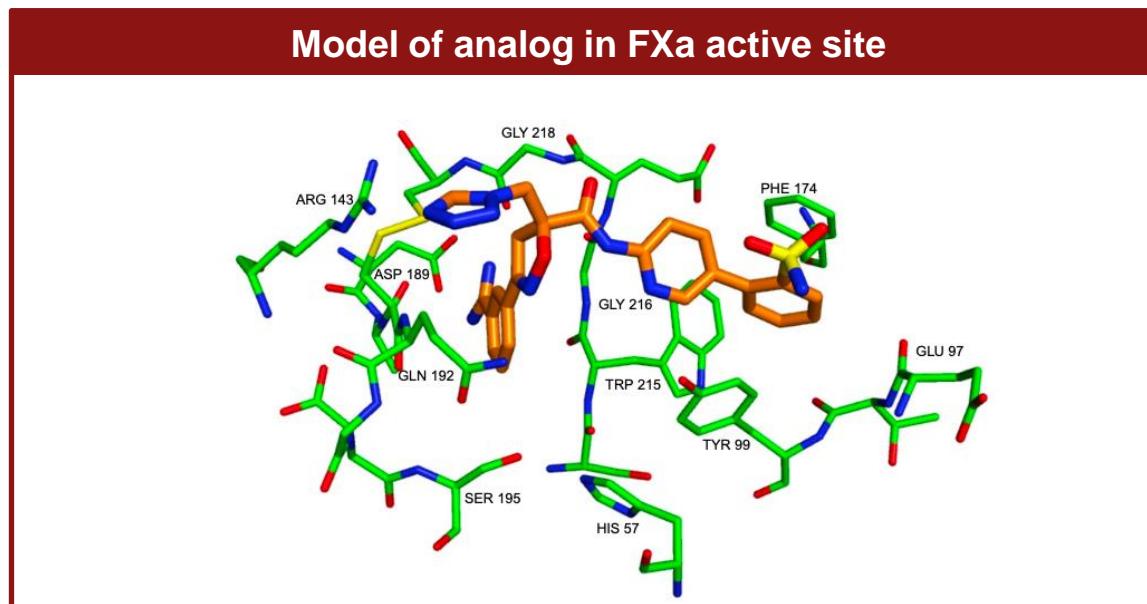
Screening Hit
 $K_i = 38.5 \mu\text{M}$

^a J. Med. Chem. 2007, 50, 5339 ^b J. Thromb Thrombolysis, 2011, 31, 478.

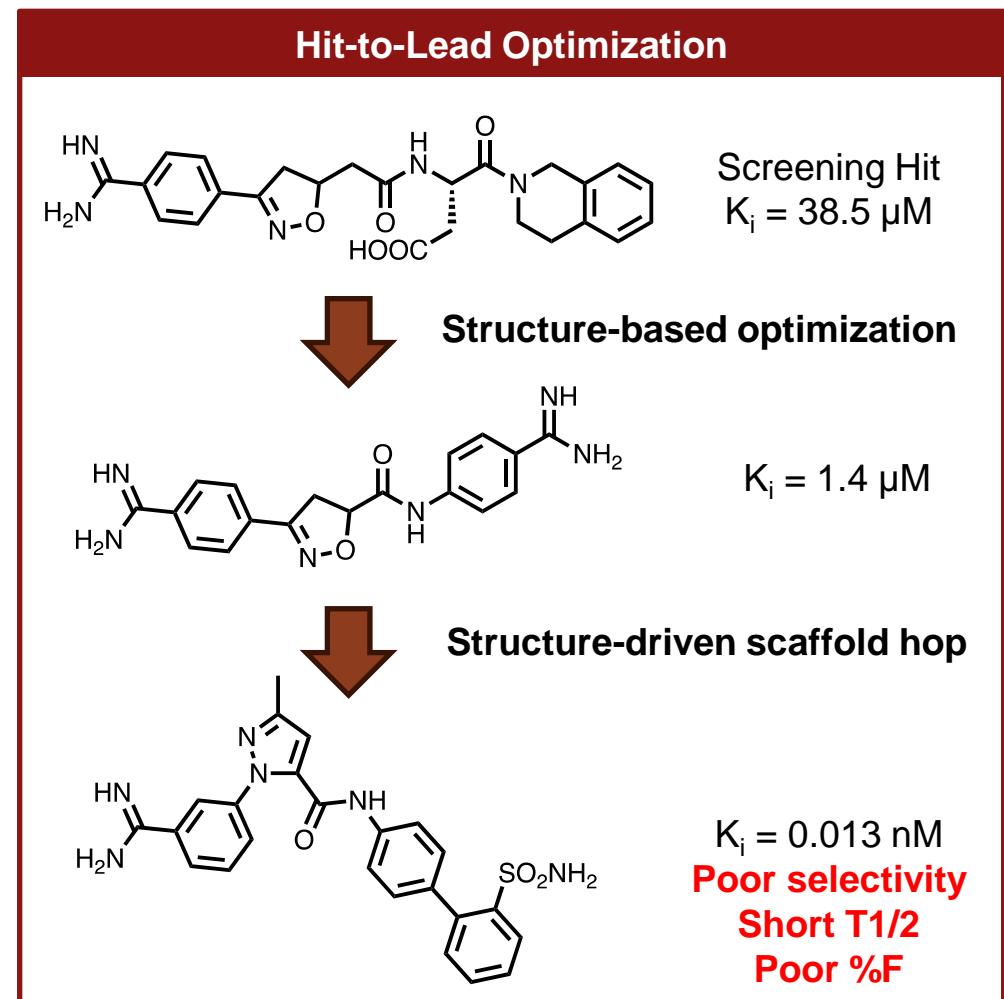
Case Study 2: Apixaban For The Treatment of Thrombosis

Hit to Lead:

- Combination of structure- and ligand-based drug design used to improve potency
 - X-ray structure of FXa with inhibitor known
 - Molecular modeling used to optimize analog potency



^a J. Med. Chem. 2007, 50, 5339 ^b J. Thromb Thrombolysis, 2011, 31, 478.

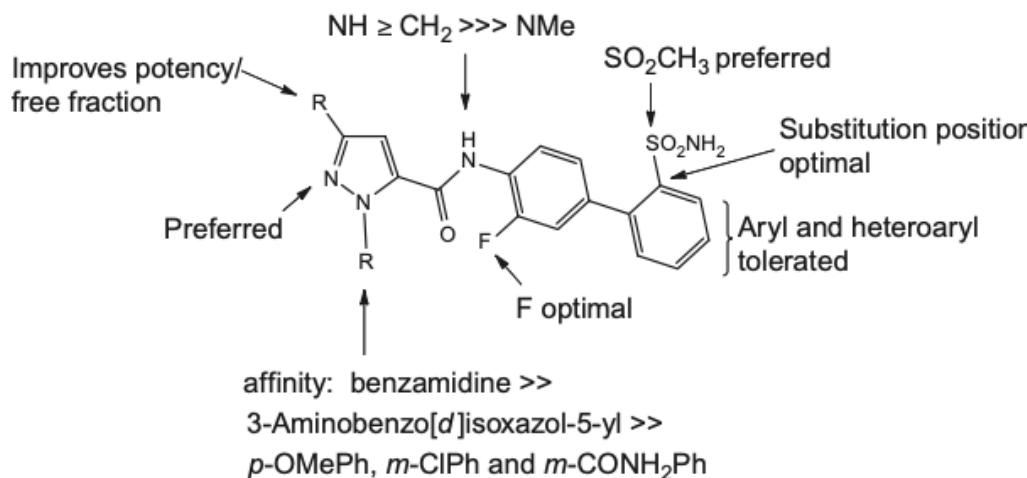


Case Study 2: Apixaban For The Treatment of Thrombosis

Lead Optimization:

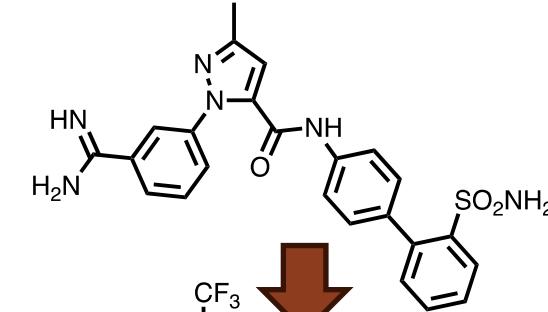
- Several generations of candidate molecules came out of lead optimization prior to the discovery of Apixaban
- Backup series were aggressively pursued

SAR Used For Lead Optimization

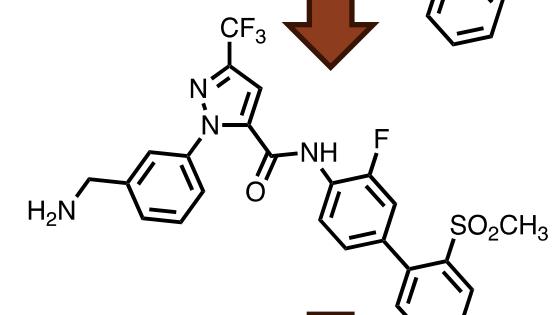


^a J. Med. Chem. 2007, 50, 5339 ^b J. Thromb Thrombolysis, 2011, 31, 478.

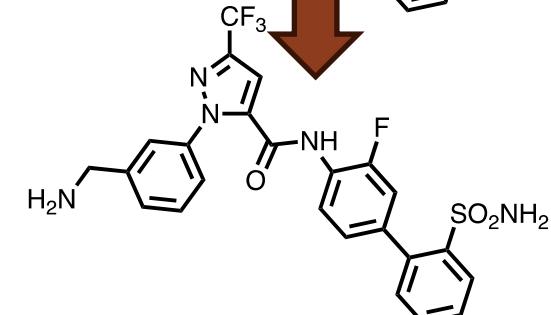
Lead Optimization



$K_i = 0.013 \text{ nM}$
Poor selectivity
Short $T_{1/2}$
Poor %F



DPC423 – Phase I
Safety – selectivity

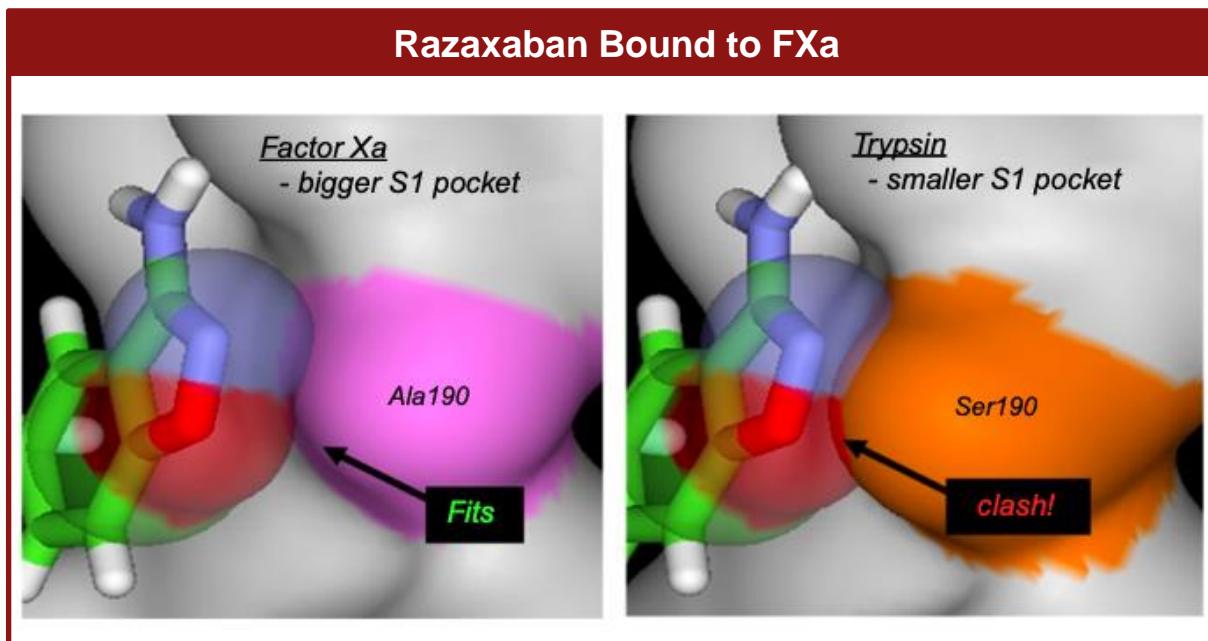


DPC602 – Preclinical
Chemical stability

Case Study 2: Apixaban For The Treatment of Thrombosis

Lead Optimization:

- Basicity of amine identified as liability for selectivity and chemical stability



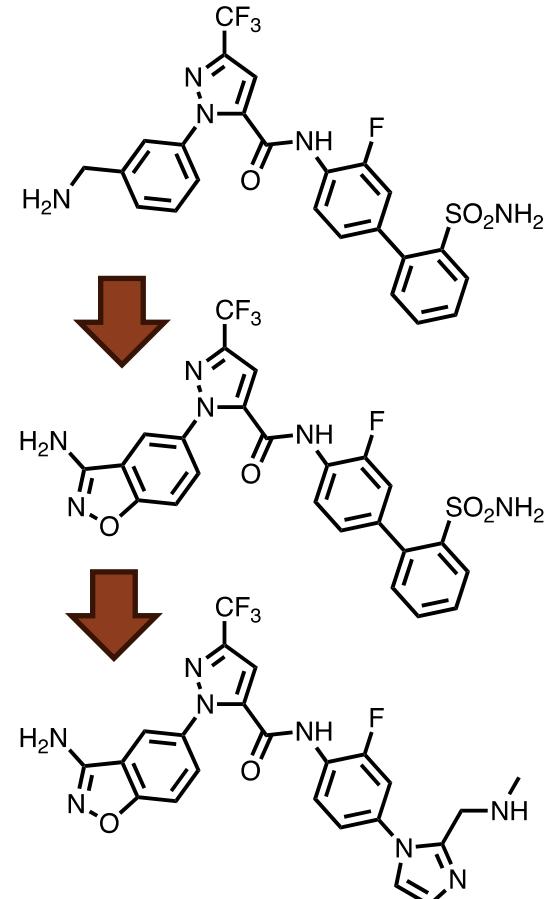
Hit-to-Lead Optimization

Less basic analogs sought

Scaffold hop

Optimization

Razaxaban – Phase 2
Safety – selectivity

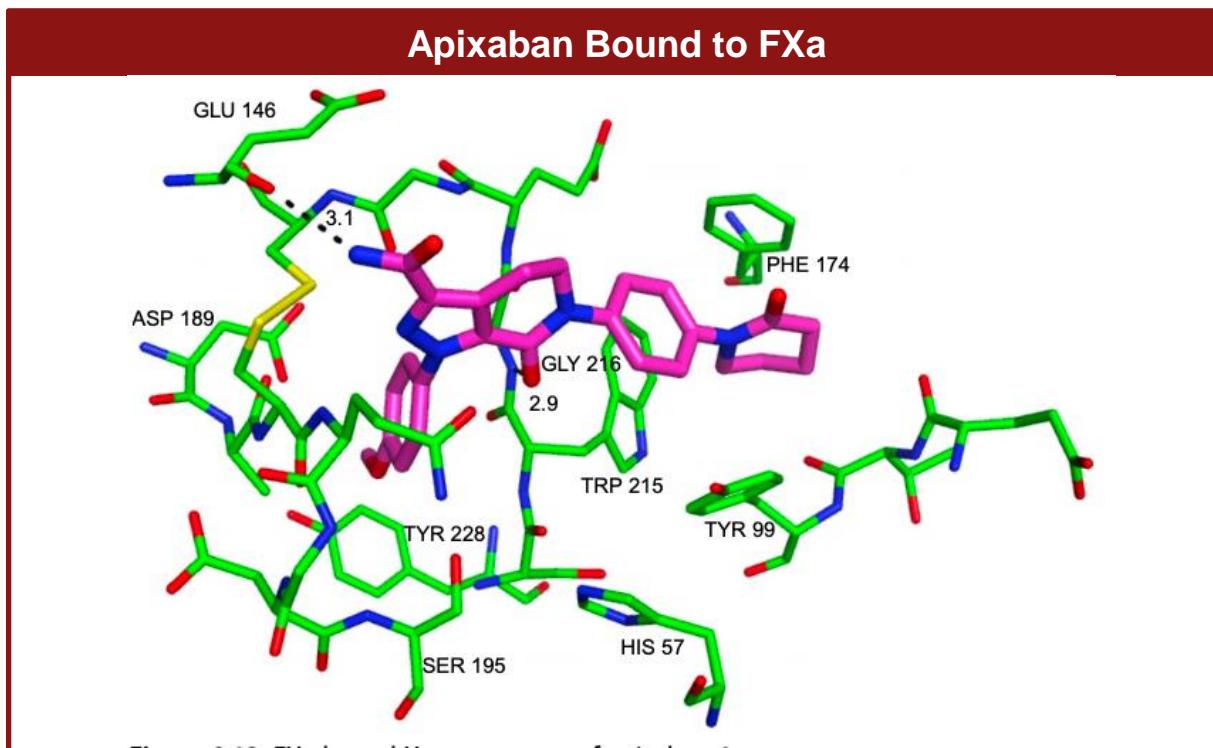


^a J. Med. Chem. 2007, 50, 5339 ^b J. Thromb Thrombolysis, 2011, 31, 478.

Case Study 2: Apixaban For The Treatment of Thrombosis

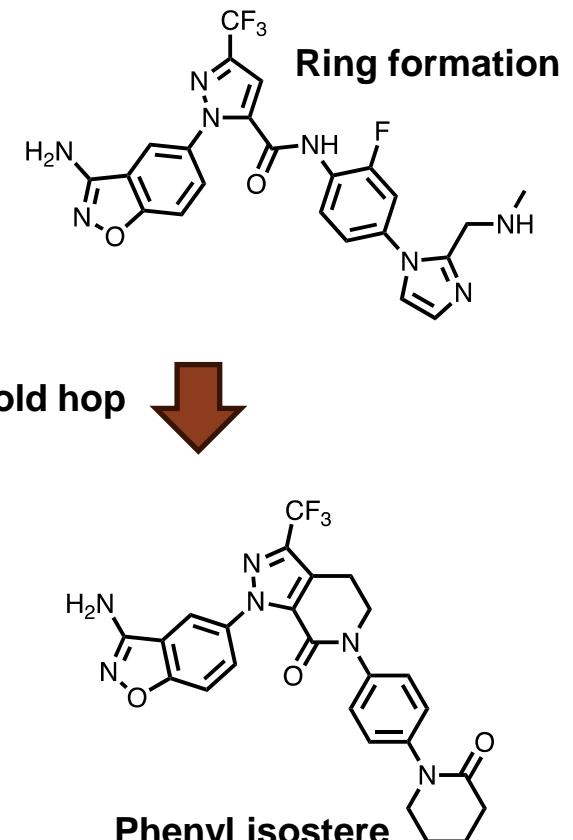
Lead Optimization:

- Prior to Razaxaban being discontinued, efforts to identify structurally distinct backups were initiated



Hit-to-Lead Optimization

Razaxaban – Phase 2
Safety – selectivity



Apixaban

Phenyl isostere

^a J. Med. Chem. 2007, 50, 5339 ^b J. Thromb Thrombolysis, 2011, 31, 478.



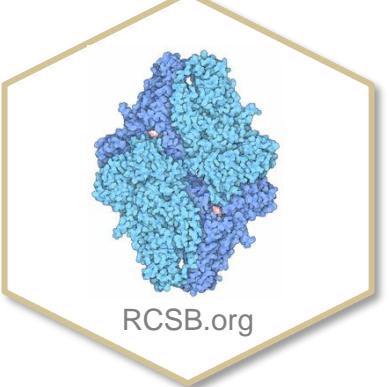
Case studies: Large molecules

Pick your Modality: Large molecules

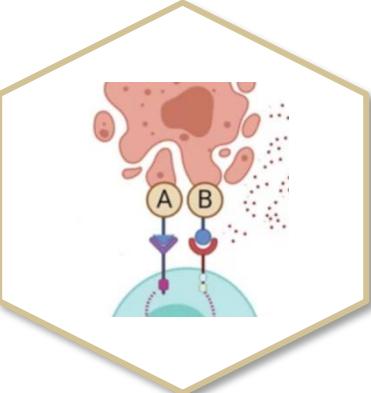
Other modalities



Vorob'yeva et al, 2019

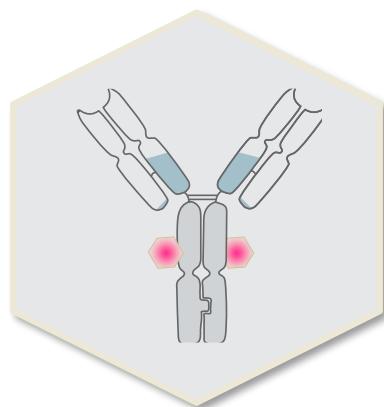


RCSB.org

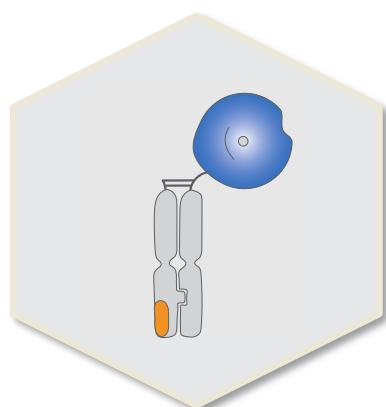


CAR T /Cell
immunotherapy

Large molecule fusions



ADC



Protein Fusions

Non-antibody
scaffolds

Protein/enzymes

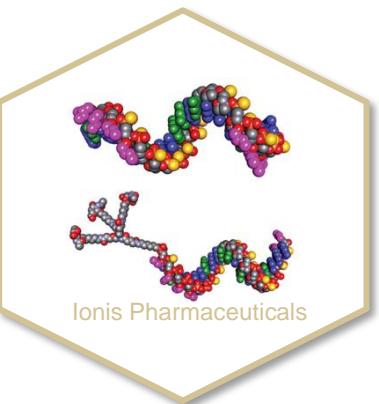
CAR T /Cell
immunotherapy

ADC

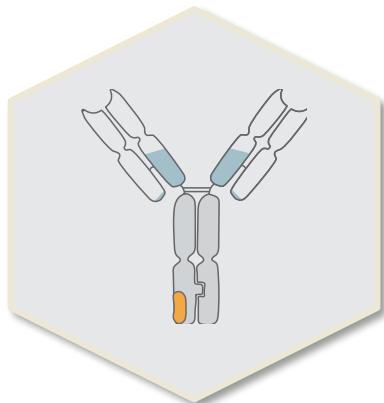
Protein Fusions



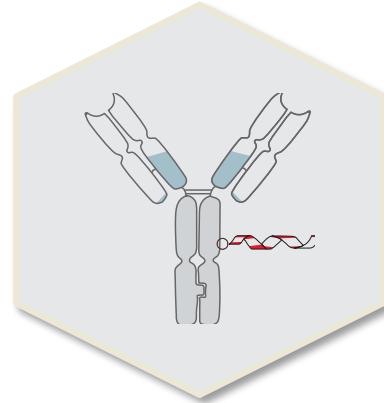
Viral Vectors



Nucleotides

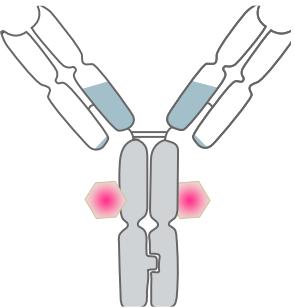


Modified Fc



Nucleic acid
conjugates

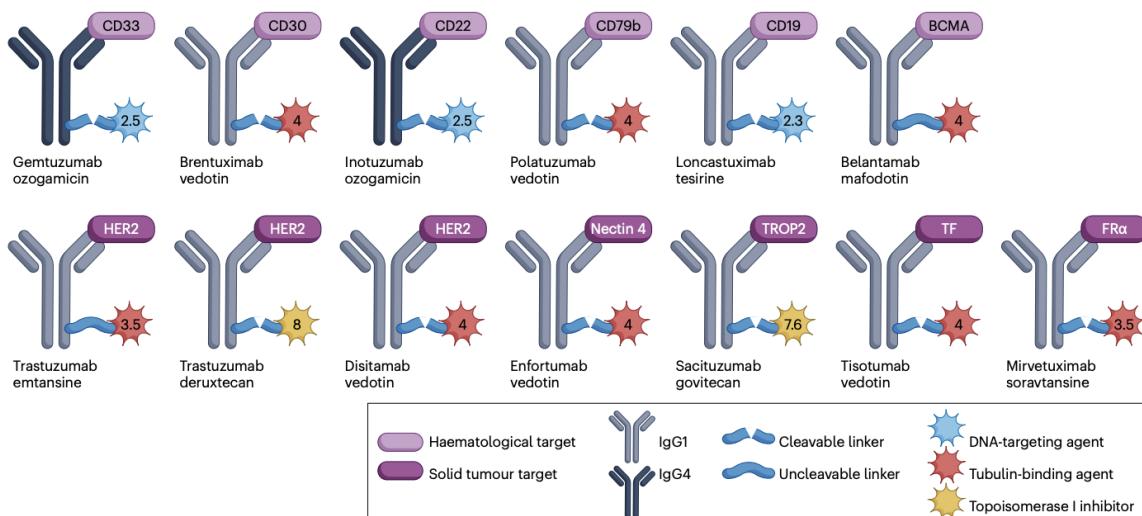
Case study 1: Antibody drug conjugates (ADCs): Best of both worlds?



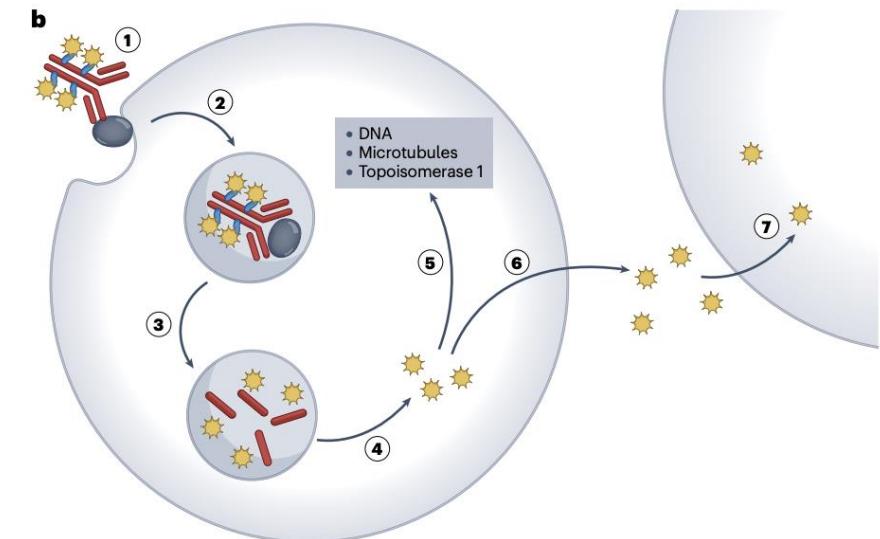
Chemically conjugate small-molecule onto an antibody

- Antibody targets delivery drug to a specific location through target binding where small molecule is released
- Small molecule can diffuse into the cell
- Can increase the specificity half-life of the small molecule.

FDA Approved ADCs (2023)



Dumontet C, Reichert JM, Senter PD, Lambert JM, Beck A. Antibody-drug conjugates come of age in oncology. Nat Rev Drug Discov. 2023 Aug;22(8):641-661. doi: 10.1038/s41573-023-00709-2. Epub 2023 Jun 12.



Mylotarg: first approved ADC: anti-CD33 with a conjugated cell death drug
- For acute myeloid leukemia (AML) cancer



MYLOTARG™
gemtuzumab ozogamicin INJECTION
4.5 mg single-dose vial

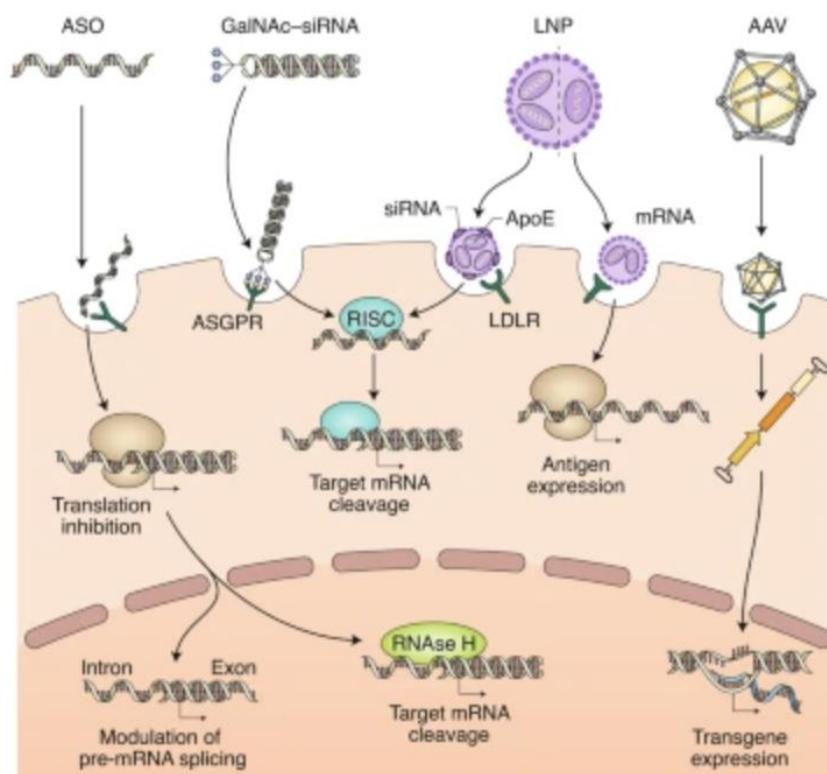
Case study 2: Nucleotide, Virus and Virus-like therapeutics

Antisense oligonucleotide (ASO):

- Can downregulate, upregulate and alternatively splice mRNA
- Functions through RNase H in the nucleus

siRNA:

- Silence mRNA in the cytosol
- Functions through the RISC complex



Kulkarni JA, Witzigmann D et al 2021 Jun;16(6):630-643. doi: 10.1038/s41565-021-00898-0. Epub 2021 May 31. Erratum in: Nat Nanotechnol. 2021 Jul;16(7):841.

Lipid nanoparticles:

- Efficient delivery of DNA/RNA to tissues by lipid carriers

Viral delivery: AAV: adeno-associated viruses

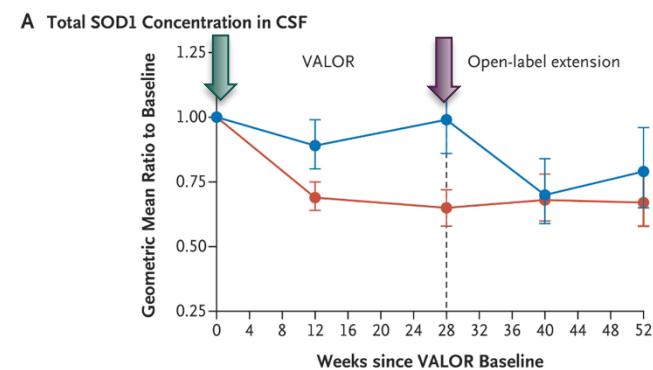
- Deliver your gene of interest

Delivery:

- ASO and siRNA are heavily modified for stability, avoid degradation
- Systemic: Intravenous/subcutaneous/intramuscular
- Central nervous system: intrathecal, eye

Tofersen: (Qalsody)

- Approved ASO for Amyotrophic lateral sclerosis ALS in 2023
- Intrathecal delivery to brain/CNS



- Knock down of specific mutated form of superoxide dismutase 1 (SOD1)

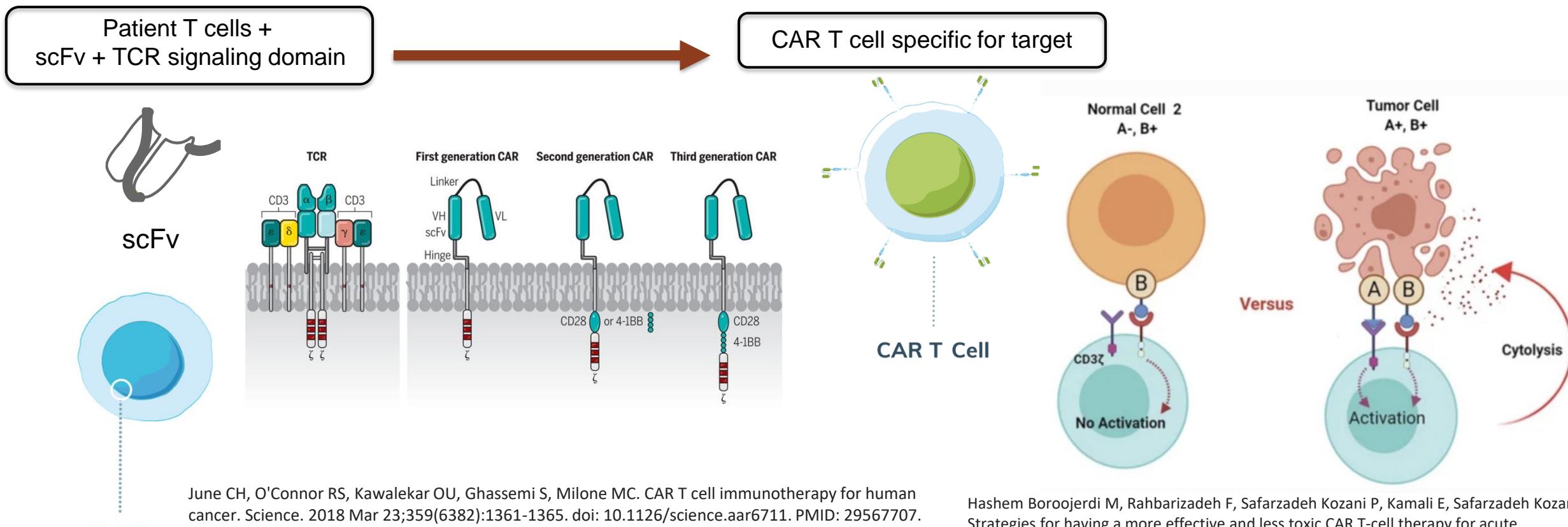


Modified with arrows from Miller TM et al N Engl J Med. 2022 Sep 22;387(12):1099-1110. doi: 10.1056/NEJMoa2204705.

Case study 3: CAR T cell to tumor delivery (cancer immunotherapy)

Chimeric antigen receptor (CAR) T-cell therapy involves engineering a patient's T cells to express a synthetic receptor that binds a tumor antigen

- Two recent FDA approvals of CAR T cells directed against the CD19 protein for treatment of acute lymphoblastic leukemia and diffuse large B-cell lymphoma



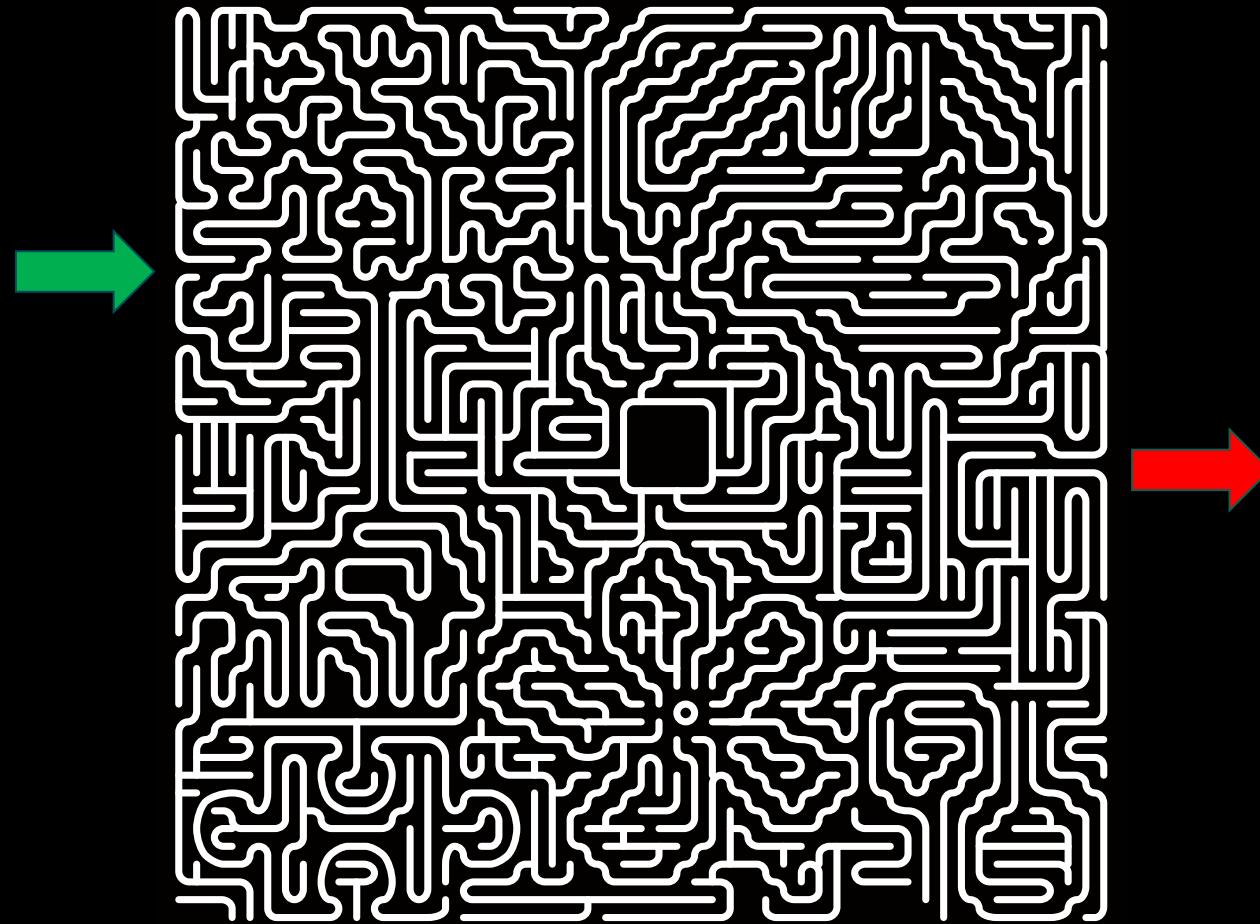
June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. *Science*. 2018 Mar 23;359(6382):1361-1365. doi: 10.1126/science.aar6711. PMID: 29567707.

Hashem Boroojerdi M, Rahbarizadeh F, Safarzadeh Kozani P, Kamali E, Safarzadeh Kozani P. Strategies for having a more effective and less toxic CAR T-cell therapy for acute lymphoblastic leukemia. *Med Oncol*. 2020 Oct 12;37(11):100. doi: 10.1007/s12032-020-01416-3.

<https://www.mesothelioma.com/treatment/immunotherapy/car-t-cell-therapy/>

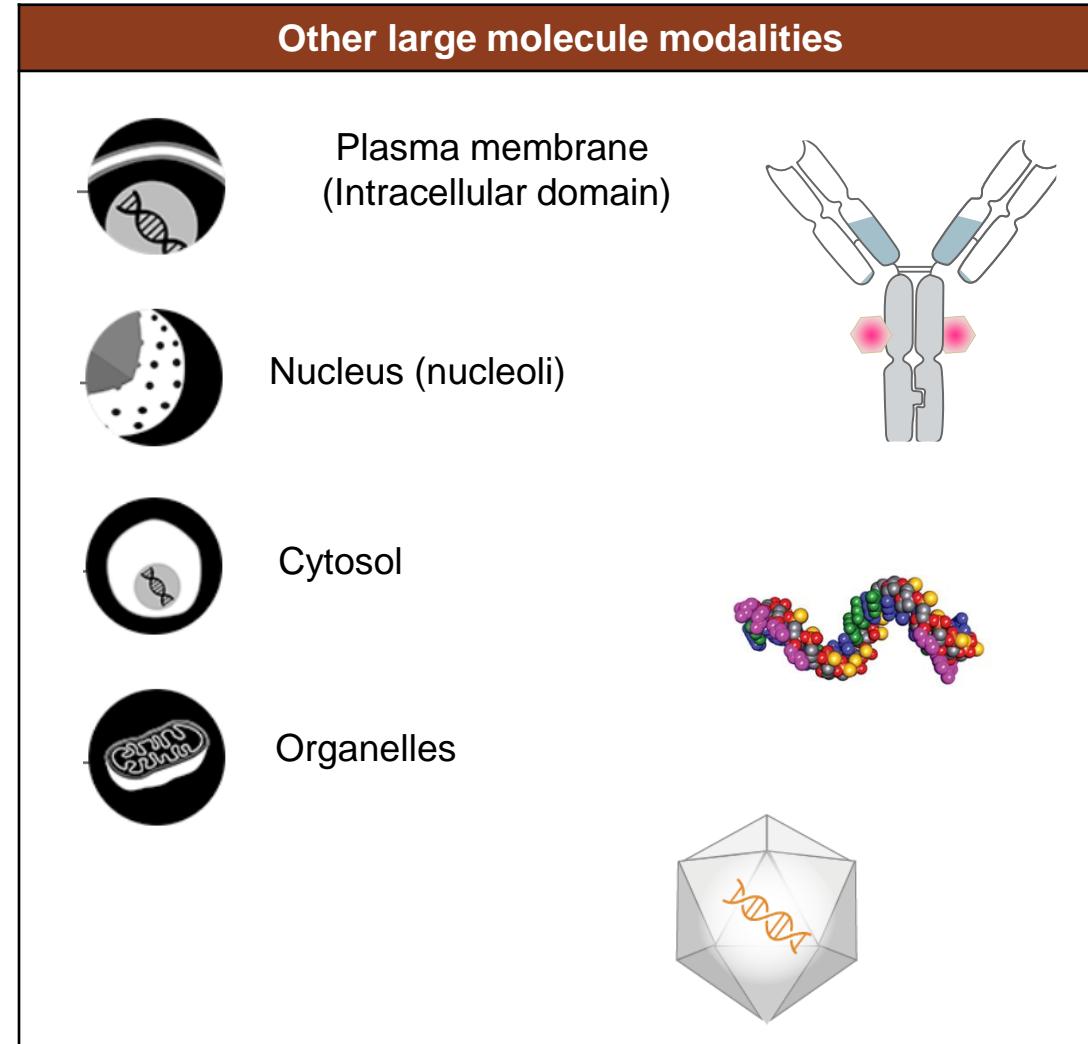
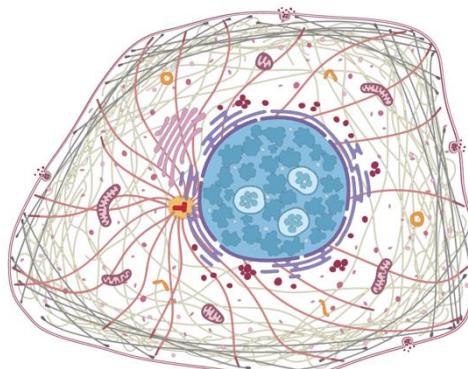
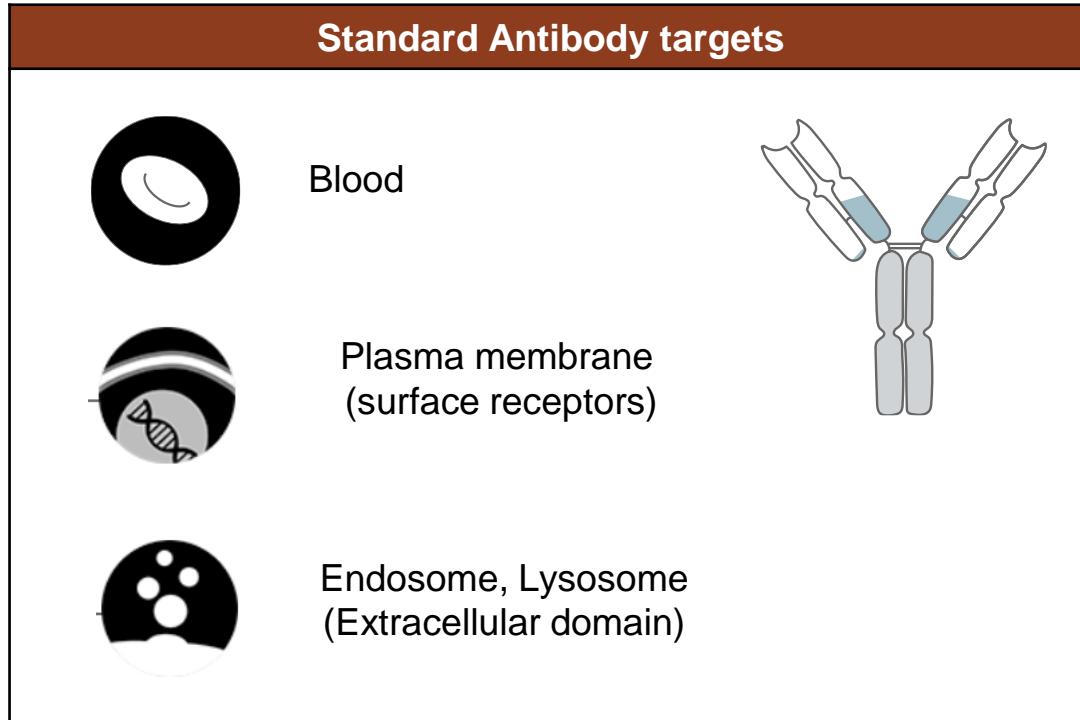


Case Study 4: Brain Delivery



What if you can't get your drug
to the site of action?

Large molecules can now deliver to almost all locations



<https://www.proteinatlas.org/humanproteome/subcellular>

The blood-brain barrier (BBB) challenge

The BBB

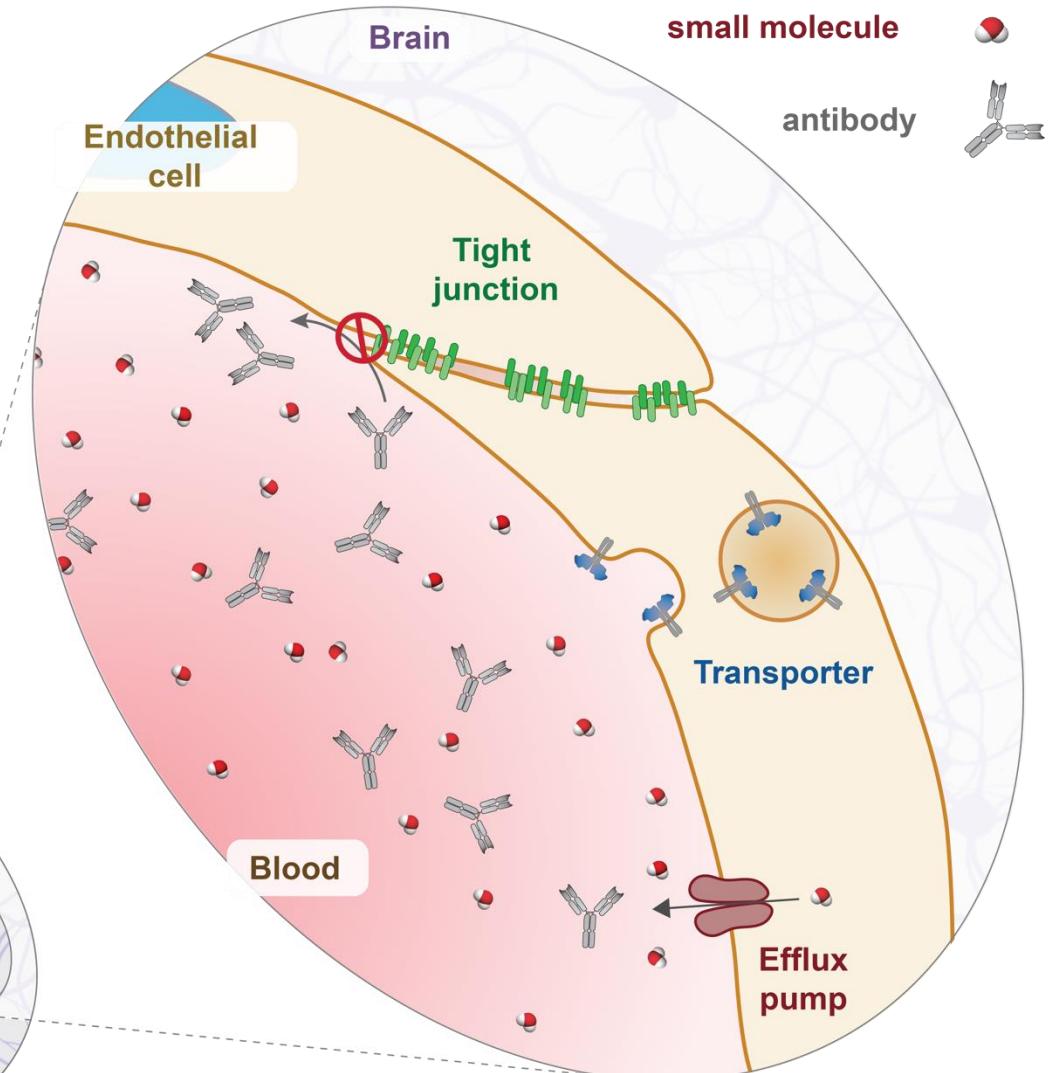
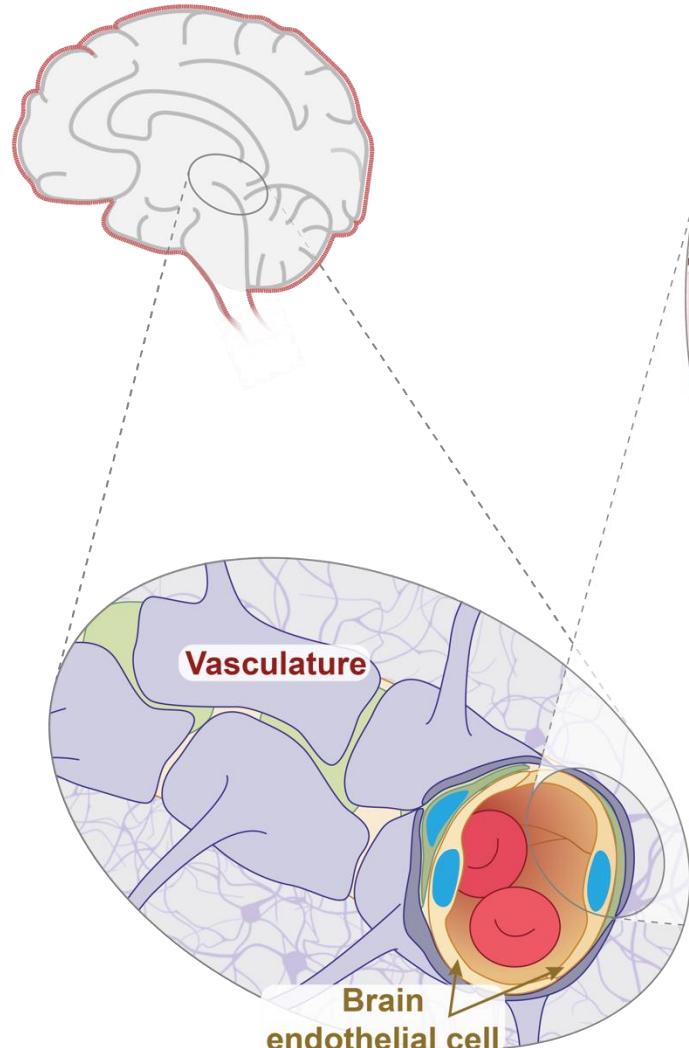
Protects brain from toxins

Tight junctions and efflux pumps keep molecules out

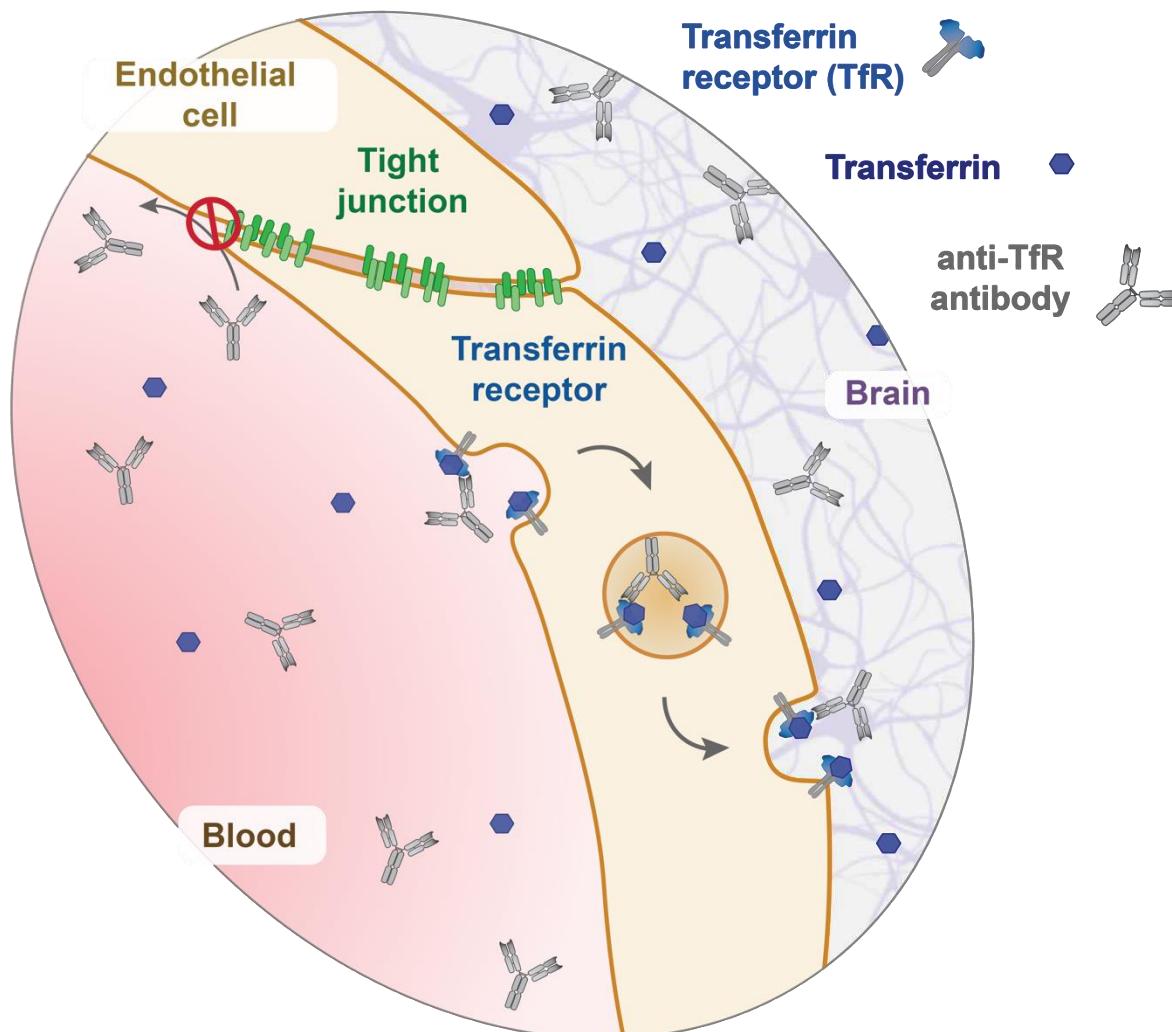
Major hurdle for drug delivery in CNS pathologies

Human brain has 400 miles of blood vessels creating a **large surface area**

BBB is **scalable** across species



INCREASING BRAIN EXPOSURE BY TARGETING TRANSFERRIN RECEPTOR



TRANSFERRIN RECEPTOR (TfR)

Native biology

- **Highly expressed** on brain endothelial cells
- **Actively transports** iron-loaded transferrin into the brain
- Plays critical role in iron homeostasis

Target for brain delivery

- Constitutive transcytosis results in **high capacity**
- High vascularization enables **ubiquitous delivery**
- Efficient transcytosis is dependent on **affinity** and **valency**

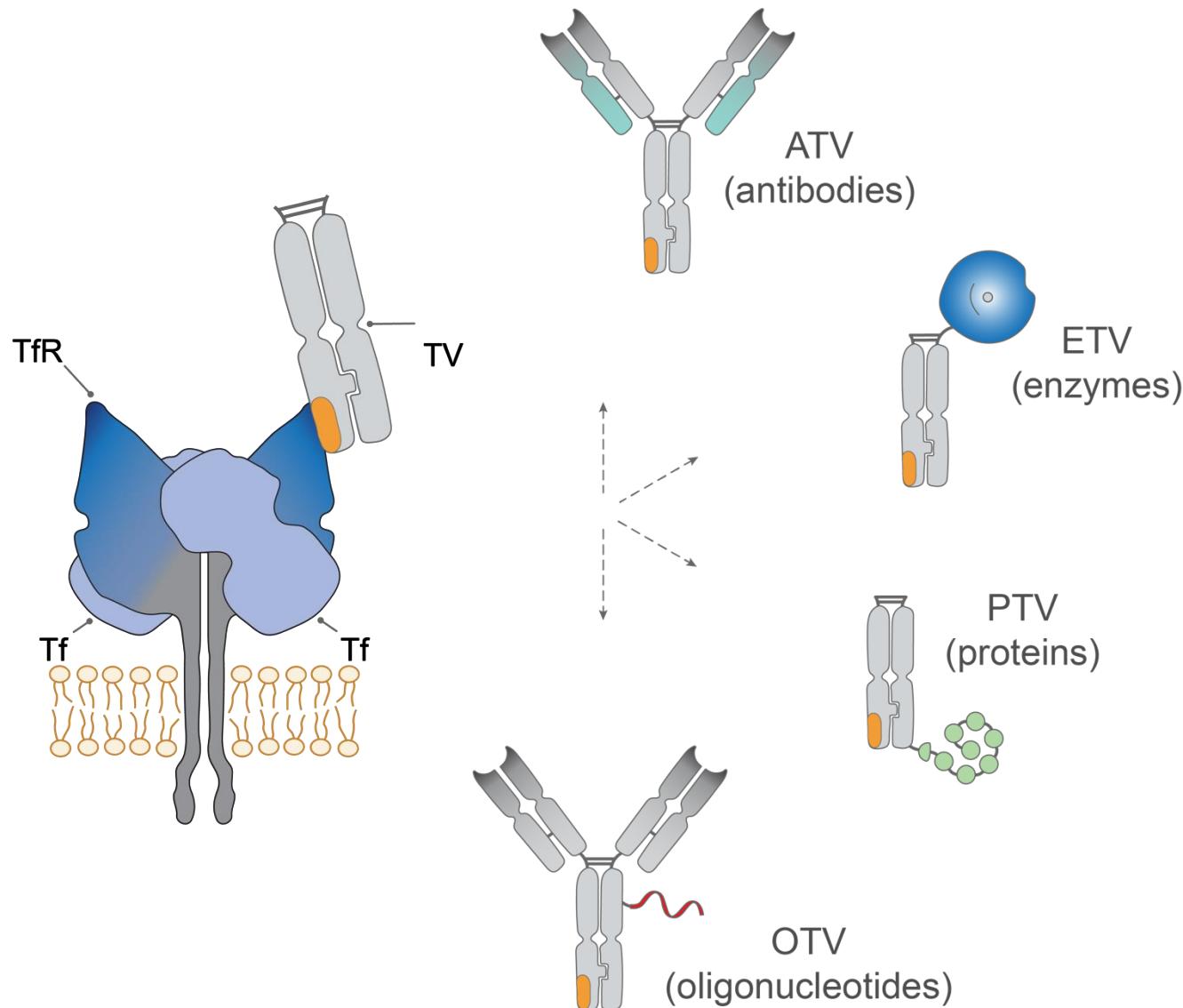
A platform to deliver biotherapeutics to the brain

The Transport Vehicle (TV)

Goal: With minimal mutations, deliver any antibody, enzyme, or protein to the brain

Advantages

- No linkers or appended domains
- Retain native IgG stability and PK
- Maximizes Fab functionality
- **Modular** – can be fused to Fabs, proteins, enzymes, and oligonucleotides

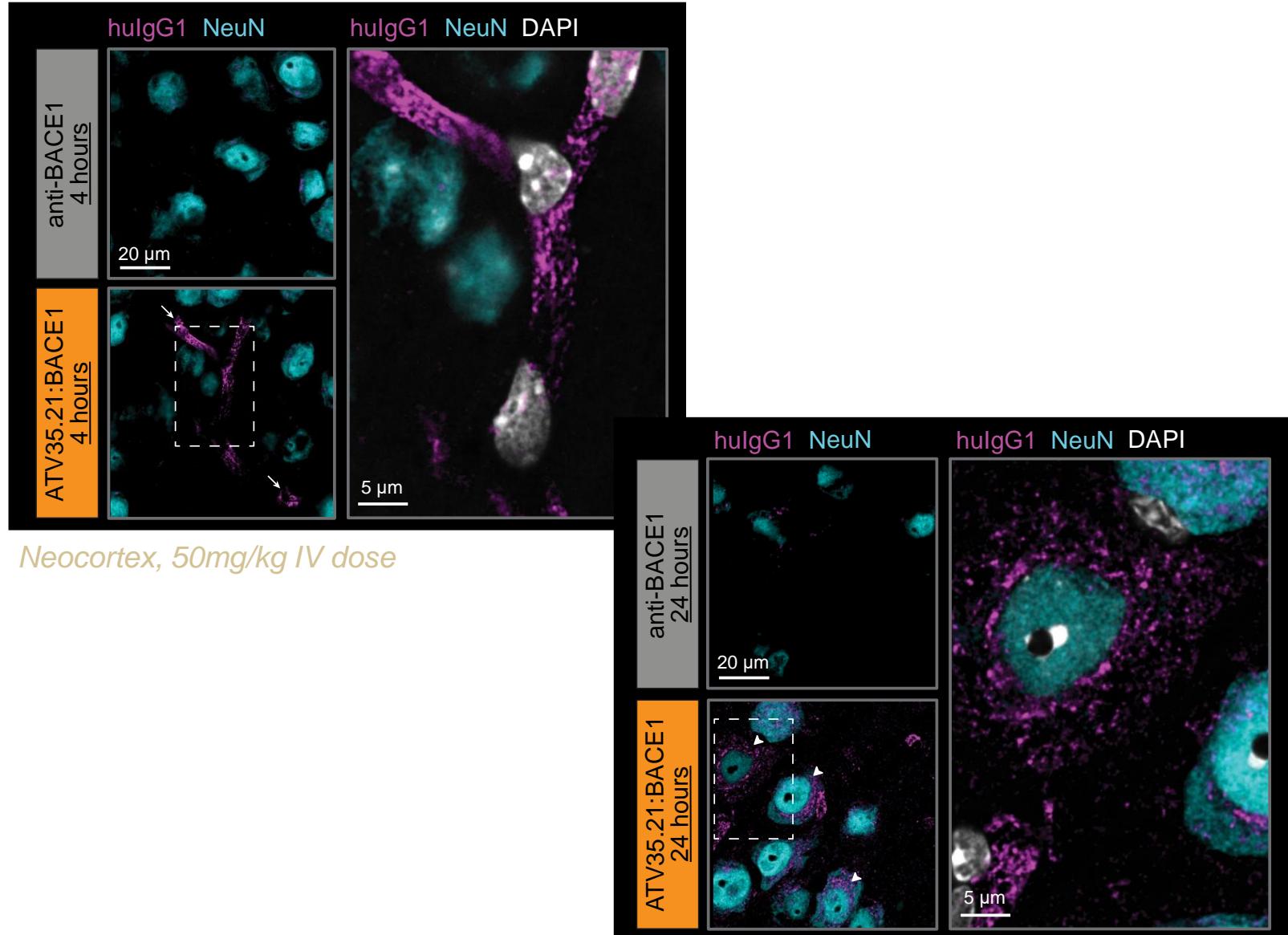


Brain uptake of ATVs in humanized mice

TV Characterization

Brain uptake in humanized mouse

Broad distribution across brain regions after IV dosing



Brain uptake and pharmacodynamic response in NHP

TV Characterization

Brain uptake in humanized mouse

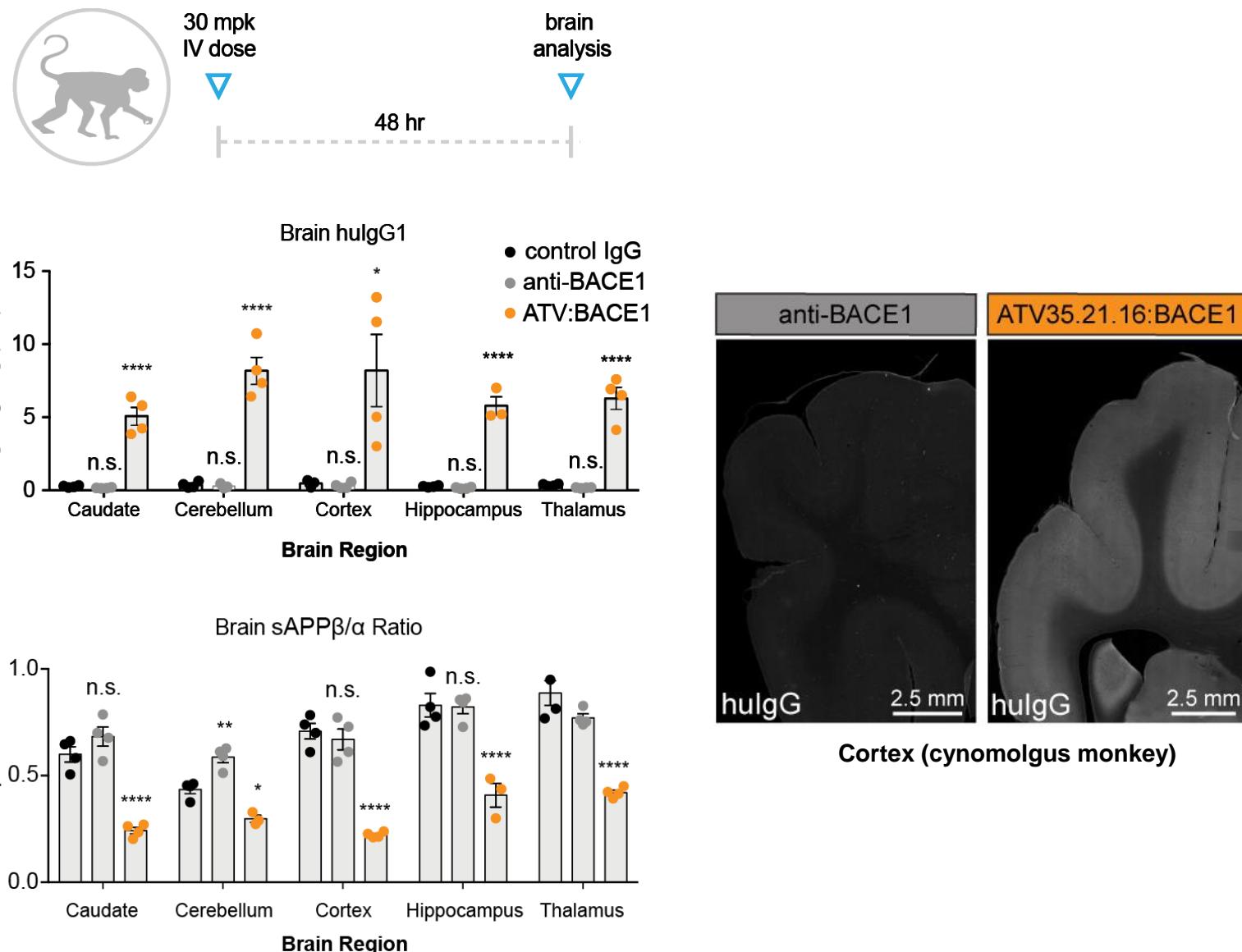
Broad distribution across brain regions after IV dosing

NHP proof-of-concept

non-ATV mAb show 0.01% brain concentrations relative to blood

20 – 25 fold increase in ATV exposures across brain regions over mAb

Pharmacodynamic response demonstrates broad parenchymal delivery





Helpful Databases

Uniprot database – Find your gene of interest

<https://www.uniprot.org/>

UniProt BLAST Align Peptide search ID mapping SPARQL

Release 2023_03 | Statistics Help

Find your protein

Advanced List Search Examples: Insulin, APP, Human, P05067, organism_id:9606

UniProt is the world's leading high-quality, comprehensive and freely accessible resource of protein sequence and functional information. [Cite UniProt](#) **

Sequence & Isoformsⁱ

BLAST 3 isoforms Align 3 isoforms

Sequence statusⁱ Complete Sequence processingⁱ The displayed sequence is further processed into a mature form.

This entry describes 3 isoformsⁱ produced by Alternative splicing.

P02768-1

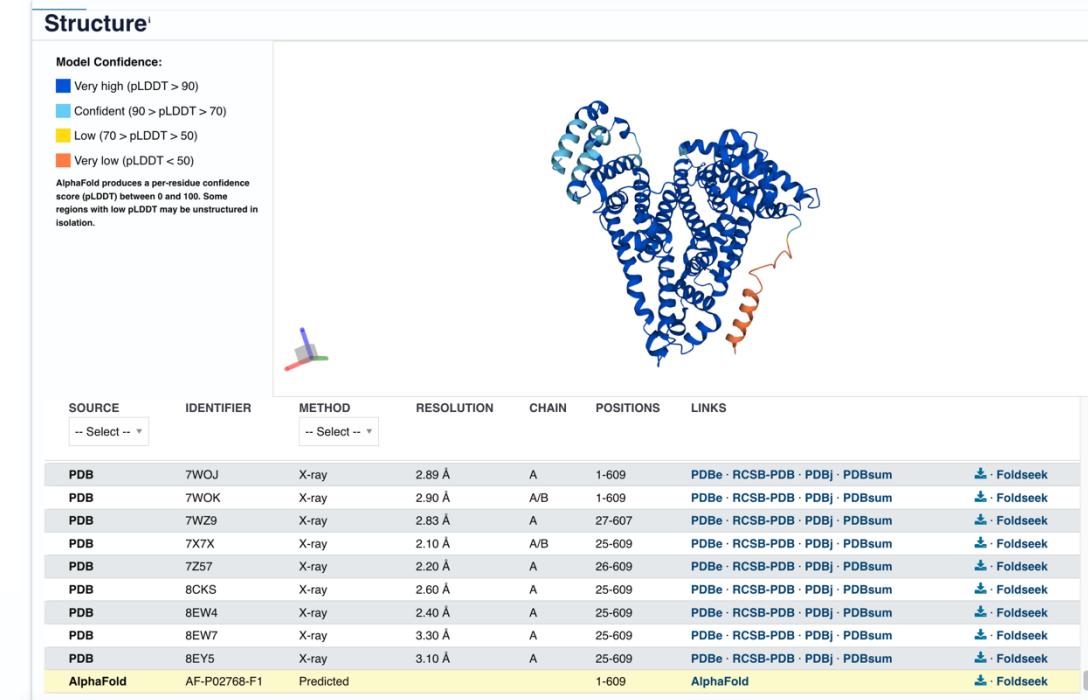
This isoform has been chosen as the canonical sequence. All positional information in this entry refers to it. This is also the sequence that appears in the downloadable versions of the entry.

Name 1 See also sequence in UniParc or sequence clusters in UniRef

Tools Download Add Highlight Copy sequence

Last updated 1990-04-01 v2
Checksumⁱ F88FF61DD242E818

Length	609	Mass (Da)	69,367																
10	MKWVTFISLL	20	FLFSSAYSRSR	30	VFRRDHKSE	40	VAHFRFKDLGE	50	ENFKALVLIA	60	FAQYLQQCFF	70	EDHVKLVNEV	80	TEFAKTCVAD	90	ESAENCDSL	100	HTLFGDKLCT
110	VATLRETYGE	120	MADCCAKQEP	130	ERNECFQHKK	140	DDNPNLPRLV	150	RPEVDVMCTA	160	FHDNEETFLK	170	KYLYEIARRH	180	PYFYAPELLF	190	FAKRYKAAFT	200	ECCQAADKAA
210	CLLPKLDLR	220	DEGKASSAKQF	230	RLKCASLQKF	240	GERAFKAWAV	250	ARLSQRFFKA	260	EFAEVSKLVT	270	DLTKVHTECC	280	RADLAKYICE	290	NQDSISSLK	300	
310	ECCEKPLLEK	320	SHCIAEVEND	330	EMPADLPSLA	340	ADFVESKDVF	350	KNYAEAKDVF	360	LGMPFLYEYEAR	370	RHPDYSVVLL	380	LEKCCAAADP	390	HECYAKVDFE	400	
410	FKPLVVEPNQN	420	LIKQNCELFE	430	QLGEYKFQNA	440	LLVRYTKVKP	450	QVSTPTLVEV	460	SRNLGKGSK	470	CCKHPKERRQRM	480	PCADEYLSVV	490	LNQLCVLHEK	500	TPVSDRVTKC



Links to AlphaFold – Model of every human protein plus more updates.

-Structures are not validated, but the vast majority are very helpful.

Tabs – Find your Therapeutic antibody of interest

https://tabs.craic.com/users/sign_in

The screenshot shows the homepage of the Tabs database. At the top is a red header bar with the logo 'Tabs - Therapeutic Antibody Database'. Below the header is a navigation menu with ten items: Home, Antibodies, Antigens, Conditions, Clinical Trials, Sequences, Structures, Papers, Patents, Companies, Biosimilars, and Help. The 'Home' item is highlighted with a light blue background. The main content area has a white background. It features a 'Welcome to Tabs' message, a note that 'TABS now contains data on 8,000 human therapeutic mAbs!', and a message about new data: 'New Data: 205 records added in the last 7 days (580 in last 30 days)'. Below these messages are two links: 'Quick Start Guide' and 'Help Pages'. At the bottom of the page is a footer bar with the Craic Computing logo, the text '© 2010-2021 Craic Computing LLC', and a sign-in status message: 'Signed in as Rob Wells Sign out'.

Requires registration and has a cost

AMA drug finder – Find your approved drug of interest

https://searchusan.ama-assn.org/finder/usan/search/*/rele

The screenshot shows the USAN Drug Finder homepage. At the top, there's a purple header bar with the AMA logo and a sign-in dropdown. Below it, the main title "United States Adopted Name (USAN) Drug Finder" is centered. A search bar contains the term "LECANEMAB". On the left, there are two filter panels: "Refine Search" and "1 Results". The "Refine Search" panel includes filters for "SPONSOR" (Eisai, Inc.) and "YEAR APPROVED" (2019). The "1 Results" panel shows "1 Results" sorted by "Most Relevant". The result for "LECANEMAB" is listed, providing details like USAN File Number (GH-07), CAS Registry Number (1260393-98-3), UNII (12PYH0FTU9), and WHO Number (11194). At the bottom, there are links for "Copyright 1995 – 2023 American Medical Association. All rights reserved." and "Terms of Use", "Privacy Policy", "Code of Conduct", and "Website Accessibility".

STATEMENT ON A NONPROPRIETARY NAME ADOPTED BY THE USAN COUNCIL

USAN (GH-07) LECANEMAB

PRONUNCIATION lek' an' e mab

THERAPEUTIC CLAIM Treatment of Alzheimer's disease

CHEMICAL NAMES

1. Immunoglobulin G1, anti-(human β -amyloid protofibril) (human-*Mus musculus* monoclonal BAN2401 heavy chain), disulfide with human-*Mus musculus* monoclonal BAN2401 light chain, dimer
2. immunoglobulin G1-kappa, anti-(*Homo sapiens* Amyloid-beta precursor protein (Alzheimer disease amyloid protein, Cerebral vascular amyloid peptide, ABPP)); humanized mouse monoclonal antibody; γ 1 heavy chain (1-454) [humanized VH (*Homo sapiens* IGHV3-48*01 (90%) –(IGHD)-IGHJ4*01 (92%)) [8.8.17] (1-124) -*Homo sapiens*IGHG1*03 (125-454)], (227-219)-disulfide with κ light chain (1'-219') [*Mus musculus* V-KAPPA (IGKV1-117*01 (91%) –IGKJ1*02 (91%)) [11.3.9] (1'-112') -*Homo sapiens* IGKC*01 (113'-219')], dimer (233-233":236-236")-bisisulfide

STRUCTURAL FORMULA

Heavy chain	LVQPGGGSLRLCSASGFTFS	SFGMHWWRQA	PGKGLEWVAY	50
EVQIVESGGG	IVQPGGGSLRLCSASGFTFS	SFGMHWWRQA	PGKGLEWVAY	50
ISSGSSTIY	GDTVKGRFTI	SRDNAKNSLF	LQMSSLRAED	100
GYYYGRSYYT	MDYWQQGTTV	TVSSASTKGP	STSGGTAALG	150
CLVKDYPFEP	VTWSNNSGAL	TSGVHTFPAV	LQSSGLYSLS	200
GTQTYIICNVN	HRSNPKTKVDK	RVEPKSCDKT	HTCPCCPAP	250
PPKPKDTLM	SRTPEVTCVV	VDSHEDPEV	LLGGPSVFLF	300
EQYNSTYRVV	SVLTVLHQDW	LNGKEYKCKV	KFNWYVDGEV	350
REPQVYTLPP	SREEMTKNQV	SLTCLVKGFY	VHNAKTKPRE	400
TFPVLDSDGS	FFLYSKLTVD	KSRWQQGNVF	KTISAKAKQP	450
SPGK			SCSVMHEALH	NGQPENNYKT
			NHYTQKSLSL	454

Light chain	DVVMTQSPLS	LPVTPGAPAS	ISCRSSQSV	HSGNNTYLEW	Y1QKPGQSPK	50'
LLIYKVSNRF	SGVPDRFSGS	GSGTDFTLRI	SRVAEEDVGI	YYCFQGSHP	100'	
PTFGPGTKLE	IKRTVAAPSV	FIFPPSDEQL	KSGTAGSVCV	LNNFYPREAK	150'	
VQWKVNDALQ	SQNSQEVSVE	QDSKDSTYSL	SSTLTLSKAD	YEKKHVYACE	200'	
VTHQGLSSPV	TKSFNRGEC					219'

Disulfide bridges location
22-96 22"-96" 23"-93' 23"-93" 139"-199" 139"-199" 151-207" 151"-207"
219"-227" 233"-233" 236-236" 268-328" 268"-328" 374-432" 374"-432"

Glycosylation sites (N)
Asn-304 Asn-304"

Google Patents



DrugBank: Database of Drugs and Drug Targets

DRUGBANK Online

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[Info Session] DrugBank Deep Dive: Connecting Conditions to Relevant Data Register Now!

Drugs

Acetaminophen 32,942

Identification Pharmacology Interactions Products Product Images International/Other Brands Brand Name Prescription Products Generic Prescription Products Over the Counter Products Mixture Products Unapproved/Other Products Categories Chemical Identifiers References Clinical Trials Pharmacoconomics Properties Spectra Targets (4) Enzymes (15) Carriers (1) Transporters (1)

Summary Acetaminophen is an analgesic drug used alone or in combination with opioids for pain management, and as an antipyretic agent.

Brand Names Acephen, Acetadryl, Allzital, Apadaz, Arthriten Inflammatory Pain, Bupap, Butapap, Cetafen, Children's Silapap, Coricidin Hbp Cold & Flu, Darvocet-N, Dayquil Sine, Diphen, Dolofin, Dologen,

Generic Name Acetaminophen **DrugBank Accession Number** DB00316

Background Acetaminophen (paracetamol), also commonly known as *Tylenol*, is the most commonly taken analgesic worldwide and is recommended as first-line therapy in pain conditions by the World Health Organization (WHO).¹⁰ It is also used for its antipyretic effects, helping to reduce fever.²³ This drug was initially approved by the U.S. FDA in 1951 and is available in a variety of forms including syrup form, regular tablets, effervescent tablets, injection, suppository, and other forms.^{15,16,23,Label}

Acetaminophen is often found combined with other drugs in more than 600 over the counter (OTC) allergy medications, cold medications, sleep medications, pain relievers, and other products.¹⁹ Confusion about dosing of this drug may be caused by the availability of different formulas, strengths, and dosage instructions for children of different ages.¹⁹ Due to the possibility of fatal overdose and liver failure associated with the incorrect use of acetaminophen, it is important to follow current and available national and manufacturer dosing guidelines while this drug is taken or prescribed.^{20,21,Label}

Type Small Molecule **Groups** Approved

Structure
Download Similar Structures

Weight Average: 151.1626 Monoisotopic: 151.063328537

Chemical Formula C₈H₉NO₂

Synonyms Acenol, Acetaminofén, Acetaminophen, Acétaminophène, APAP, Paracetamol, Paracétamol, Paracetamolum

External IDs NSC-109028, NSC-3991



C E R S I
U C S F - S t a n f o r d