DEVELOPING PHARMACEUTICALS FROM BASIC RESEARCH DISCOVERIES

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TOPICS COVERED

1 **Charles River Overview**

2 **Drug Discovery**

3 **Preclinical Strategy**

Charles River Overview

EVERY STEP OF THE WAY

Founded in 1947 by Dr. Henry Foster

A leading, full-service drug discovery and early-stage development company

Our scientists worked on ~85% of the drugs approved by the FDA in 2019

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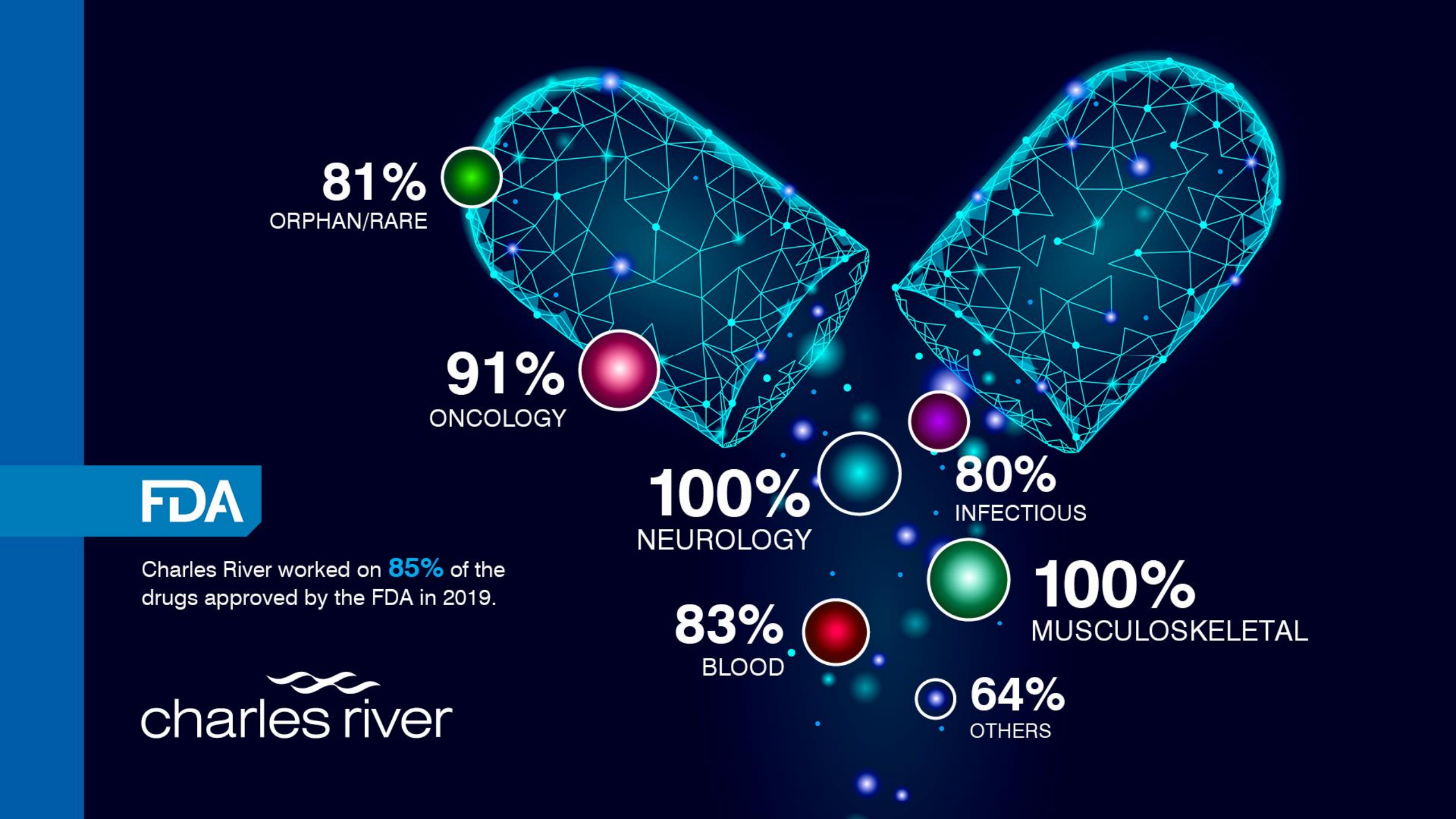

FDA

Charles River worked on **85%** of the drugs approved by the FDA in 2019.

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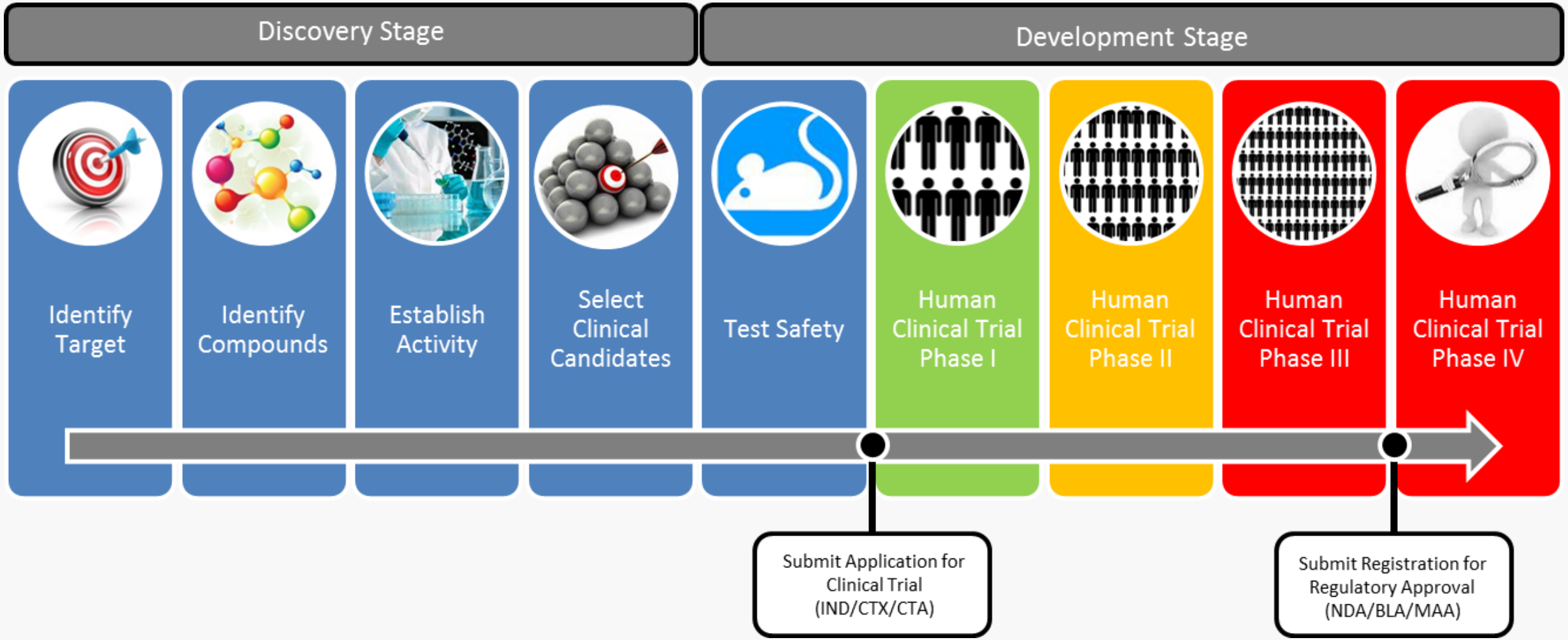
DRUG DEVELOPMENT IS …



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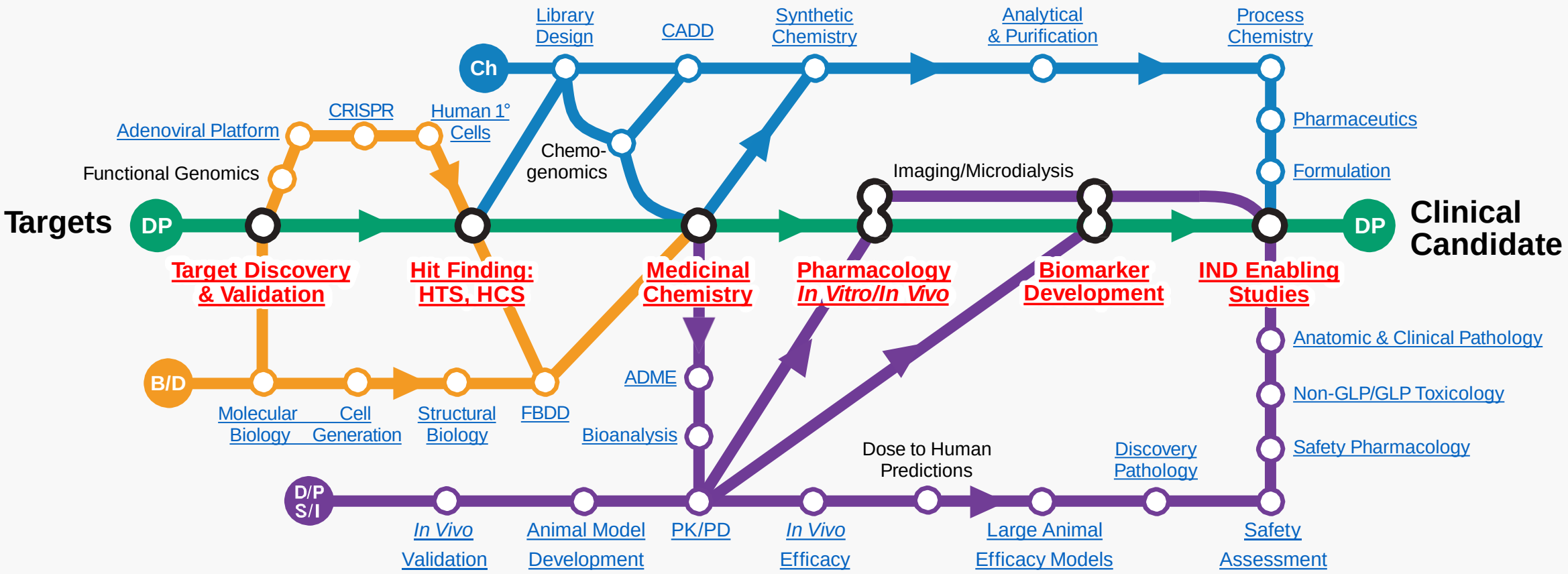
Drug Discovery

“END TO END” INTEGRATED DRUG DISCOVERY



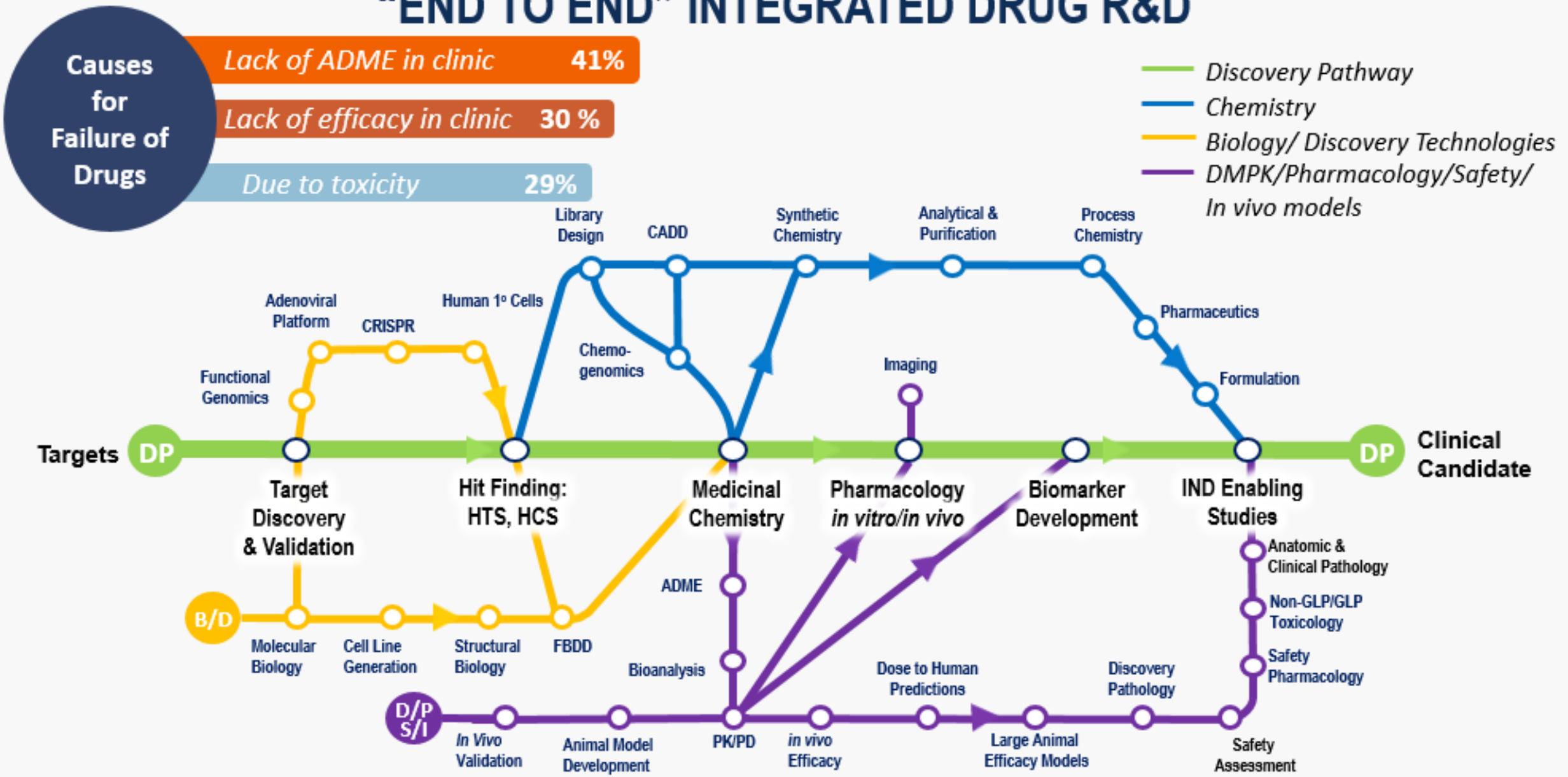
DP Discovery Pathway

Ch Chemistry

B/D Biology/Discovery Technologies

D/P S/I DMPK/Pharmacology/Safety/*In Vivo* Models

"END TO END" INTEGRATED DRUG R&D



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FIVE R'S FROM ASTRAZENECA

Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework

David Cook, Dearg Brown, Robert Alexander, Ruth March, Paul Morgan, Gemma Satterthwaite and Menelas N. Pangalos

Abstract | Maintaining research and development (R&D) productivity at a sustainable level is one of the main challenges currently facing the pharmaceutical industry. In this article, we discuss the results of a comprehensive longitudinal review of AstraZeneca's small-molecule drug projects from 2005 to 2010. The analysis allowed us to establish a framework based on the five most important technical determinants of project success and pipeline quality, which we describe as the five 'R's: the right target, the right patient, the right tissue, the right safety and the right commercial potential. A sixth factor — the right culture — is also crucial in encouraging effective decision-making based on these technical determinants. AstraZeneca is currently applying this framework to guide its R&D teams, and although it is too early to demonstrate whether this has improved the company's R&D productivity, we present our data and analysis here in the hope that it may assist the industry overall in addressing this key challenge.

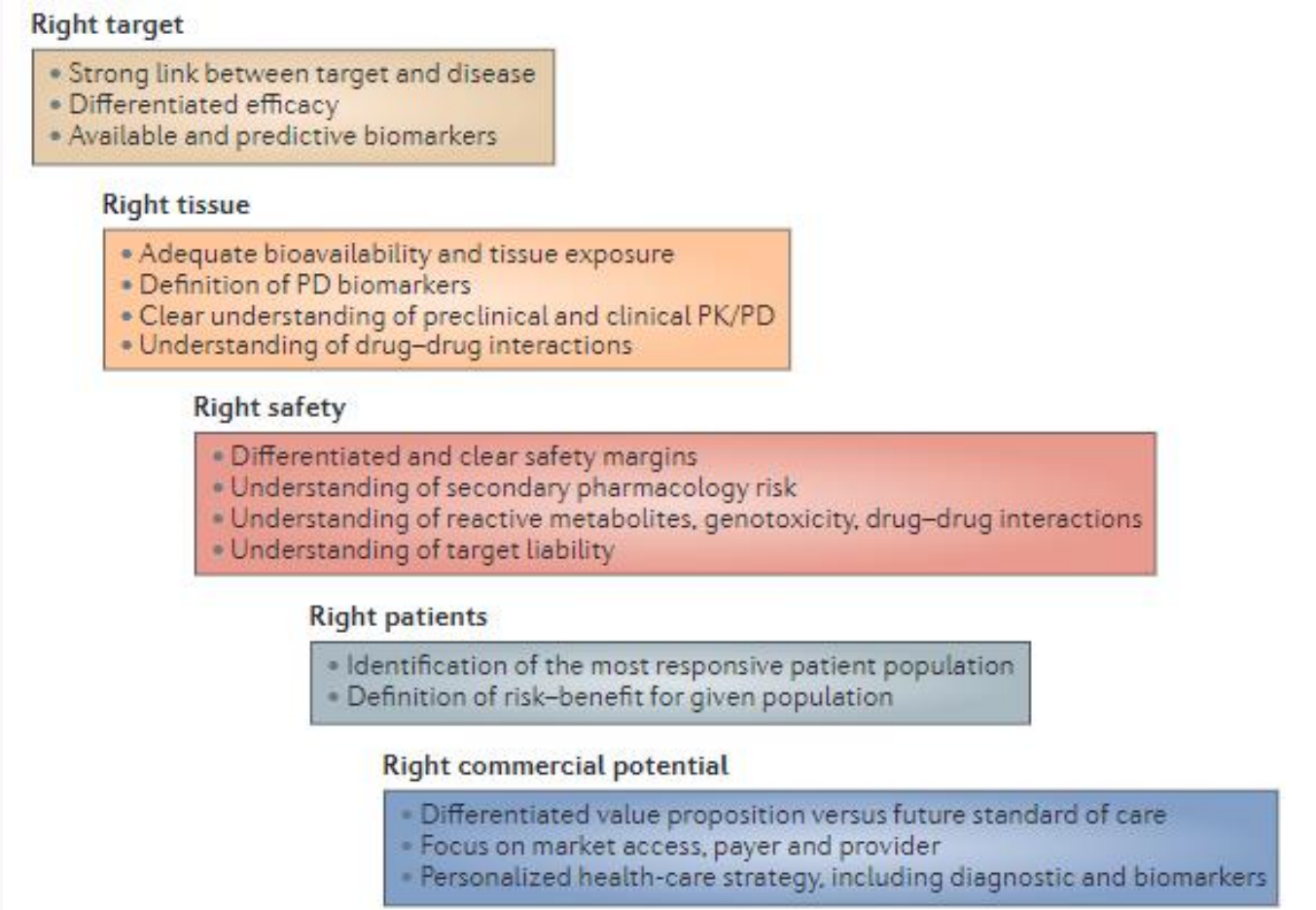

Figure 5 | **The 5R framework.** Summary of the key features of the five-dimensional framework that can be used to describe a drug discovery and development project. PK/PD, pharmacokinetics/pharmacodynamics.

Nature Reviews Drug Discovery (2014) 13: 419-431

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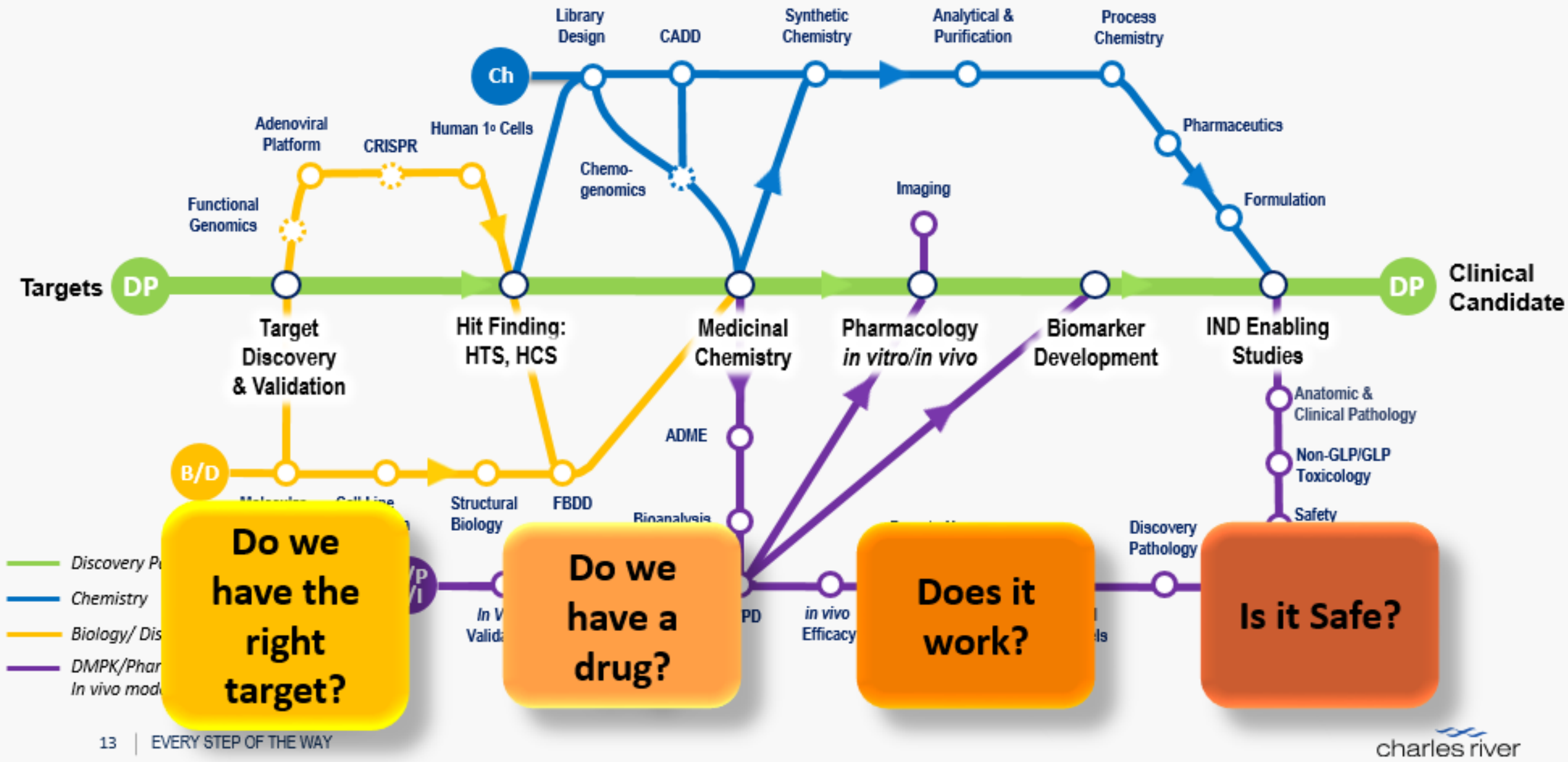
‘END TO END’ INTEGRATED DRUG DISCOVERY



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GROWING NUMBER OF OTHER MODALITIES

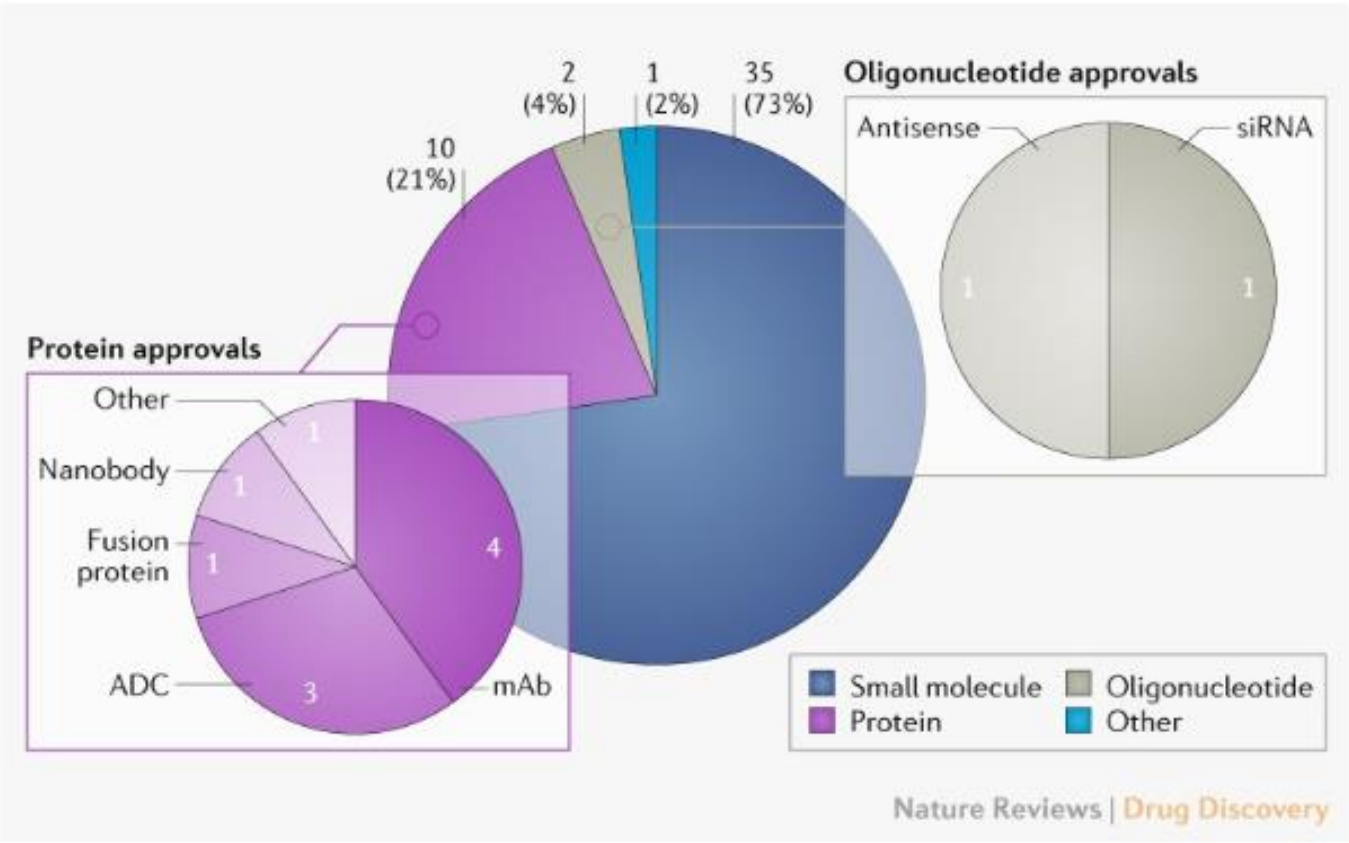
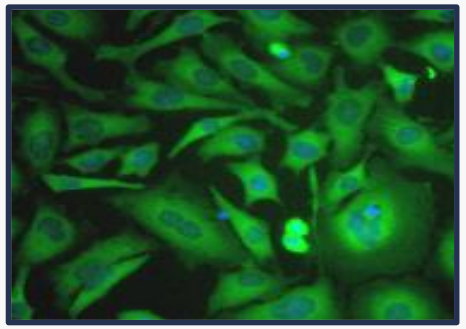


Fig. 4 | **CDER approvals by modality.** 'Small molecules' includes all peptides of up to 40 amino acids in length. Small molecules and oligonucleotides are approved as new molecular entities (NMEs). Protein-based candidates are approved as biologics license applications (BLAs). ADC, antibody–drug conjugate; mAb, monoclonal antibody. Source: *Nature Reviews Drug Discovery*.

Mullard, A. (2020) Nature Reviews Drug Discovery

Cell Therapy

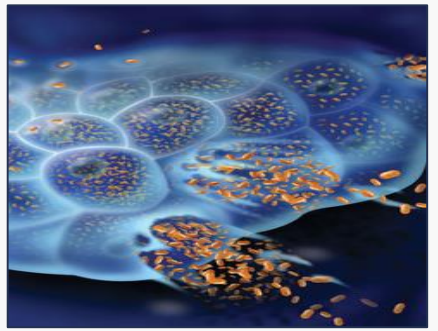


Regenerative Medicine
CART / TCR-T
Genetically-modified Cells

Gene Therapy

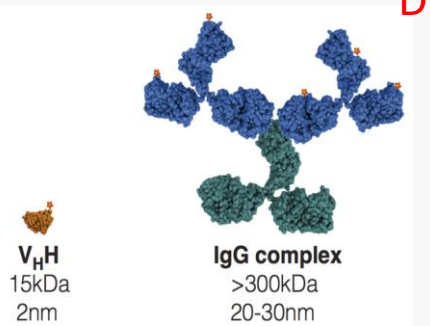
Gene (protein) replacement
Gene silencing
Gene Editing

Oncolytic virus



Antibodies

DBIO



Selectivity

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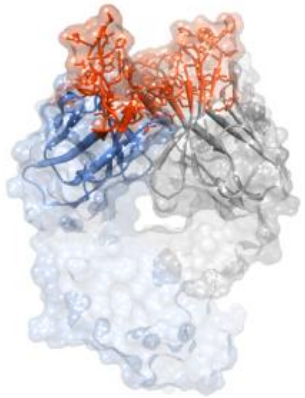
ANTIBODY THERAPEUTICS

Distributed Bio – a Charles River Company

Clever approach to getting Abs into brain and to targets

SuperHuman 2.0 scFv Phage Display



superhuman2.0

DIVERSITY

* 76 billion unique fully human antibodies
* >5000 enriched clones against any target panned
* Hits on GPCRs, ion channels, pMHC, rare epitopes, etc
  * 100% success rate on ALL targets panned
* Unprecedented, fully-natural CDR diversity
* Computationally optimized CDR fitness

DEVELOPABILITY

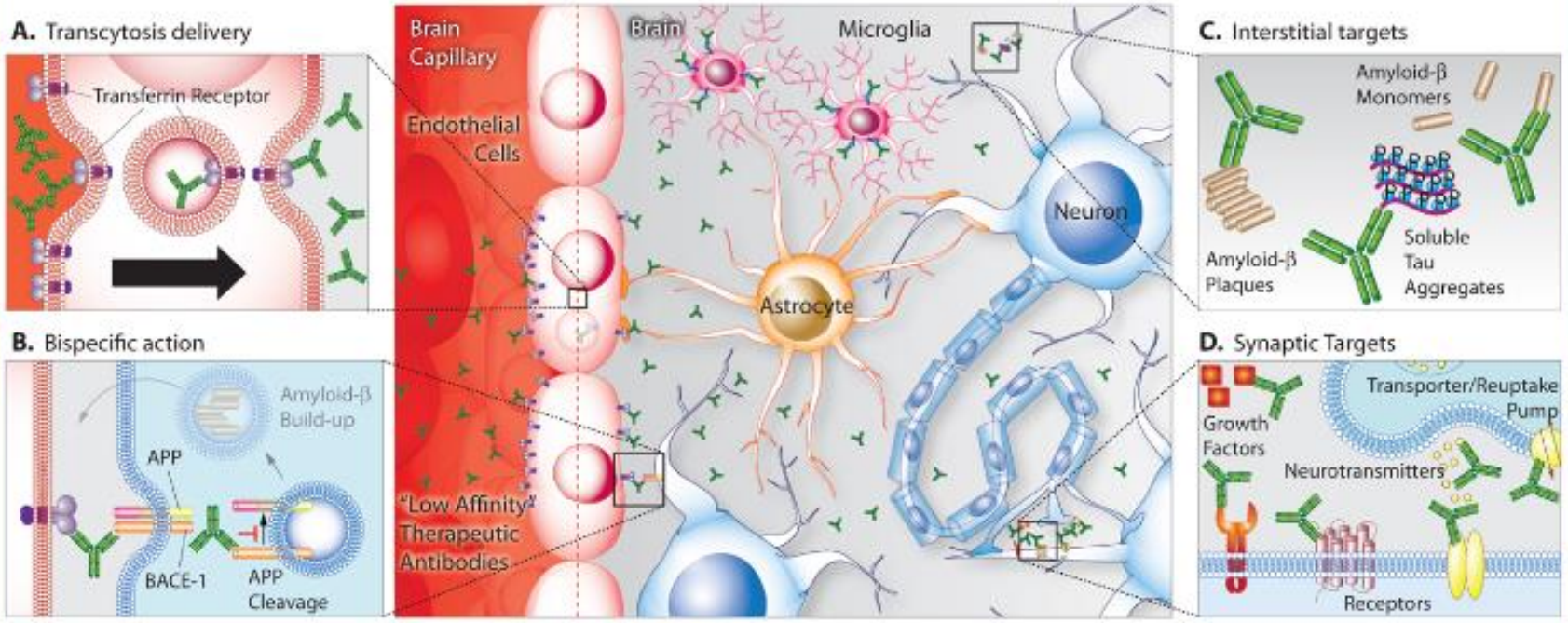
* Drug-worthy scaffolds
* Naturally selected CDR diversity
  * 100% germline frameworks
* Enhanced thermostability, minimized immunogenicity
* Depleted biochemical liabilities

SPEED

* 2-month discovery + 3-month optional optimization
* Single-pass multi-parameter optimization with Tumbler
* Easy affinity maturation; routinely to 100-300 pM
* Easy cross-species optimization; no surrogate needed
* Refine specificity and selective activation
* Machine Learning to further optimize wet-lab identified variants

A decade of computational immunology Big Data distilled into a revolutionary antibody library

Transferrin-Iron Complex Through Receptor-Mediated Endocytosis



Adapted from *Steven M. Paul Sci Transl Med 2011;3:84ps20*

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PRECLINICAL STUDIES WITH CELL & GENE THERAPY PRODUCTS

Key takeaways from the FDA Guidance Document

- Studies should be guided by traditional pharmacology/toxicology principles…BUT
- There is no standardized preclinical testing paradigm, each therapy is evaluated on specific product characteristics
- Proof-of-Concept study objectives:
  - Consistent and well-characterized product is mandatory, when possible use product intended for patients
  - Use animal models of disease to provide insight into dose, activity and toxicity
  - Characterize MOA and establish biomarkers relevant to pharmacology
  - Establish effective dose range, timing of product administration and dosing schedule
  - Optimize biodistribution and fate of product (engraftment, migration, differentiation, tumorigenicity)
  - Identify toxicities that might arise in a clinical setting
  - Incorporate appropriate safety endpoints that capture full spectrum of acute and delayed-onset toxicities
  - Reports; sufficiently comprehensive to allow for independent interpretation of the study results

DIFFERENCES FROM DRUGS

Addressing Toxicology/Safety Concerns

- Less concern for systemic toxicity
- Certain efficacy endpoints can be measured in a relevant animal model of disease
- GLP study may be done in one animal model of disease
- Immunogenicity concerns / Infection
- Cell migration *in vivo*
- Cell phenotypic stability *in vivo*
  - Tumorigenic potential / Ectopic tissue formation
- Irreversibility
  - Cells can rarely be removed
- Tissues also retained for Q-PCR, ISH, IHC evaluations

- Route of administration emulating clinical regimen
- Incorporate appropriate controls
- Animal model robust enough to endure chronic toxicity for the life of the model
- Dose-range study before the main study
- Standard toxicological evaluations

Milasen: The drug that went from idea to injection in 10 months

A custom antisense oligonucleotide drug has set records for both personalization and speed in drug development – CLN7 fatal neurodegenerative condition called Batten disease

by Ryan Cross

OCTOBER 16, 2019 | APPEARED IN VOLUME 97, ISSUE 42

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DO WE HAVE A DRUG?

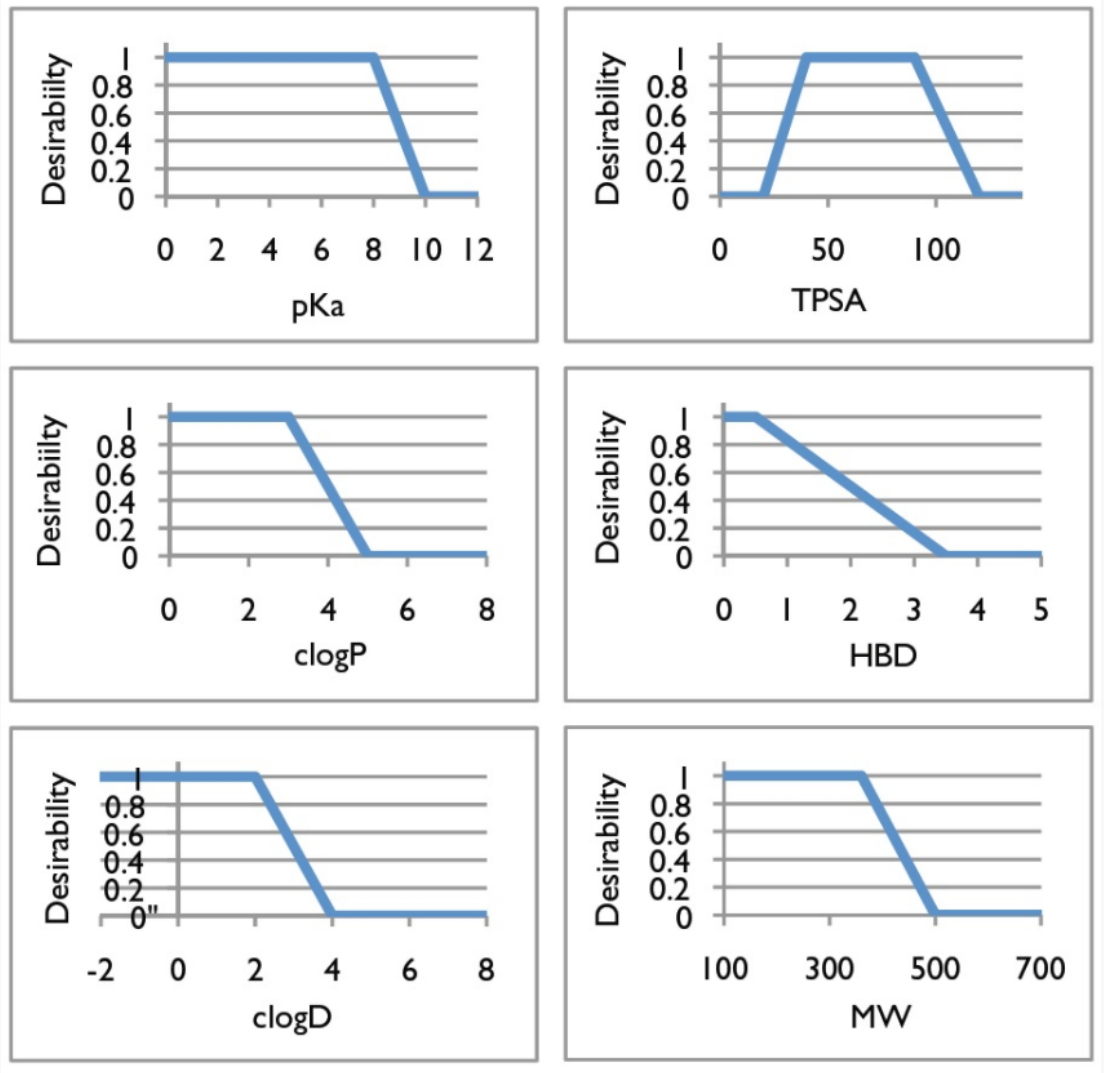
Drug likeness Metrics

Lipinski's Rule of 5 – developed for oral drugs based on solubility and permeability

Lipophilicity (logP and logD), molecular weight (MW), number of hydrogen bond donors and acceptors (HBD and HBA), polar surface area (PSA), acid dissociation co-efficient (pKa)

A similar approach was proposed by Wager et al (2010) for the selection of compounds with an improved chance of success as a drug intended for a target in the central nervous system (CNS).
(MW, logP, logD, PSA, HBD and pKa of the most basic nitrogen) The **'CNS MPO score'** is calculated by adding the desirabilities of the individual properties to give a number between 0 and 6.

Really just a starting point
Most screening libraries are 'lead like'



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BEGIN WITH THE END IN MIND!

Properties of Drug-Like Molecules

nM – not uM

- Potent
  - Modulate the target in a predictable way
- Selective
  - How selective is selective enough?
- Formulatable
  - Reasonable synthetic route or method of production
- Good safety profile
  - Understanding of toxicity
  - No such thing as a "magic" therapeutic index

On target on tissue
On target off tissue
Off target on tissue
Off target off tissue

Must allow for target engagement

- Well-behaved pharmacokinetics
  - PK relates to the pharmacodynamics
  - Amenable to QD dosing (BiD acceptable in some cases)
  - Rapid, predictable onset of action
  - Consistent metabolism
  - Clearance is important
  - No accumulation
  - Understand drug-drug interactions
- Available biomarker

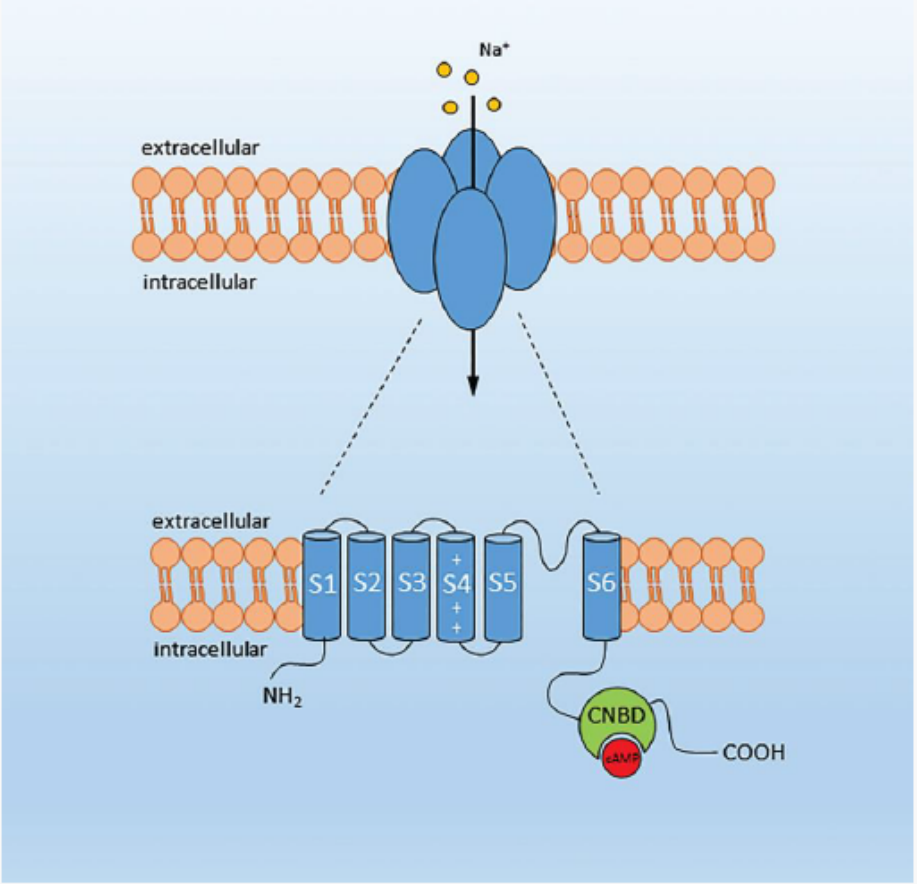
Relevant to clinic

HCN ION CHANNELS

Hyperpolarisation-activated Cyclic Nucleotide-gated channels

- HCN ion channels are expressed in a range of excitable cells
  - Function in peripheral and central neurons as well as in cardiac cells
- Four members of the family HCN1-4 with approx. 60% sequence identity
  - Higher identity within the pore
- Inward current carried by sodium ions
- Channels are opened by hyperpolarisation
  - For HCN2 and HCN4, channel opening by hyperpolarisation is enhanced by intracellular cAMP
- Subunits of HCN1-4 form tetramers in the cell membrane
  - HCN1 and HCN4 have an important role in pacemaker activity in the heart
  - HCN2 has significant expression in pain-sensing nerve fibres

Selectivity targeting ion channels is tough



Tsantoulas *et al*, *Biochemical Journal*, **2016**, *473(18)*, 2717

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HCN2 CHANNELS AS "THE PACEMAKERS OF PAIN"

Target validated

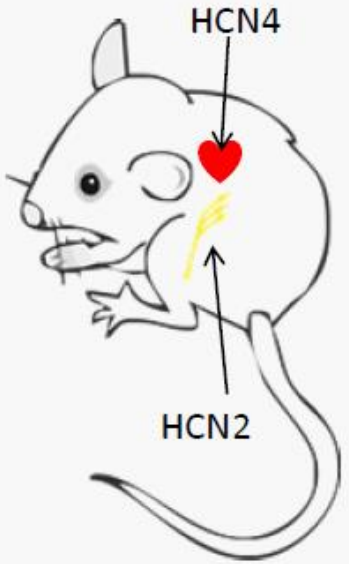
Peter McNaughton

HCN2 Ion Channels Play a Central Role in Inflammatory and Neuropathic Pain

Edward C. Emery,[1*] Gareth T. Young,[1*] Esther M. Berrocoso,[1,2] Lubin Chen,[1] Peter A. McNaughton[1†]

Science
AAAS

9 SEPTEMBER 2011 VOL 333



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EFFECTS OF HCN2 BLOCK IN CNS

- HCN2 is expressed in the central nervous system
- Genetic deletion of HCN2 in mice causes epileptic seizures
- Similar effects are observed in humans with HCN2 loss of function mutations
- Partially brain penetrant HCN2 blockers dosed at high concentrations caused tremors

On target Off Tissue Tox

Restriction from the CNS is required for an HCN2 blocker for neuropathic pain

ORAL ION CHANNEL BLOCKER PROJECT FOR NEUROPATHIC PAIN

Academic project funded by the Wellcome Trust

Objective

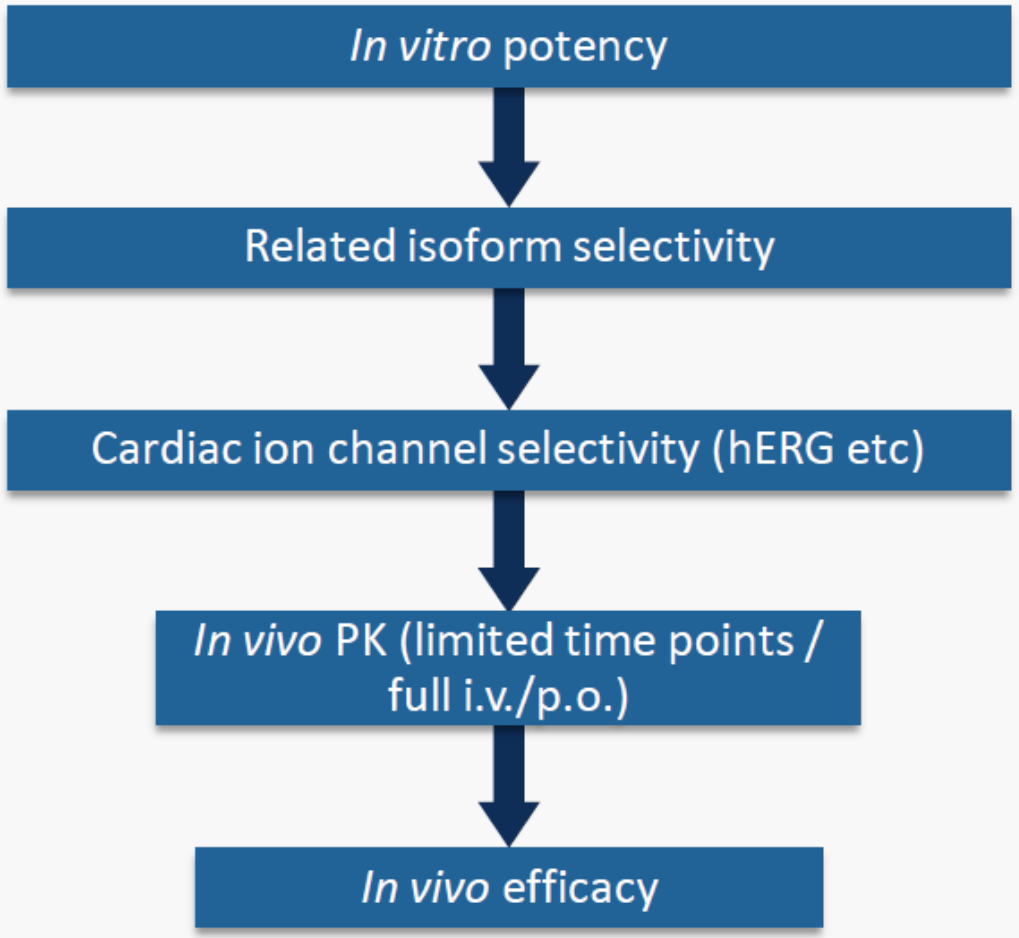
- Potent HCN2 blockers with selectivity over related isoforms to provide a window between analgesia and bradycardia
- High analgesic efficacy, equal or superior to existing treatments such as gabapentin
- High selectivity over cardiac ion channels
- Restriction from the CNS to avoid possible on-target neurological effects
- *In vivo* half-life and oral bioavailability to support once or twice daily dosing

Medicinal chemistry starting points

- Virtual screening
- Knowledge-based design

ORAL ION CHANNEL BLOCKER PROJECT

ADMET properties:
PPB, microsomal stability
CYP inhibition, MDCK-MDR1
permeability and efflux



nM

HCN1 and 4 versus HCN2

Nav1.5, hERG

Off target effects:
CEREP, cardiac and pain ion channels etc

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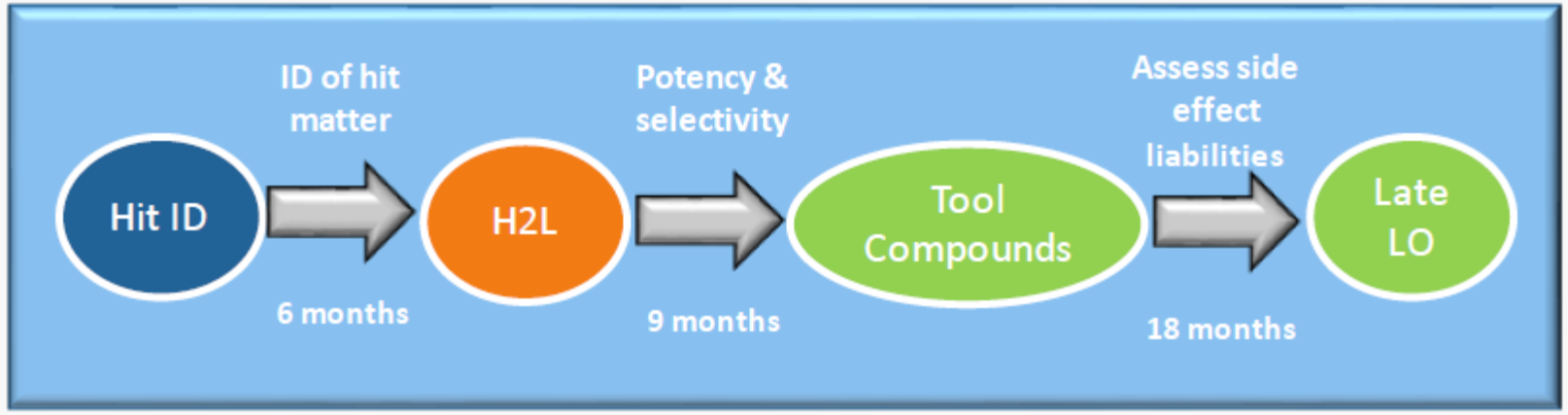
OUTCOMES

Project went smoothly but took 3 years



Merck gives King's College London, Wellcome Trust up to $340M in deal to develop non-opioid painkillers
March 8, 2019


Peter McNaughton

- Excellent ion channel activity with good selectivity over isoforms
- High selectivity over off-target cardiac ion channels
- Significant reduction in bradycardia compared to tool compound
- Lead compound is peripherally restricted with 235-fold window over neurological side effects
- *In vivo* PK profile requires further optimisation for once or twice daily dosing
- Three patents have recently been filed covering the area

Potent and selective analogues identified with *in vivo* proof of concept

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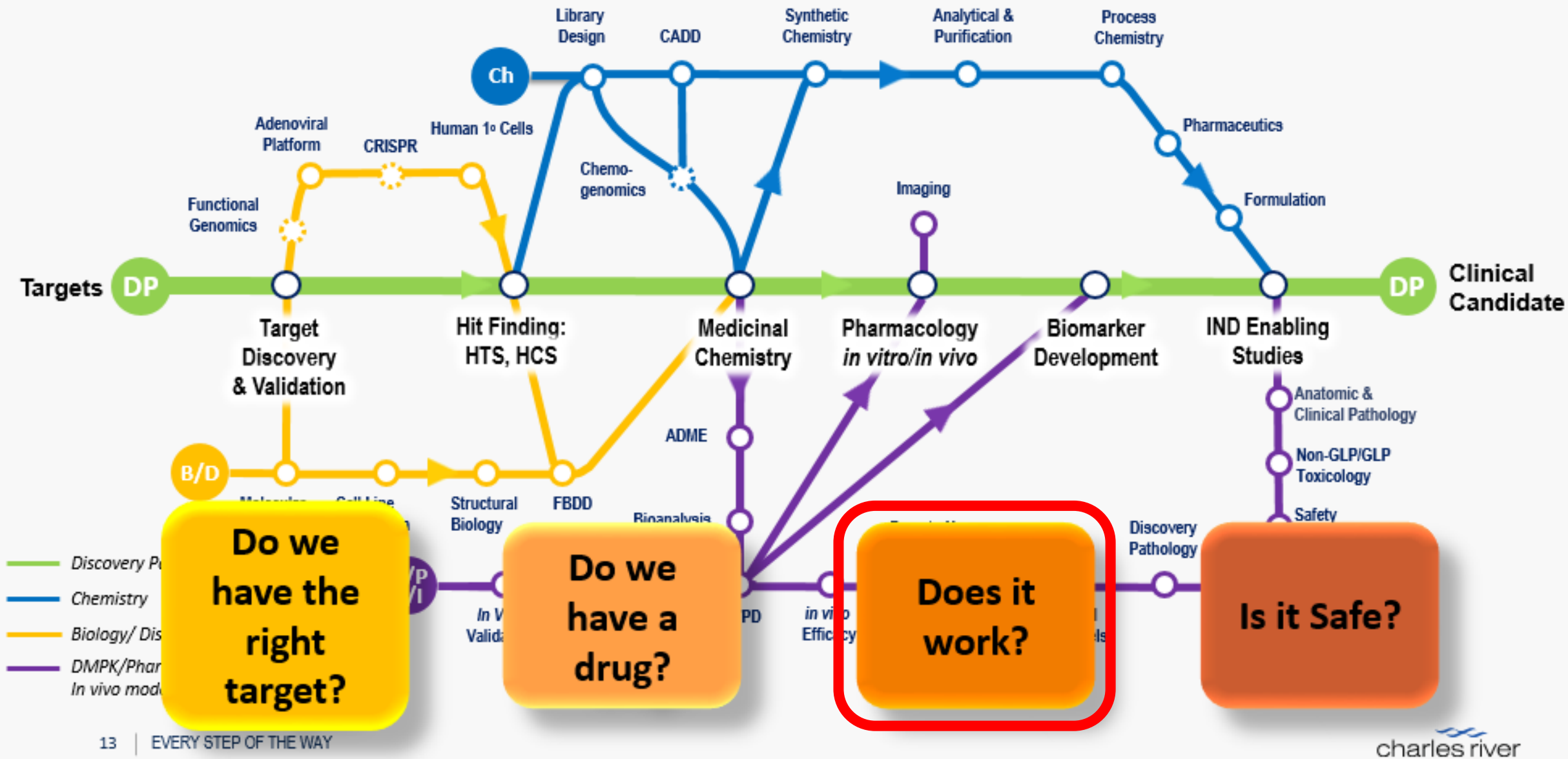
‘END TO END’ INTEGRATED DRUG DISCOVERY



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DOES IT WORK?

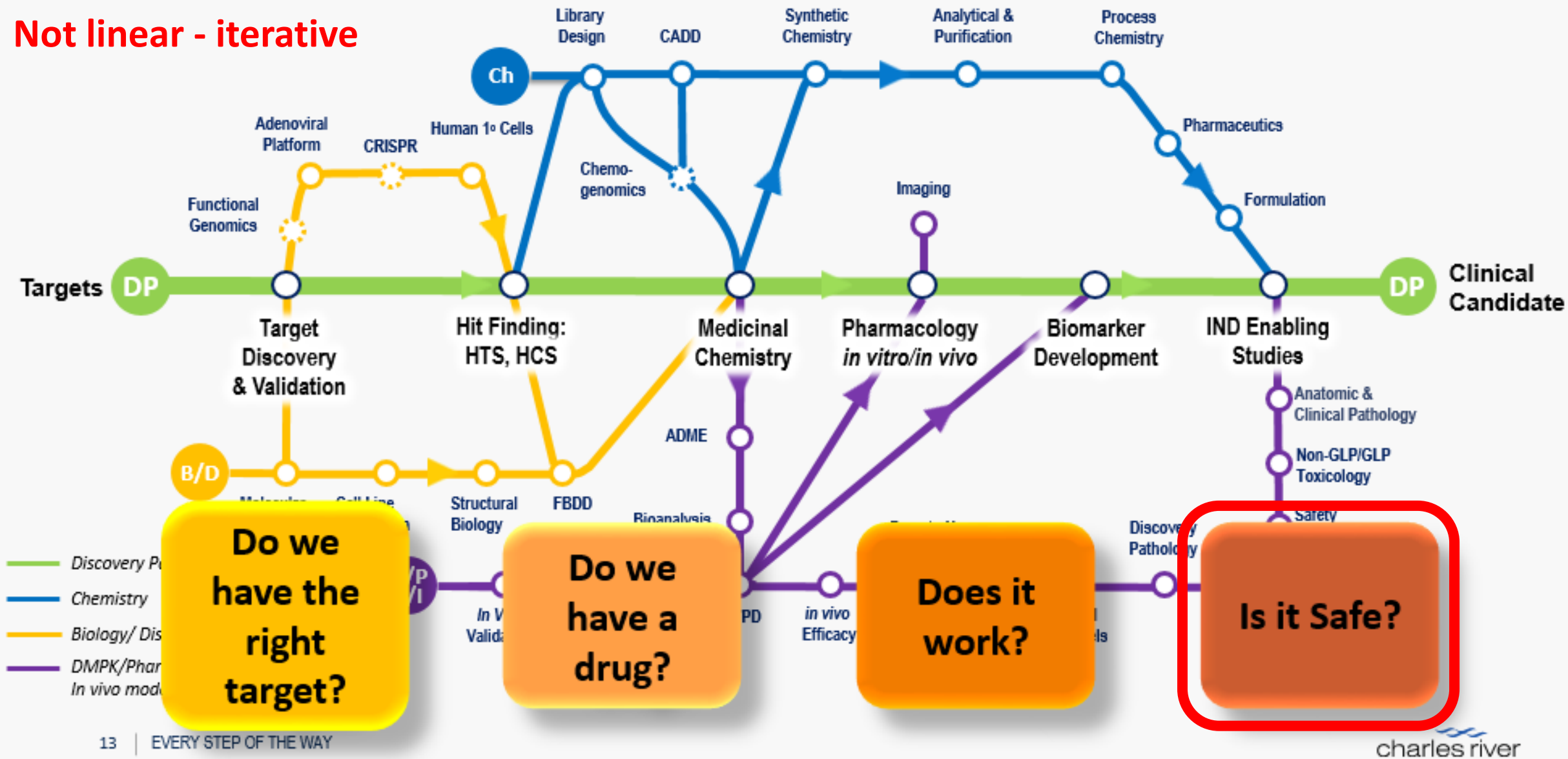
Not everyone has the luxury to start at the beginning….or return to the beginning

A chemical tool is a fit for purpose compound and may be good enough to get **POC**

- Might come from literature
- Likely never to become a drug
- Should serve as a starting point for a synthetic chemistry strategy but doesn't have to
- Must establish target engagement in vivo - think BIOMARKER i.e. HTT – can be imaging/behavior too…
    - Go with highest tolerated dose –MTD
    - Highest exposure over prolonged period (PK –route and frequency of dosing) – therapeutic or prophylactic
    - Right tissue? PD – is it getting to brain - to the cells, target if inside cell? (MDCK-Pgp/Brain to plasma/ISF-microdialysis)
- Choose the right model
    - TAT and cost of running model – AD vs PD for neurodegeneration
    - Predictive validity verses construct and face validity – Tg2576 has been cured in mice >300 times
        - Why have trials failed – pain, stroke, AD
    - All animal models are bad but some are useful
        - Replicate previous result or show robust effect across models – be careful not to over anthropomorphize model or over interpret

‘END TO END’ INTEGRATED DRUG DISCOVERY

Not linear - iterative



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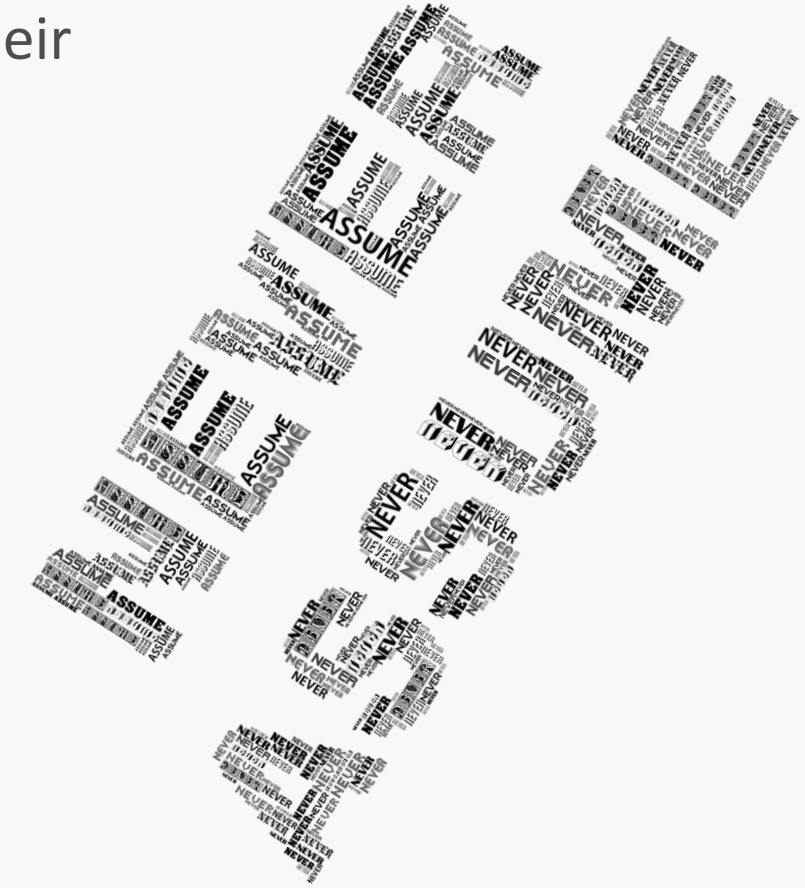
CASE STUDY: STARTING WITH THE WRONG SPECIES!

Company “A” wants to use CRL to perform GLP studies for their small molecule program.

During discussions, client provide summary of data so far:

- In vitro efficacy studies
- In vivo pharmacology studies
- Pharmacokinetic studies in rats and dogs
- Maximum tolerated studies in rats and dogs
- 2-week toxicology studies in rats and dogs



However, they lacked in vitro metabolite data!

In vitro metabolite data revealed dog lacked major human metabolite. The dog was **NOT** an appropriate toxicology model!

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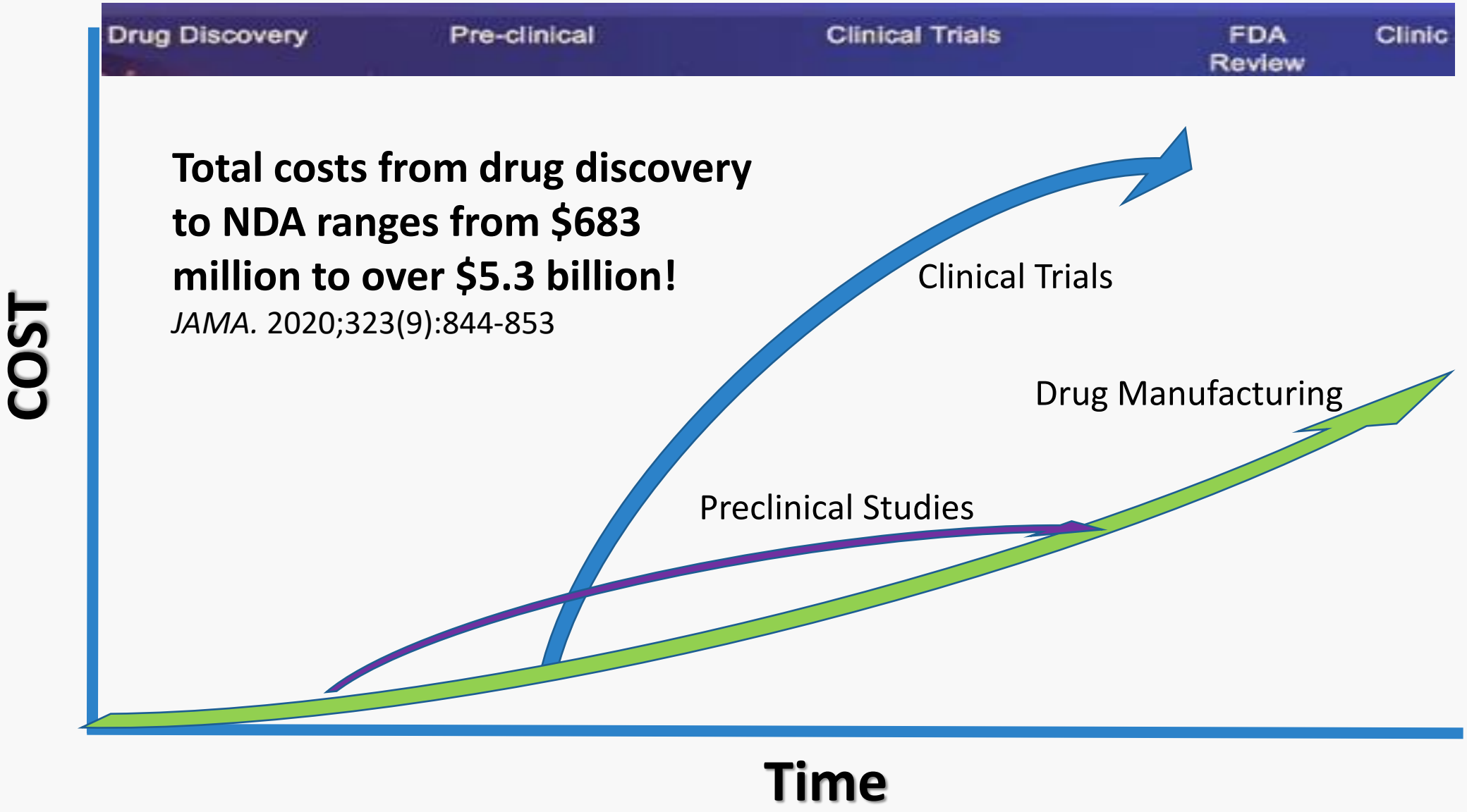
Preclinical Strategy

Deciding On the Right Approach

COST DURING DRUG DEVELOPMENT



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PRECLINICAL DEVELOPMENT IS AN EXPENSIVE INVESTMENT

Item	Pre-Clinical		Phase I
	Range-Finding	GLP Toxicology	
API Needed	50 - 200 grams	0.20 - 2.0 kilograms	2 - 10 kilograms

API Need For	Amount, grams
Dose Range Finding Studies	100
Salt Screen	10
Polymorph Screen	10
GLP Toxicology Studies	1,000
Formulations research	100
Reference Standard	50
Phase I	3,000
Stability Study	25
ST	4,295
Program Contingency @ 25%	1,074
ST	5,369
Mass Contingency @ 25%	1,342
Program Total	6,711

Budget for Preclinical package:

$1 to 2 million US… or more!

(depends on complexity of the program!)

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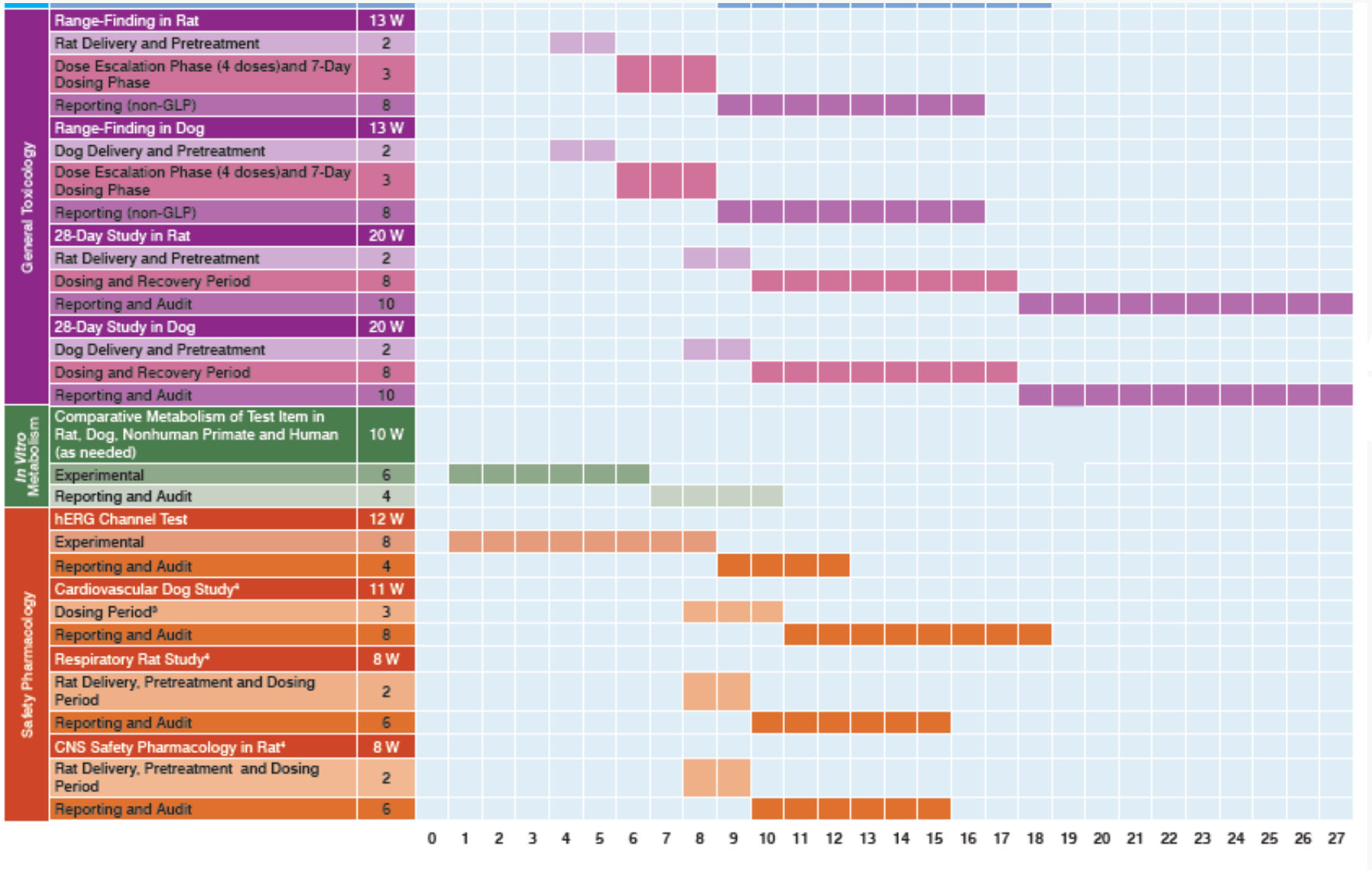
SMALL MOLECULE INVESTIGATIONAL NEW DRUG PROGRAM TIMELINE

Continued…



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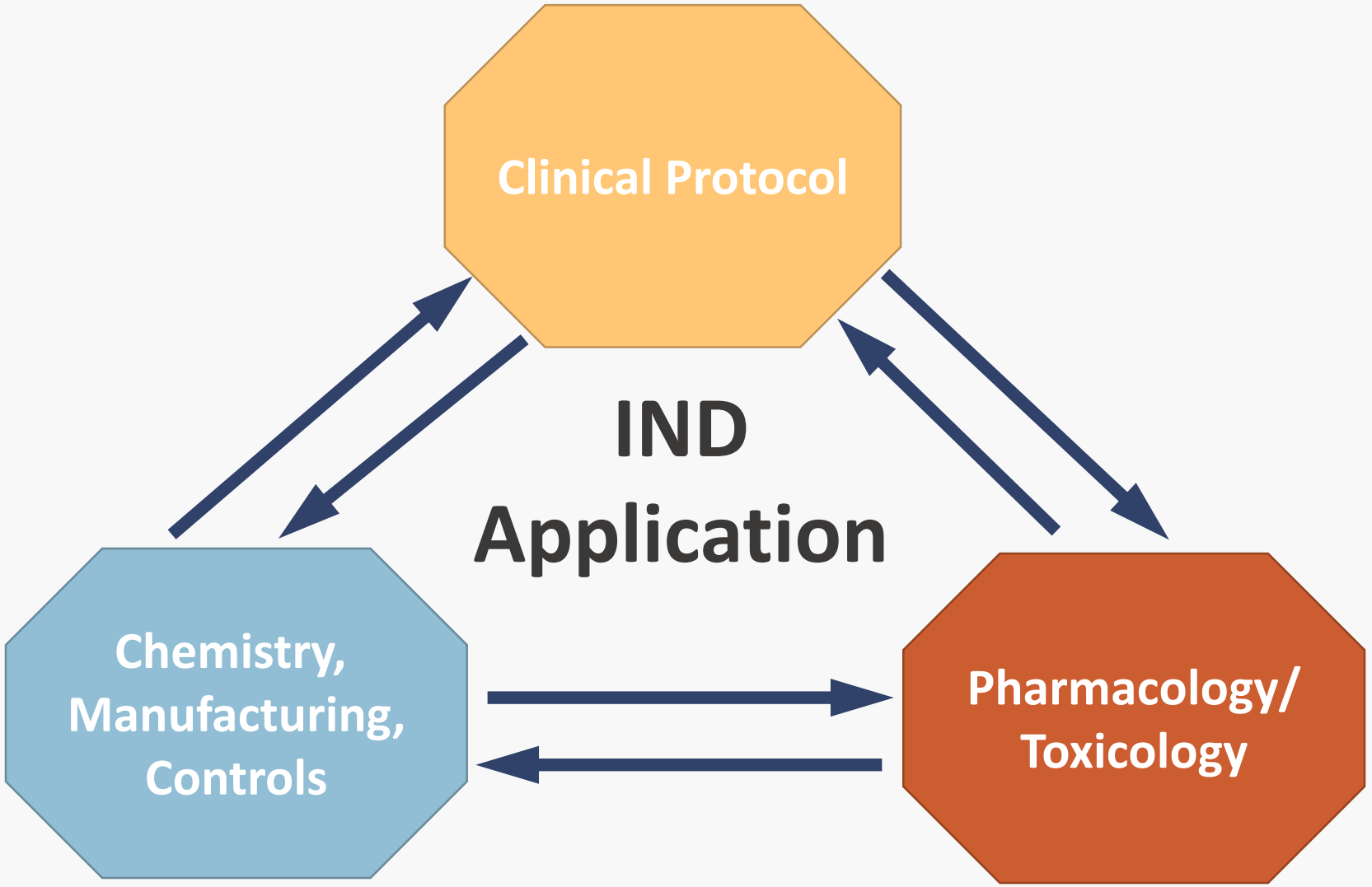
KEY ELEMENTS OF THE IND SUBMISSION



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CHEMISTRY, MANUFACTURING AND CONTROLS

Sufficient information to assure:

- Proper identification, quality, purity and strength
- Whether batches can be adequately produced and consistently supplied

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CASE STUDY- “COMMITTING BEFORE PLANNING”

Company Management:

- CEO- former professor creating company to commercialize antibody discovery
- CFO- Finance background only

Situation: BOD-approved time - 15 month time line

Current status: Protein expression only at transient cell line stage

Major Steps Needed to accomplish to reach IND: Total= 22 to 28 months!

- Cell line development and manufacturing
  - Scheduling 3-5 months
  - Cell line development 3-5 months
  - Protein (drug) production 4-6 months
- Toxicology program 12 months

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CLINICAL TRIAL PROTOCOL

- Describes the objective(s), design, methodology, statistical considerations, and organisation of the trial.
- The primary objective will dictate the primary end-point.
- Primary end point measurement will be used to calculate sample size.
- Specify how to ensure safety of the subjects/patients in the study (#1 reason INDs are placed on clinical hold)
- **Need this first to appropriately design supporting toxicology program**

DURATION OF IND-ENABLING TOXICOLOGY STUDIES

General design considerations

- Dosing duration
  - Typically 1 month to support Ph. 1, 3 months to support Ph. 2, 6 or 9 months to support Ph. 3 and beyond
  - ICH M3 recommendations

Maximum Duration of Clinical Trial	Recommended Minimum Duration of Toxicology Studies	
	Rodents	Non-rodents
≤ 2 weeks	2 weeks	2 weeks
2 wk. < X < 6 mo.	Same as clinical duration	Same as clinical duration
> 6 mo.	6 mo.	9 mo.

CASE STUDY: APPLES VS ORANGES

When 4 doses ≠ 4 doses

IND-Enabling Study Design:

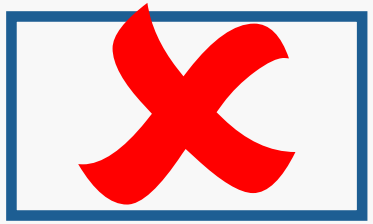
- 4 doses given weekly followed by a 4 week recovery period
- 4 dose groups (1 control and 3 test article-dosed groups)

Phase I Clinical Trial Design:

Phase 1A: Single Ascending Dose (SAD)

Phase 1B: Multiple Ascending Dose (MAD)

4 doses given monthly



NONCLINICAL TOXICOLOGY

Study Objectives

- The data from IND-enabling studies are needed to:
  - Estimate a safe starting dose for clinical trials
  - Identify potential biomarkers for monitoring toxicity in clinical studies
  - Identify potential target organs for toxicity
  - Assess reversibility of toxicities
  - Establish margin of safety
- Prior to registration/marketing, data from animals should be used to identify potential long-term risks
  - Toxicology studies range from months to near life-time
  - Potential for adverse developmental and reproductive effects
  - Carcinogenic potential

CNS DRUG CLASSES SUBJECT TO FDA REGULATION (CSA)

Any drug with direct or indirect actions on effects on other neurotransmitter systems associated with abuse potential

- CNS Depressants (sedatives, tranquilizers and hypnotics)
- CNS Stimulants (amphetamines)
- NMDA
- Opioids
- GABA analogs
- Norepinephrine
- Acetylcholine
- Cannabinoids

PHARMACOLOGY - PRECLINICAL

Neuropharmacological Characterization

- Receptor Binding Assays
- Animal behavioral studies
  - Reinforcing Effects (Self-Administration)
  - Discriminative Effects (Drug Discrimination)
  - Physical Dependence (Withdrawal)
  - Tolerance
  - Locomotor Activity (Kinder Scientific)

  - Need for positive control stimuli
  - Species
    - Rodents
    - Nonhuman primate

ABUSE LIABILITY ASSESSMENT PACKAGE

New Drug Application (NDA) package needs to include

- Preclinical pharmacology
- Human Pharmacology
- Clinical trial data
- CSA scheduling proposal (Schedule I to V)
- Data on Overdose

NOVELTIES OF BOTH CELL AND GENE THERAPY PROGRAMS

- **Customized design for each product/indication - "CASE BY CASE"**
  - Evaluate the quality attributes of the product
  - Consider the indication and proposed clinical trial design
- **Biomarkers are key to pharm/tox translation**
  - Utilize tools, models, assays available and make product specific
  - Gene/protein expression, surface proteins, impacts on disease state
- **Source, manipulations and indication drive extent of risk assessment**
- **"Process is the product"**
  - Consider using the intended clinical product to avoid process changes

SIMILAR TO TRADITIONAL DRUG TOXICOLOGY

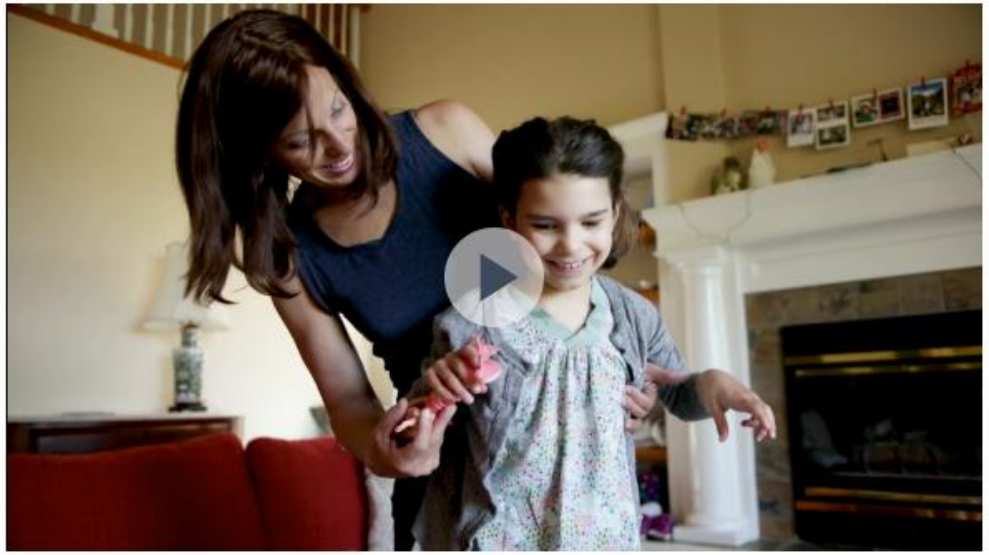
- **Support clinical trials – must be reasonably safe**
  - Preclinical data provides information for potential clinical toxicities
- **Consistent and well-characterized product is mandatory**
- **Biomarkers related to pharmacology of the product must be established**
- **Route of administration and regimen must mimic clinical conditions**
- **Dose-response established**
- **Exposure must be defined (biodistribution / fate over time)**
  - Quantitative Polymerase Chain Reaction (Q-PCR), Immunohistochemistry (IHC) and/or in-situ hybridization (ISH)

RARE DISEASE RESEARCH FOR DRUG DEVELOPMENT

Regulatory considerations

Recent advances in antisense oligonucleotide (ASO) and cellular therapies offer hope to the 300 million patients living with rare disease.

Click on picture to watch Mila's Story and how Boston Children's Hospital and CRL teamed up to develop an ASO and preclinical strategy to treat Mila from concept to treatment in just months!



Click on picture to read about Jaci's story and how Project ALS and CRL are worked together to develop a drug to uniquely treat Jaci at break-neck speed!

NONCLINICAL SAFETY IN DRUG DEVELOPMENT

Considering Regulatory Guidelines (recommendations)

- Understand regulatory context and expectation
  - ICH M3 guideline on non-Clinical safety studies for the conduct of human clinical trials for pharmaceuticals - addresses general principles for the development of non-clinical strategies and on the timing of toxicity studies in relation to the conduct of clinical trials
  - FDA (December 2002): estimating the safety starting dose in clinical trials for therapeutics in adult healthy volunteers
  - ICH S6 guidance for Preclinical safety evaluation of biotechnology-derived pharmaceuticals - Covers protein and other biological therapeutics produced by biotechnology method
  - **FDA Guidance: Assessment of Abuse Potential of Drugs**
  - …. other guidance documents may impact your drug development program

CONCLUSIONS

- Charles River has an extensive range of services and capabilities to support CNS drug development from Early discovery and target validation to the pre-market approval
- Drug discovery screening platforms should be created to ensure selection of highly potent selective safe and efficacious drugs to increase the chances of clinical success
- Safety assessment (IND-enabling programs) should be carefully planned and designed to minimize unnecessary timing delays and ensuring proper characterization of the drug for regulatory approval
- Drug products that have CNS activity and produce euphoria (or other changes in mood), hallucinations, and effects consistent with CNS depressants or stimulants will likely need to undergo a thorough assessment of its abuse potential.

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APPENDIX

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BIOMARKERS

"A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"

- NIH Biomarkers Definitions Working Group, 1998

Usually a biological molecule, such as a protein, gene, mRNA or secreted agent

Measured in the body or its products, such as disease tissue, blood, urea, cerebrospinal fluid (CSF), or other bodily fluid

Indicates a disease: **diagnostic**
Influences outcome**: prognostic**
Predicts treatment efficacy: **predictive**

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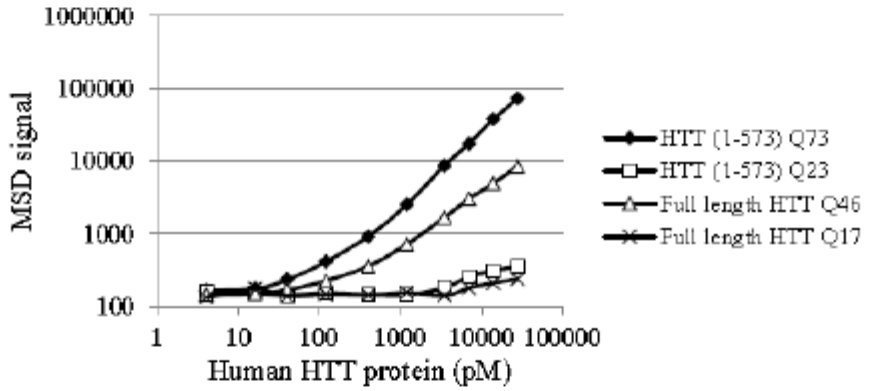
IMMUNOASSAYS FOR HUNTINGTON'S DISEASE (HD)

Developed immunoassays on the MSD platform to quantify different species of HTT protein

- Expanded vs. Non-expanded
- Human vs. Rodent
- Aggregated vs. soluble

Used for:

- Patient derived cells (lymphoblasts, fibroblasts, macrophages
- Rodent in vivo samples (brain, CSF, PBMC)
- Clinical samples (CSF, PBMC, post-mortem brain)



Recombinant HTT proteins used as standard

Quantification Assays for Total and Polyglutamine-Expanded Huntingtin Proteins

Douglas Macdonald[1*], Michela A. Tessari[2*], Ivette Boogaard[4], Melanie Smith[3], Kristiina Pulli[2], Agnieszka Szynol[4], Faywell Albertus[4], Marieke B. A. C. Lamers[3], Sipke Dijkstra[4], Daniel Kordt[5], Wolfgang Reindl[5], Frank Herrmann[5], George McAllister[3], David F. Fischer[4*], Ignacio Munoz-Sanjuan[1*]

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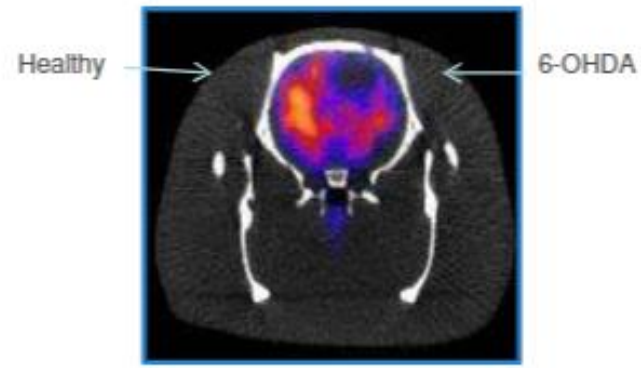
DIAGNOSTIC: SPECT IMAGING OF PARKINSON'S DISEASE STATE

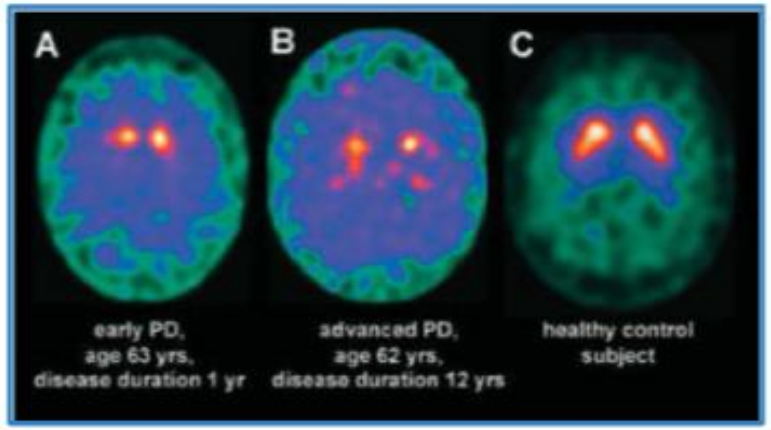
Parkinson's Disease is characterized by a loss of dopamine (DA) neurons in the substantia nigra (SN)

DA-selective lesions of the rodent SN result in loss of DA transporter (DAT) in the ipsilateral striatum, leaving the non-lesioned "healthy" side unaffected



SPECT: Dopamine transporter (DAT) in healthy rat brain hemisphere vs. 6-OHDA treated DA-lesion

Density of DAT is reduced in the striatum of PD patients (A, B) relative to the healthy brain (C)



SPECT: Dopamine transporter (DAT) in Human PD vs healthy brain