Chemistry 259

Medicinal Chemistry of Modern Antibiotics

Spring 2012

Lecture 4: *Drug Discovery, Development & Approval*
Part II

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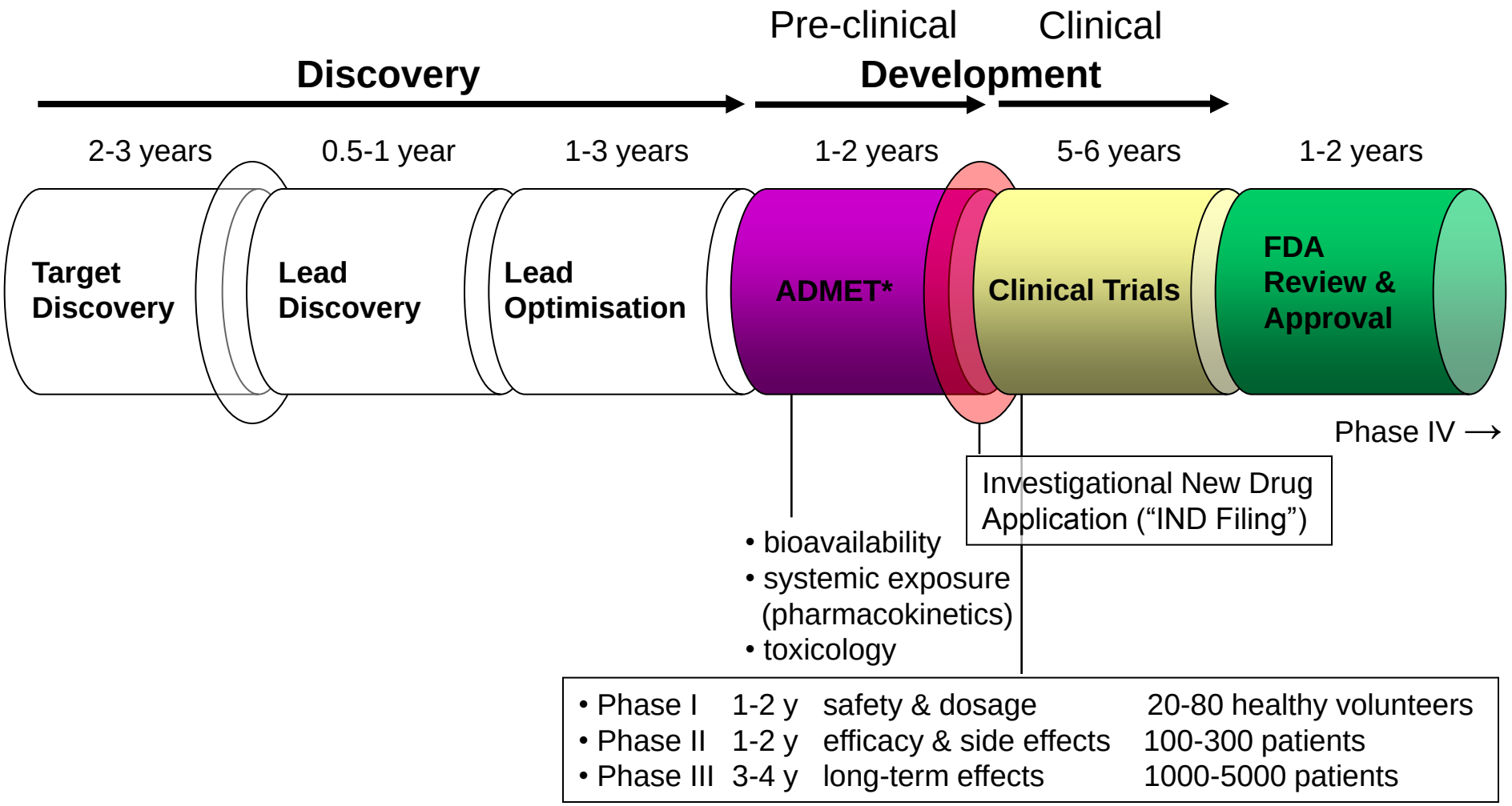
Drug Discovery & Development Process: General Overview



(*absorption, distribution, metabolism, elimination, toxicity)

Drug Discovery & Development Process: ADME-Tox

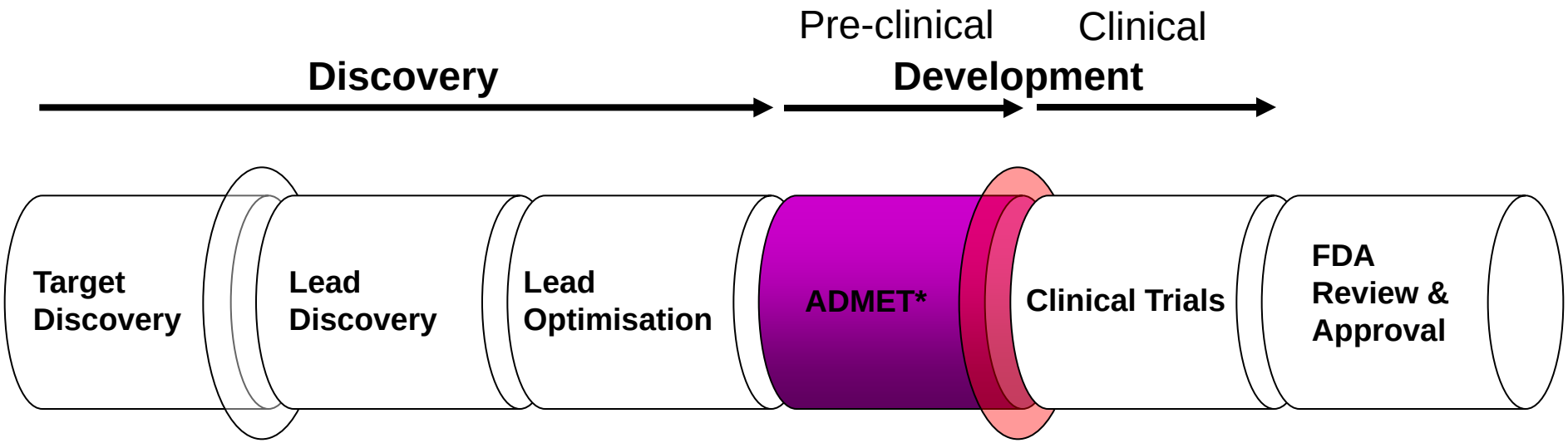
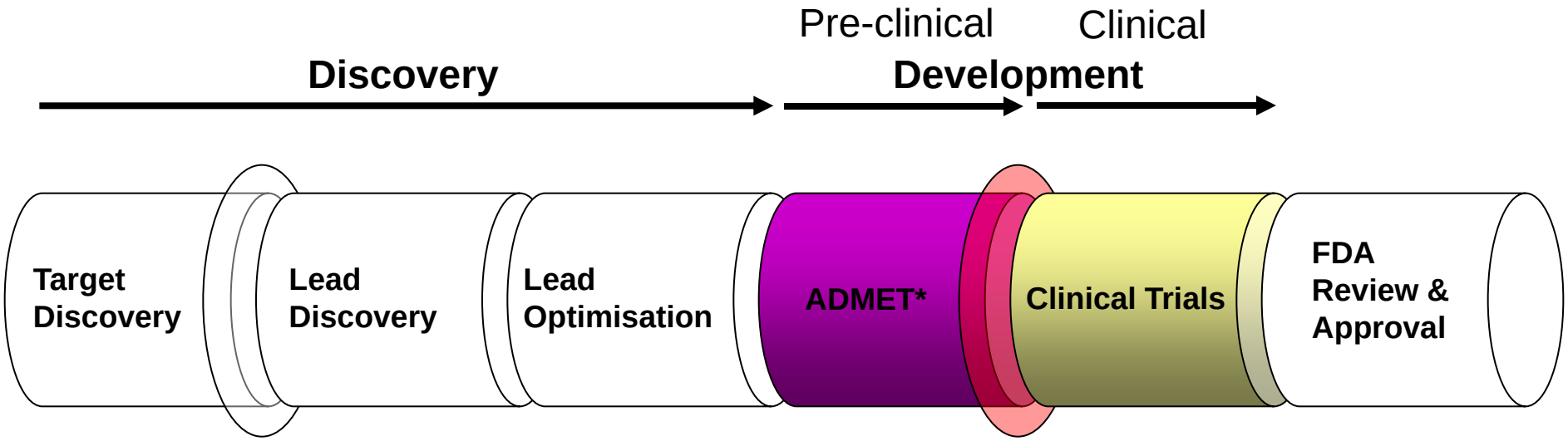


Table 1 | **Drug candidate selection: key questions and pivotal studies**

Questions	Studies that provide answers
Can the drug candidate be measured and is it stable in biological matrices?	Bioanalytical assay development.
Does the drug candidate have reasonable metabolic stability? What are the metabolites and are they active, possibly even a better drug candidates? Are there species differences in metabolism?	*In vitro* metabolism studies using animals and human hepatocytes, microsomes or human expressed enzyme systems and analysis of incubates using LC/MS/MS or LC/NMR/MS. Synthesis and pharmacology testing of metabolites.
Does the drug have sufficient oral bioavailability and persistence in the bodies of animal models?	Pharmacokinetic studies in rodent and non-rodent species after oral versus intravenous administration.
Is the drug mutagenic or cytotoxic *in vitro*?	Ames bacterial mutagenicity assay. Mammalian cell (for example, mouse lymphoma) mutagenicity assay.
What is the maximum tolerated dose (MTD) and dose-limiting toxicity?	Rising single- and repeat-dose escalation toxicology study in rodent and non-rodent species until limiting toxicity is observed.
Can active pharmaceutical ingredient (API) be synthesized at reasonable cost? Is the API stable after synthesis?	Chemical synthesis process assessment. Assay development for API purity. Chemical stability assessment. Generation of API certificate of analysis.
Can the drug be formulated for use in animal toxicology studies and early human studies?	Pre-formulation development testing. Assay development for purity and content formulated product.

Drug Discovery & Development Process: ADME-Tox

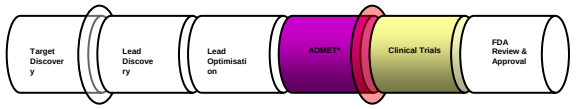


Sources of ADME-Tox data:

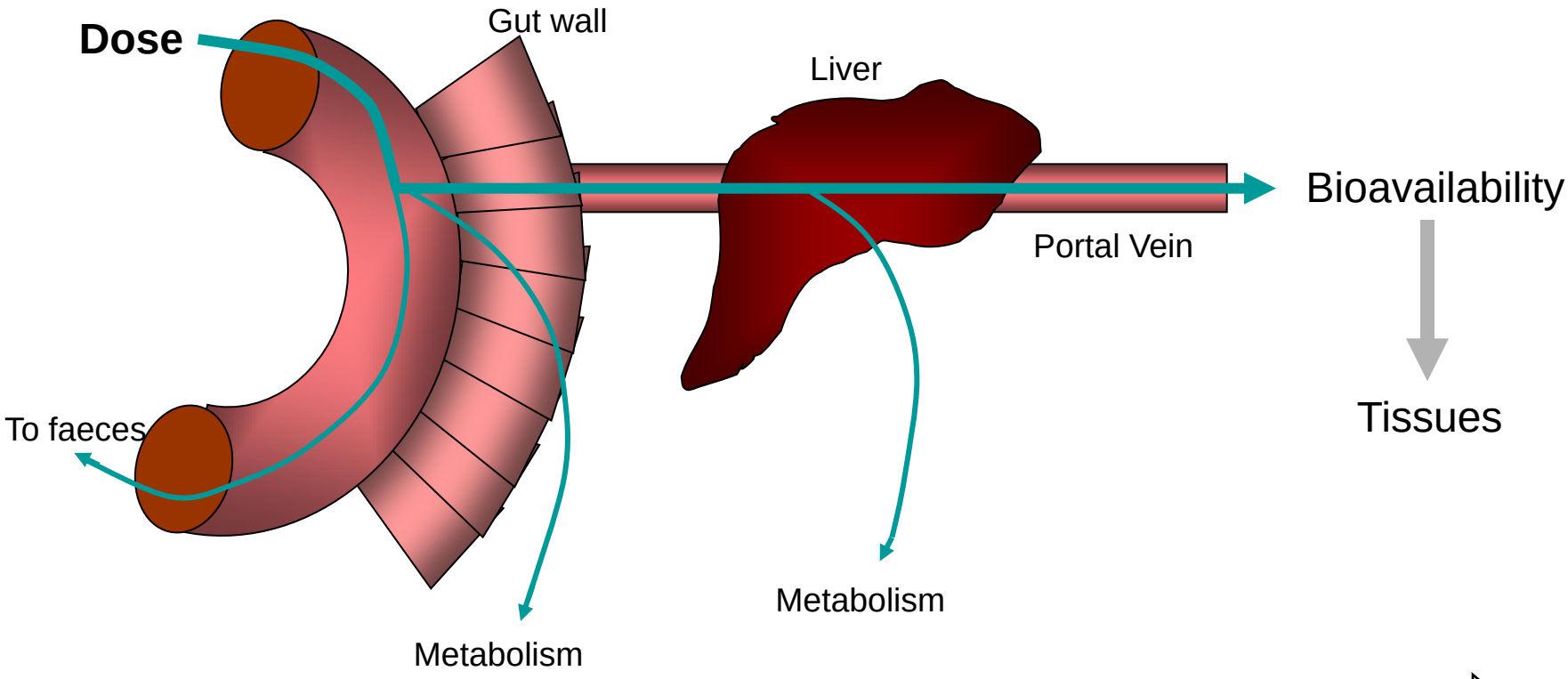
preclinical: *in vitro* assays (enzymatic, cellular)
in vivo animal studies (rats, dogs, pigs, primates):
toxicology
pharmacodynamics

clinical: toxicology & pharmacodynamics (human, Phase I)

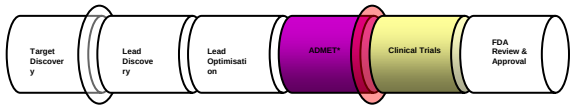
Pharmacokinetics (PK)



Study of the time course of drug concentration within the body.
It incorporates the processes of Absorption, Distribution, Metabolism and Excretion (ADME).



Pharmacokinetics (PK)



• Most drugs are given orally.

• Typically the drug dissolves in the GI tract, and is absorbed through the gut wall.

• It then passes to the liver to get into the circulation. The percentage dose reaching the circulation is called the bioavailability.

• The drug is then distributed to various tissues and organs in the body. The extent of distribution will depend on the structural and physicochemical properties of the drug.

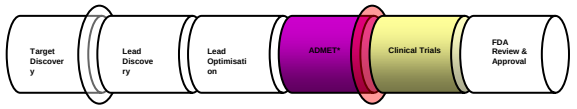
• Some drugs can enter the brain and CNS by crossing the blood-brain barrier (BBB).

• Most drugs will bind to various tissues and in particular to proteins in the blood, such as albumin.

• A good understanding of the physicochemical properties of a drug may help to predict its PK and metabolic fate.

• There is often a tension between ideal PK properties and those required for optimal binding to the target receptor.

Pharmacokinetics (PK): Definitions



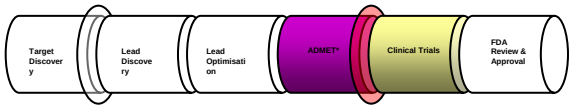
- **Clearance** is the removal of the drug from plasma and relates the rate at which a drug is given and eliminated to the resultant plasma levels (volume/time).

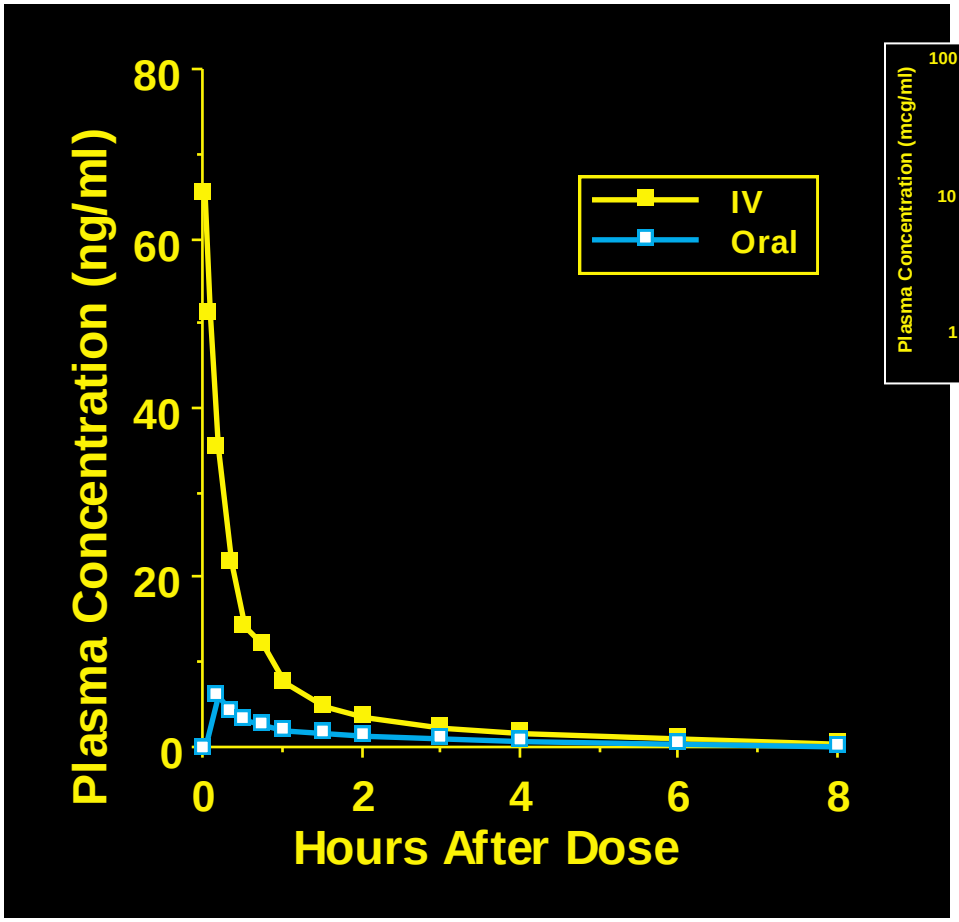
- **C_{max}** is the maximum concentration reached at the site of infection, usually taken as the peak serum level.

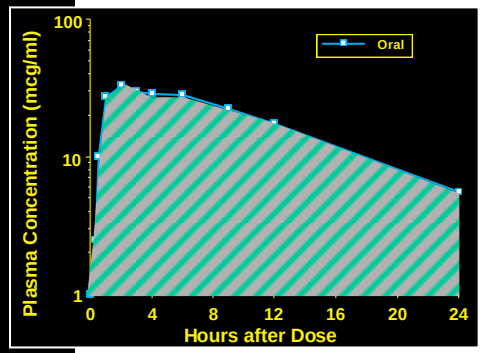
- **t_{max}** is the time taken, after dosage, to reach the C_{max}.

- **Half-life ($t_{½}$)** is the time taken for the concentration of the drug in the plasma to decrease by half. This is often used as an indicator as to how often the drug should be administered.

Pharmacokinetics: Routes and Bioavailability







Area under the curve (AUC)

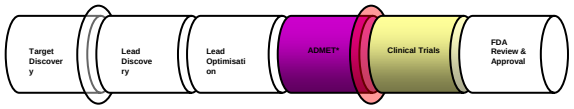
Bioavailability F [%]:

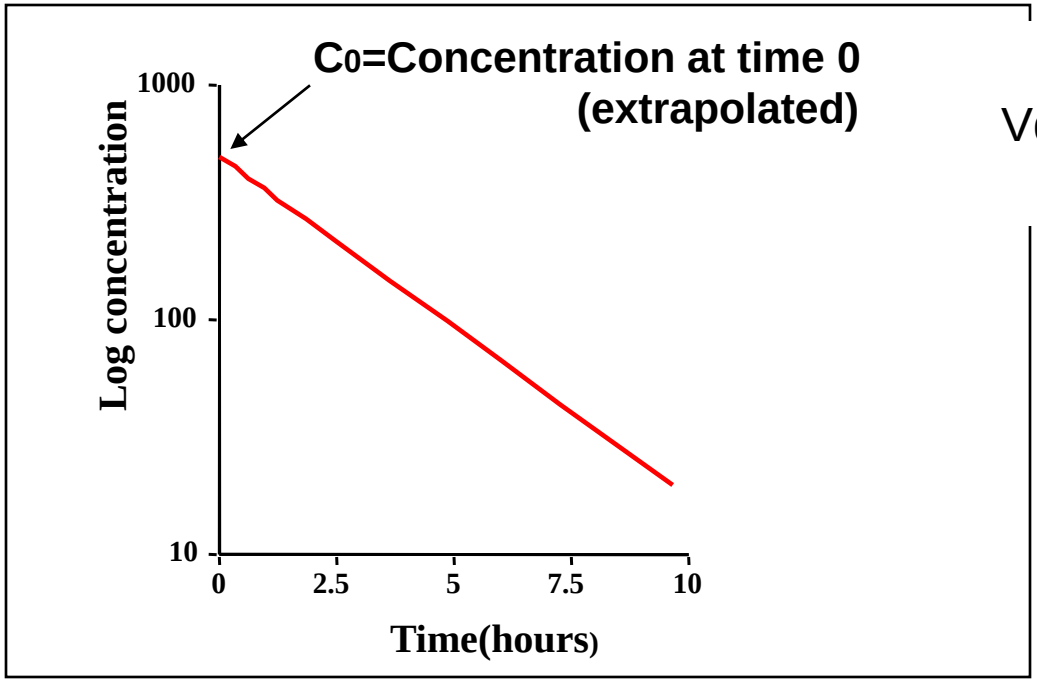
$$F = \frac{AUC_{po}/(Dose)}{AUC_{IV}/(Dose)}$$

F = 28.6%

IV: intravenous
PO: oral
SC: subcutaneous
IP: intraperitoneal
IM: intramuscular

Pharmacokinetics: Volume of Distribution (Vd)





Vd (L)=Dosis (mg) / C_0 (mg/L)

Extensive distribution (e.g. intracellular concentrations, tissue binding)leads to *low serum concentrations* and *high apparent volume of distribution*

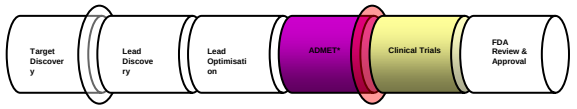
Warfarin	Vd (L)=	7
Gentamicin		16
Theophylline		35
Cimetidine		140
Digoxin		510
Mianserin		910
Quinacrine		50,000

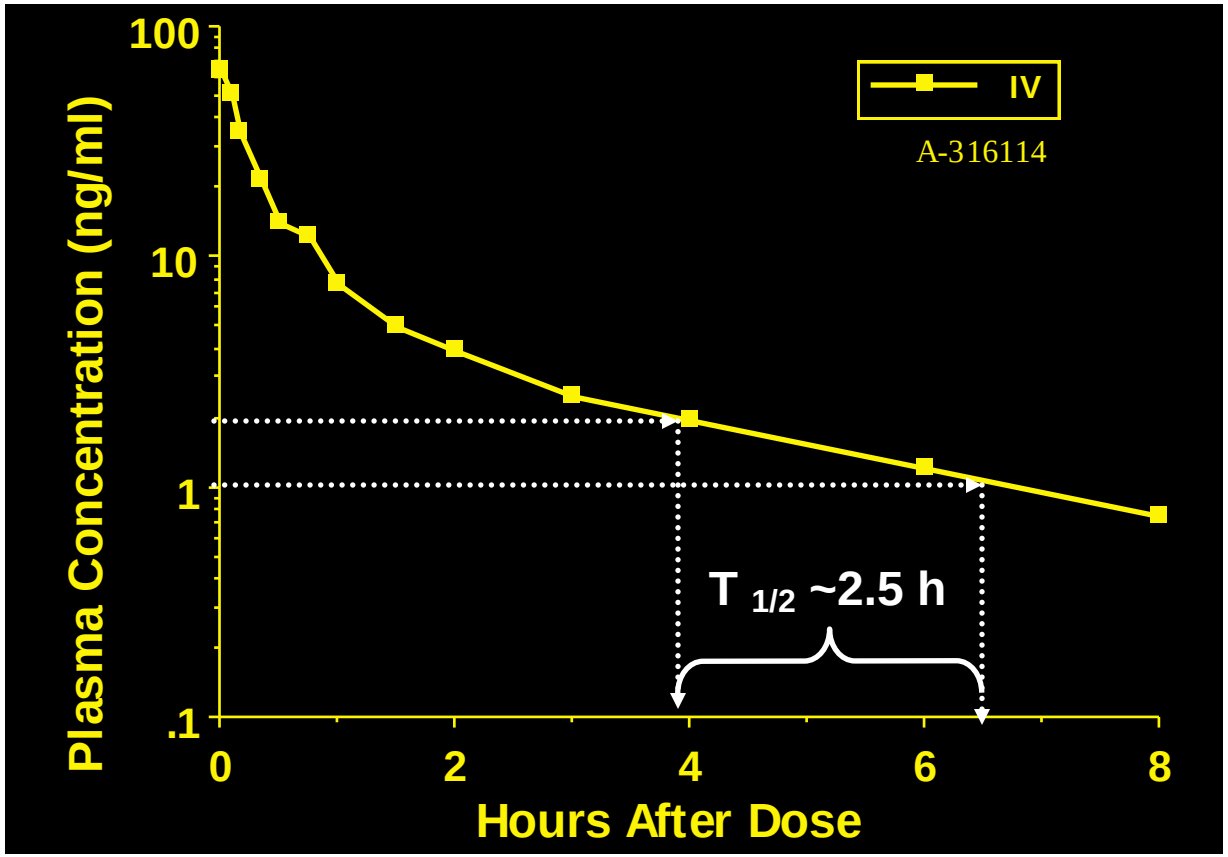
Small Vd: Mainly stays in plasma; little in tissues.

Medium Vd: Similar concentrations in plasma and tissues

Large Vd: Mainly in tissues, little in plasma.

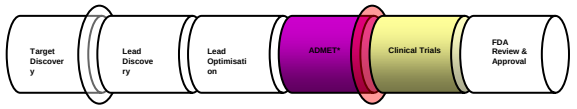
Pharmacokinetics: Half Life ($T_{1/2}$)





Species	'short'	'medium'	'long'	'very long'
Rat	<1h	1-4h	4-10h	>10h
Dog	<2h	2-6h	6-12h	>12h
Man	<3h	3-8h	8-14h	>14h

Pharmacokinetics: Half Life of Antibiotics



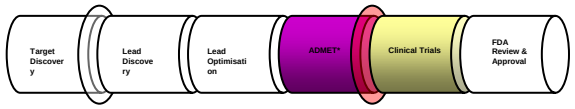
- The half-life of the early antibiotics were quite short (~ 1h). Therefore the antibiotic had to be administered many times per day.

- With oral versions, this causes problems with patient compliance and with parenteral versions, this becomes expensive in resources.

- Increasingly, the newer antibiotics have much longer half-lives, some up to > 24 h.

- This means that the patient needs to be dosed just once a day in order to maintain sufficient drug concentrations.

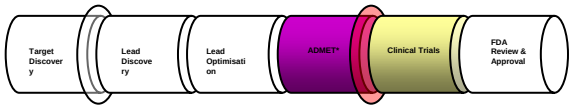
Pharmacokinetics: Clearance (Cl)

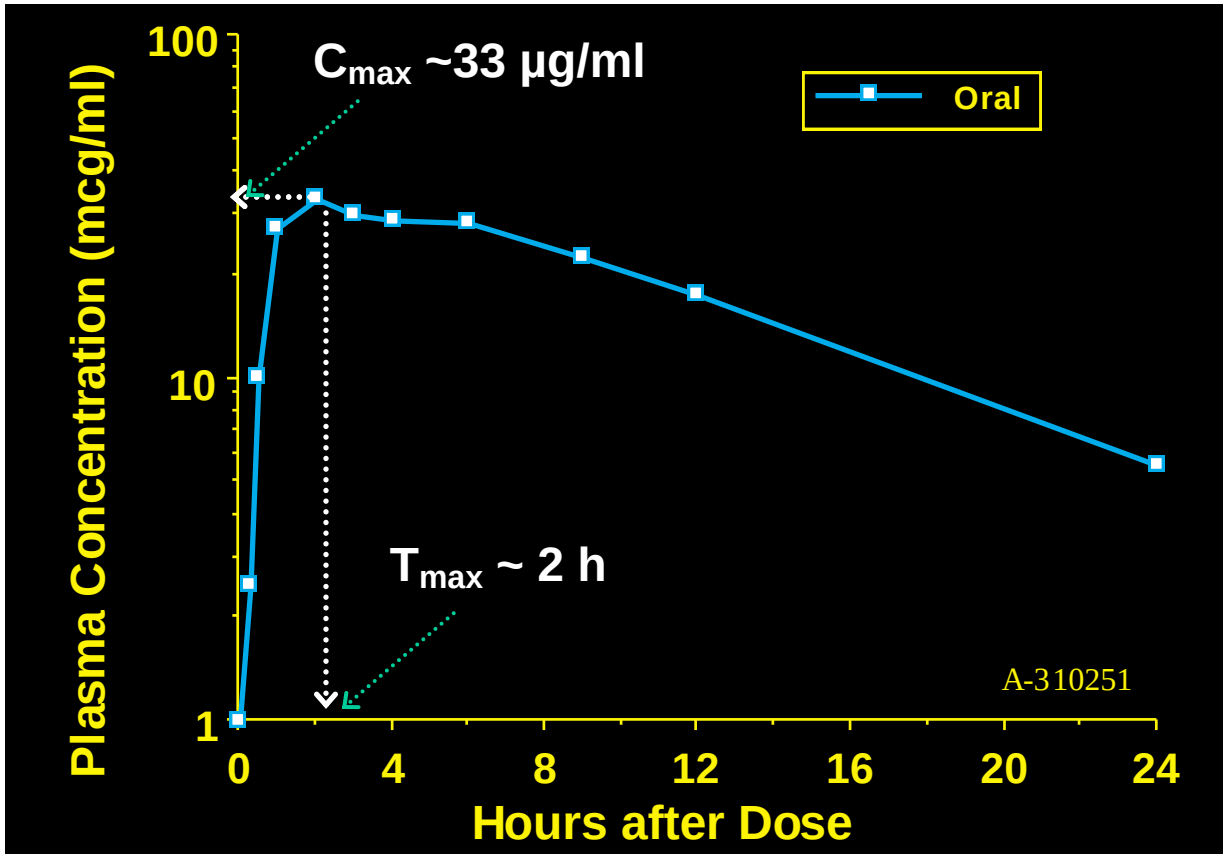


- The volume of blood/plasma that is completely cleared of drug per unit of time.
- Clearance is inversely proportional to its half-life and directly proportional to its Vd and elimination.
- Clearance reflects the rate of elimination relative to the drug concentration.

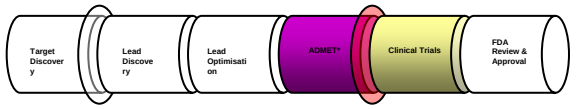
Cl = rate of elimination / concentration of the drug [ml/min]

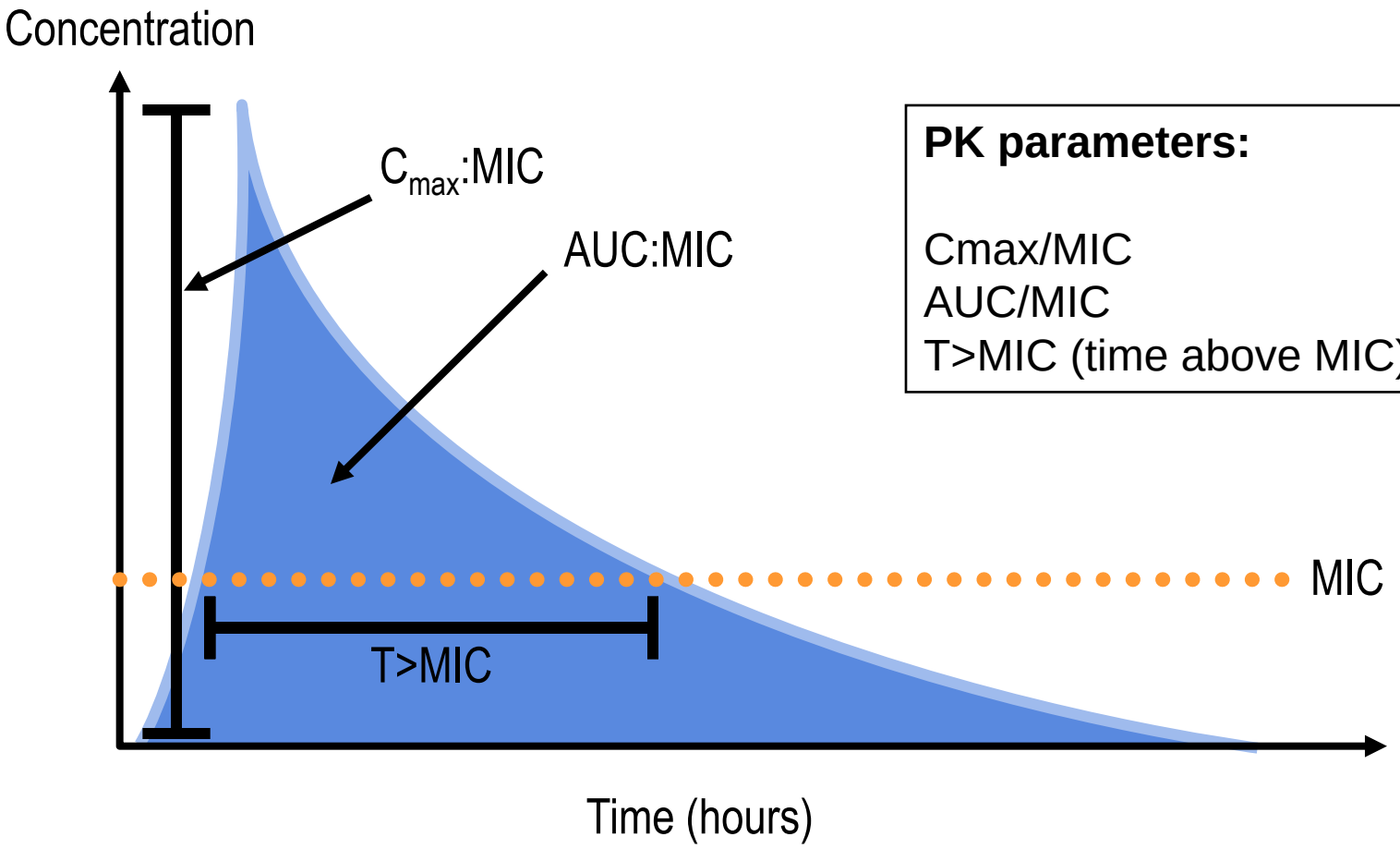
Pharmacokinetics: Peak Plasma Conc. (c_{max}/T_{max})





Pharmacokinetics: Importance for Antibiotics



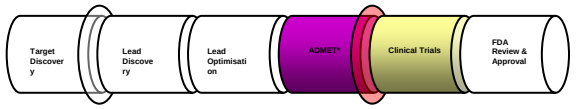


PK parameters:

Cmax/MIC
AUC/MIC
T>MIC (time above MIC)

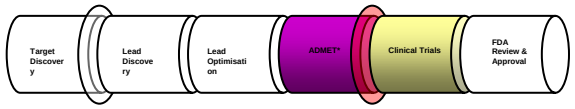
- Time-dependent killing and minimal to moderate persistent effects ➔ Time above MIC (T>MIC)
- Time-dependent killing and prolonged persistent effects ➔ AUC/MIC ratio
- Concentration-dependent killing and prolonged persistent effects ➔ AUC/MIC or Peak/MIC ratio

Pharmacokinetics: Antimicrobial Activity

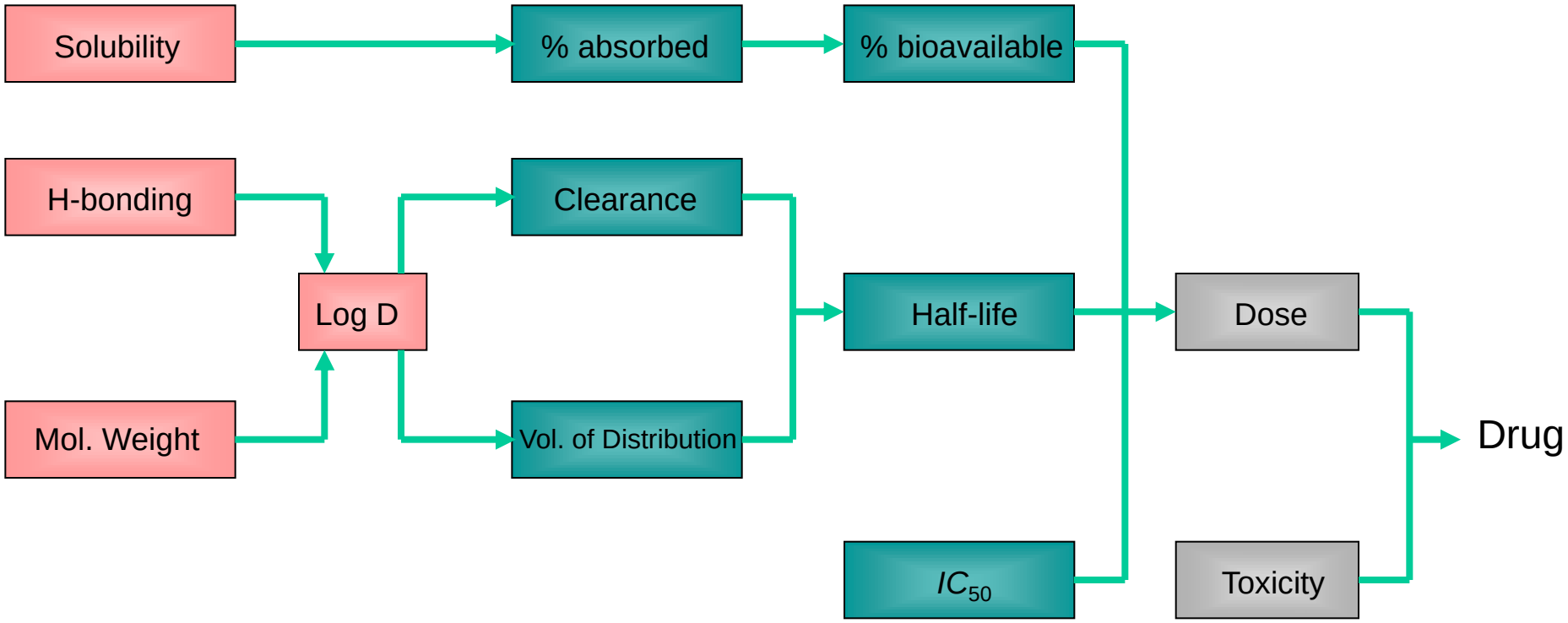


Parameter correlating with efficacy	**T>MIC**	**AUC:MIC**	**C_{max}:MIC**
Examples	Penicillins Cephalosporins Carbapenems Macrolides Oxazolidinones	Azalides Fluoroquinolones Ketolides	Fluoroquinolones Aminoglycosides
Organism kill	Time-dependent	Concentration-dependent	Concentration-dependent
Therapeutic goal	Optimise duration of exposure	Maximise exposure	Maximise exposure

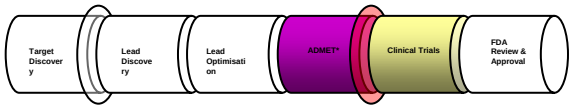
Pharmacokinetics: Putting It All Together



Physicochemical properties influence the development of compounds.



ADME-Tox: Physiological Factors

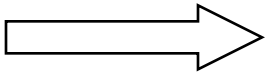


• Transporter proteins such as P-glycoprotein (P-gp) can either promote or hinder permeability. They are found in most organs and are involved in the uptake and elimination of endogenouse compounds and xenobiotics.

• Plasma-protein binding – drugs bind to a variety of particles in addition to proteins such as albumin (particularly acidic drugs) and α_1-acid glycoproteins (basic drugs).

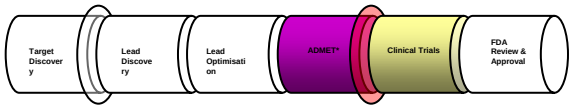
• Cytochrome P450 (CYP450) inhibition – these enzymes are primarily located in the liver and catalyse oxidation reactions to produce polar products, which are metabolised further (e.g. sulphation, glucoronidation) or excreted.

• CYP450 induction - binding of drugs to the pregnane receptor (PXR) or the constitutive androstane receptor (CAR) can induce expression of CYP450's. In particular, PXR is a key regulator of CYP3A4, which metabolises **50-60%** of all prescription drugs. PXR binding can highlight potential drug-drug interactions.



Metabolism

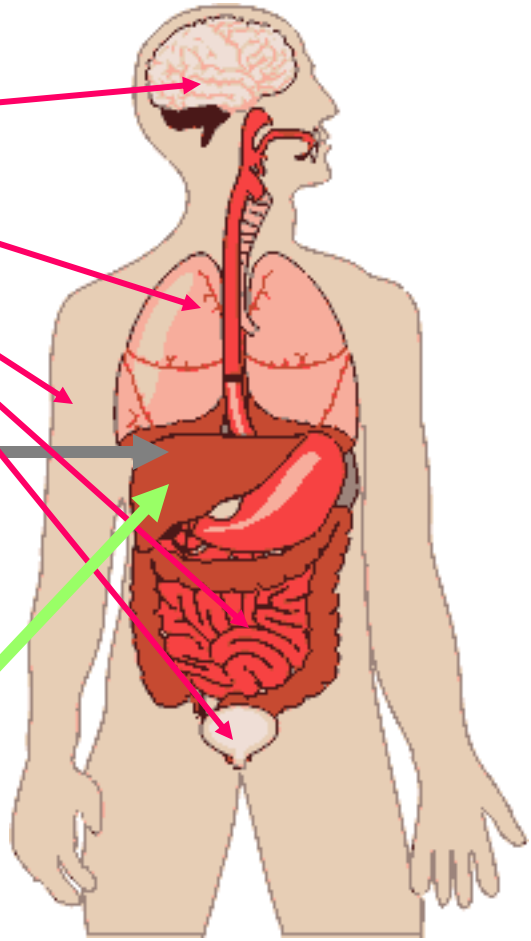
Drug Metabolism



Extrahepatic microsomal enzymes (oxidation, conjugation)

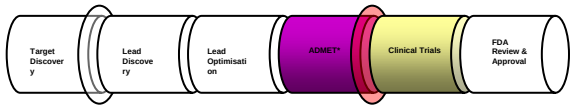
Hepatic microsomal enzymes (oxidation, conjugation)

Hepatic non-microsomal enzymes (acetylation, sulfation,GSH, alcohol/aldehyde dehydrogenase, hydrolysis, ox/red)



- Lipophilic drugs are rendered more hydrophilic, thus more easily excreted.
- Inactive, active and toxic metabolites

Drug Metabolism: Phase I & II Processes



Phase I: Mainly oxidative processes

Generate metabolites that are more polar than the parent compound.
Functional group added that can undergo phase II reaction.

Phase I reaction can lead to the formation of toxic metabolites.

Phase II: Conjugation of parent molecule or phase I metabolite

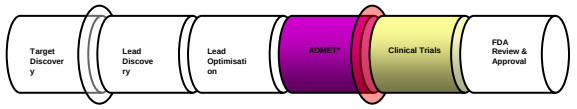
Glucoronic Acid

Glutathione

Sulfate

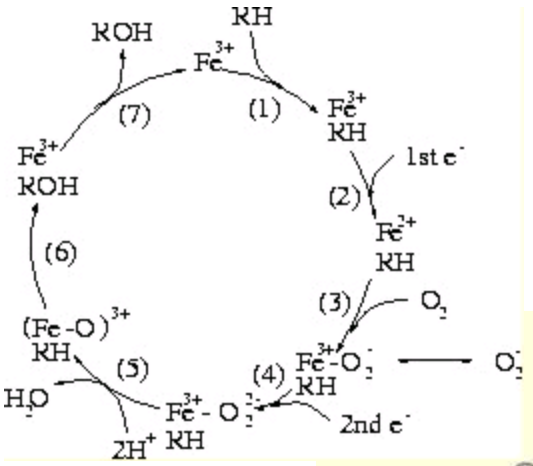
Conjugates are more water-soluble and less active (less toxic) than (phase I) nonconjugated compounds.

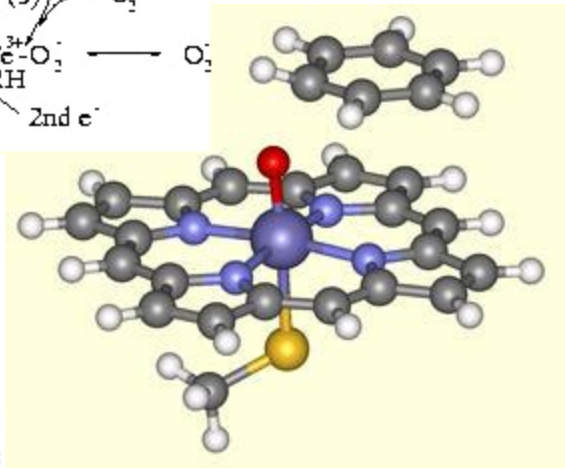
Drug Metabolism: Cytochrome P450 Substrates

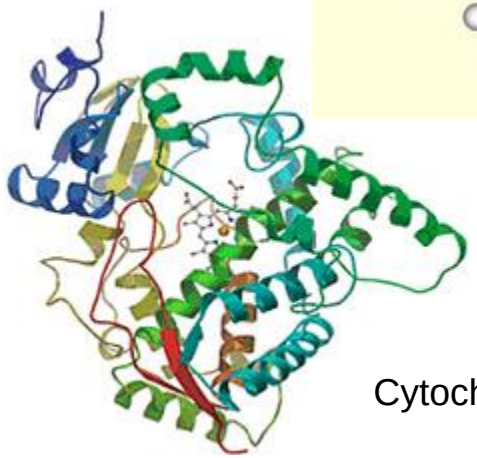


CYP450 enzymes (various isoforms) catalyse heme-dependent oxidative reactions on many different substrates – normally epoxidation or hydroxylation.

Functional Group	Product
R	R–OH
R, R', R'	R, O, R', R'
Ar, R	Ar, R, OH
R, R', R	R, R', OH
R, R'	OH, R, R'
R, X, R'	OH, R, X, R'
R, O, R'	R, O, R', OH

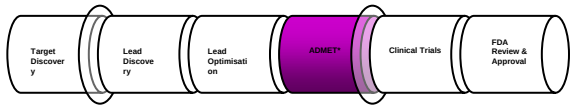






Cytochrome P450

Preclinical Development: *In vitro* ADME Tests



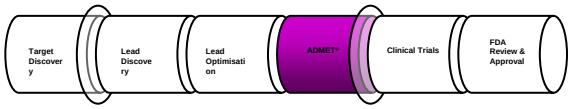
• **Metabolism** – incubation of compound with rat or human liver microsomes commonly used in combination with LC-MS analysis of incubates.

• **CYP450 inhibition** - many isoforms have been cloned and expressed. Kinetic assays are available for the most important isoforms, namely, CYP3A4, CYP2D6, CYP2C9, CYP1A2 and CYP2C19. These five enzymes are the predominant drug-metabolising enzymes in the human liver.

• **Plasma-binding** – compound activity in presence/absence of plasma proteins.

• **Absorption** – Caco-2 or Madin-Darby canine kidney MDCK monolayers to model gastrointestinal (GI) permeability (→).

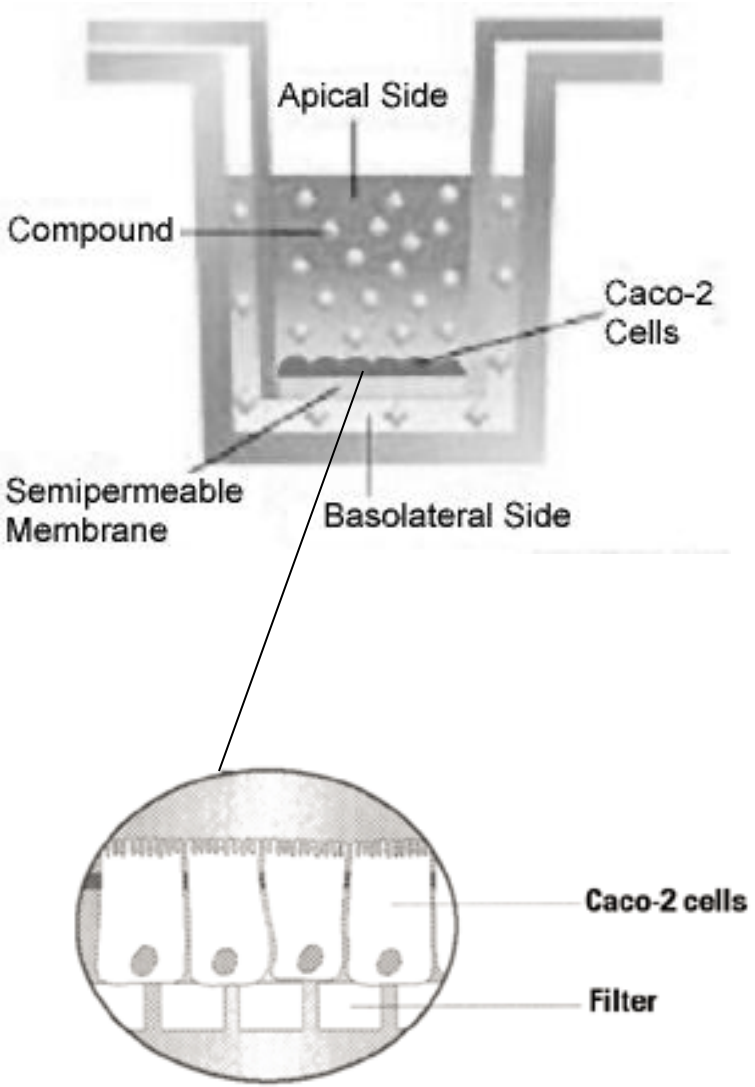
Preclinical Development: Permeability Testing



"A-to-B" setup: the solution of a drug is applied to the apical (A) side of the Caco-2 monolayer. The progress of the drug permeation through the monolayer is monitored by taking the samples of the basoleteral (B) solution and quantitating the drug by LCMS. The P_{app} then is calculated based on the kinetics curve of the parent drug appearance in the basolateral side.

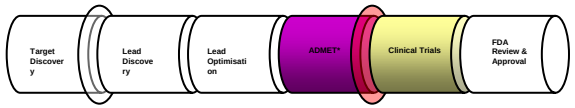
"B-to-A" setup: transport of compound from the basolateral side to the apical side by cellular efflux pumps can be determined.

Comparison of the "A-to-B" and "B-to-A" permeability provides information on active efflux processes for the studied compounds.



(caco2: cells from human colon adenocarcinoma)

Toxicity

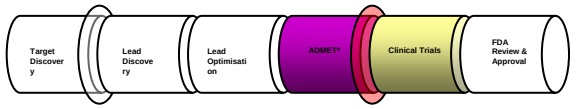


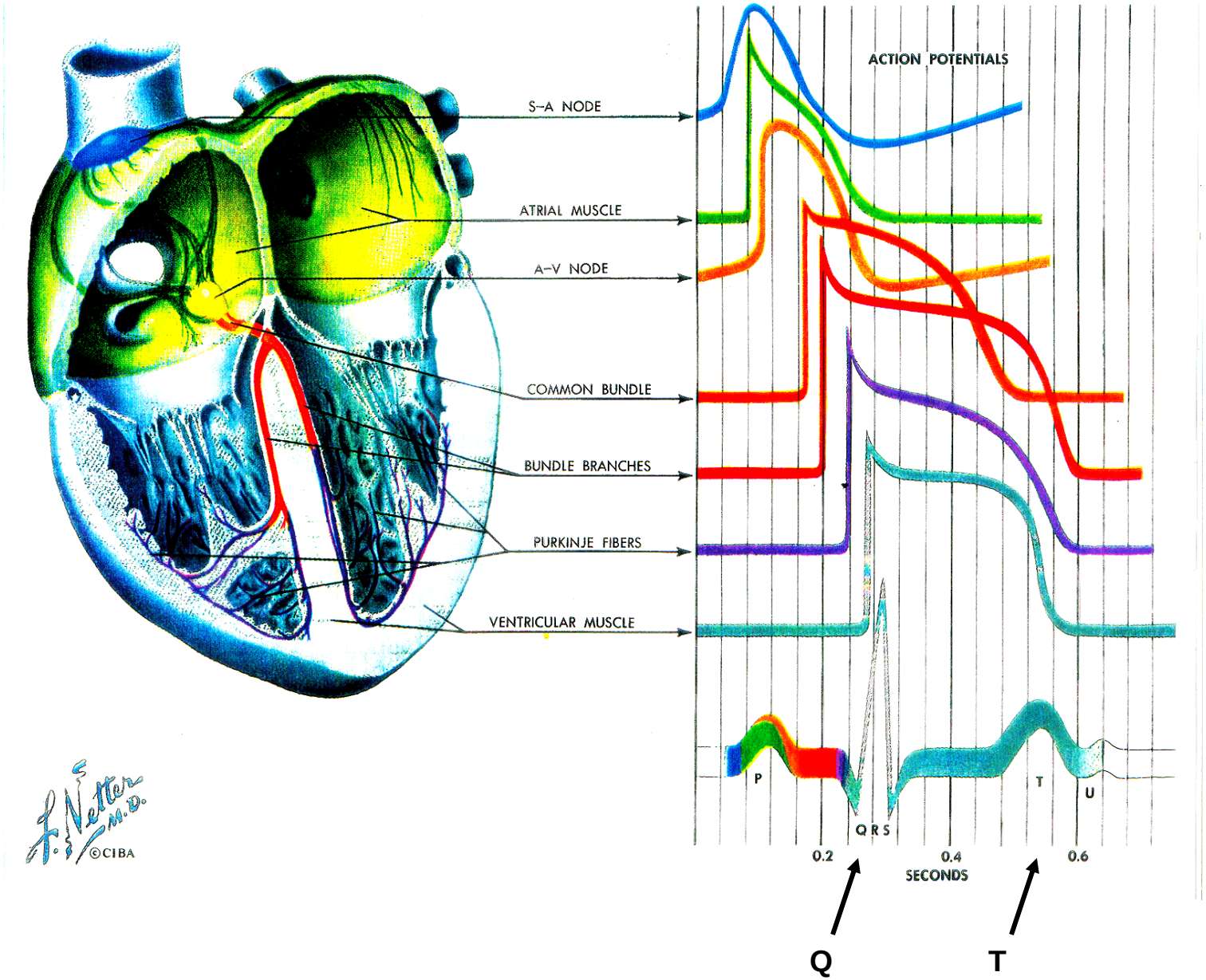
• **Cytotoxicity** – gross cell cytotoxicity can measured in mammalian cells using MTT (cell proliferation) assays.

• **Mutagenicity** – microbiological Ames test used to assess genotoxicity.

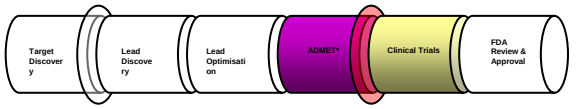
• **QT interval prolongation** – prolongation of cardiac repolarisation has been associated with specific and potentially fatal tachycardia in humans. There is a growing use of the hERG-K+ conductance assay using CHO cells to assess this (→).

QT Interval Prolongation

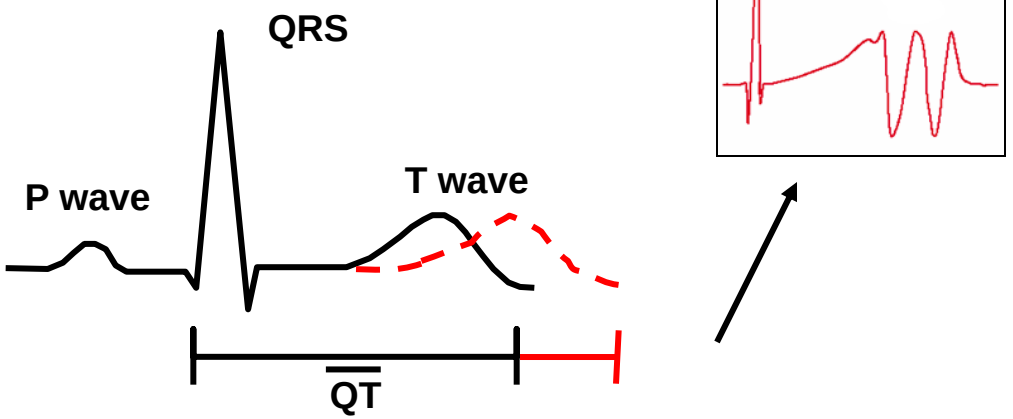


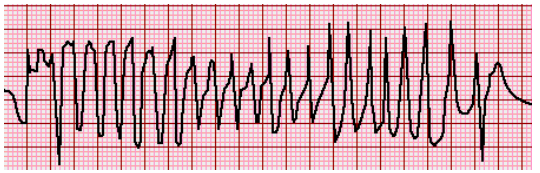


QT Interval Prolongation

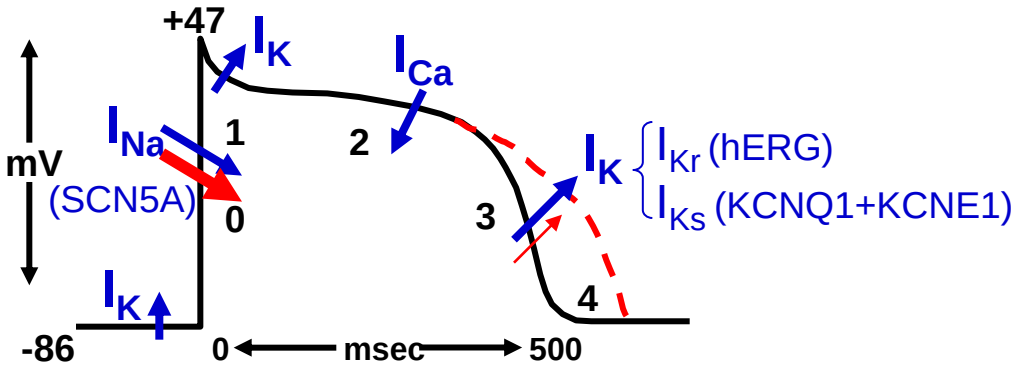


Electrocardiogram (ECG):



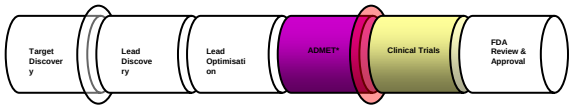


Ventricular Arrhythmia



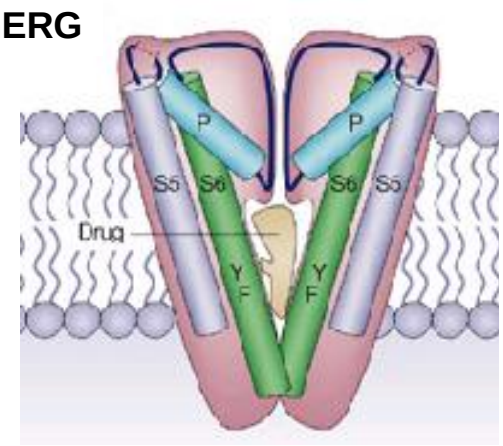
- molecular genetics of congenital Long QT Syndrome clarified mechanisms for drug-induced QT interval prolongation
- the rapid component of the delayed rectifier K^+ current, or **hERG**, is the target of most QT interval-prolonging drugs

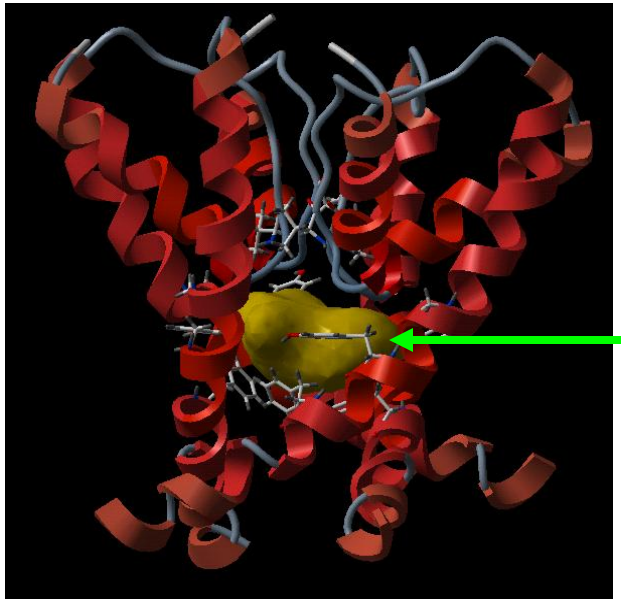
QT Interval Prolongation



- Drugs that prolong QT interval are associated with increased risk for ventricular arrhythmias (TdP) and sudden death.
- IKr/hERG K+ channel is the target for QT interval-prolonging drugs
- Nonclinical testing (electrophysiological assay using voltage clamp; ECG telemetry in animal) cannot reliably exclude clinical risk.
- In many cases drugs have to be evaluated for possible effects on the QT interval in an early clinical trial.
- Drug-induced QT interval prolongation is the most important cause of approval delays and the 2nd most important cause for approved drug withdrawal.

hERG





Homology model of hERG based on KvaP structure.

Large central cavity with hydrophobic surface critical for drug binding.

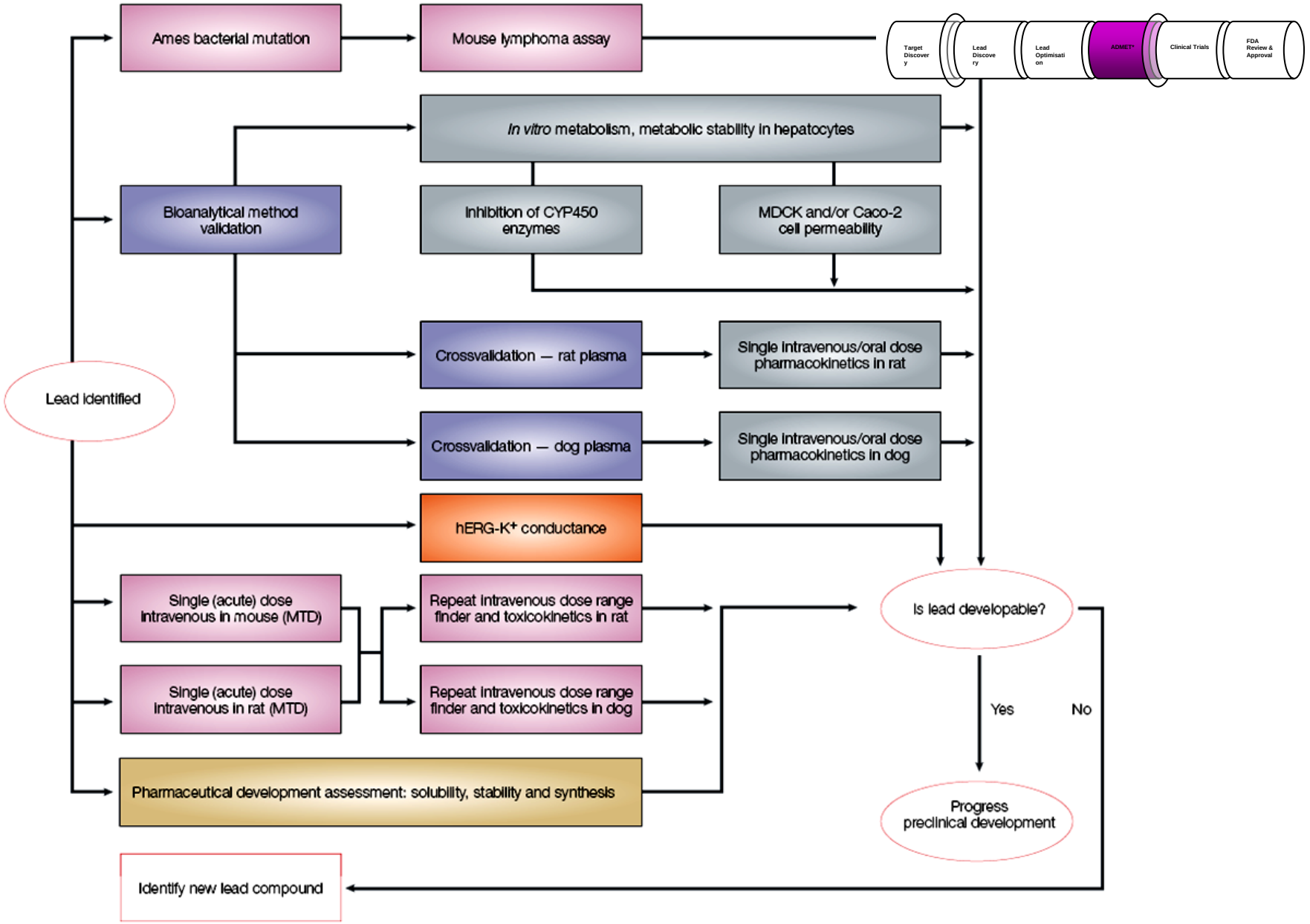
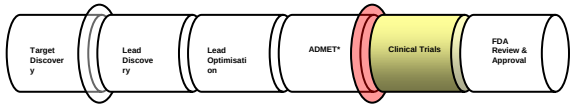
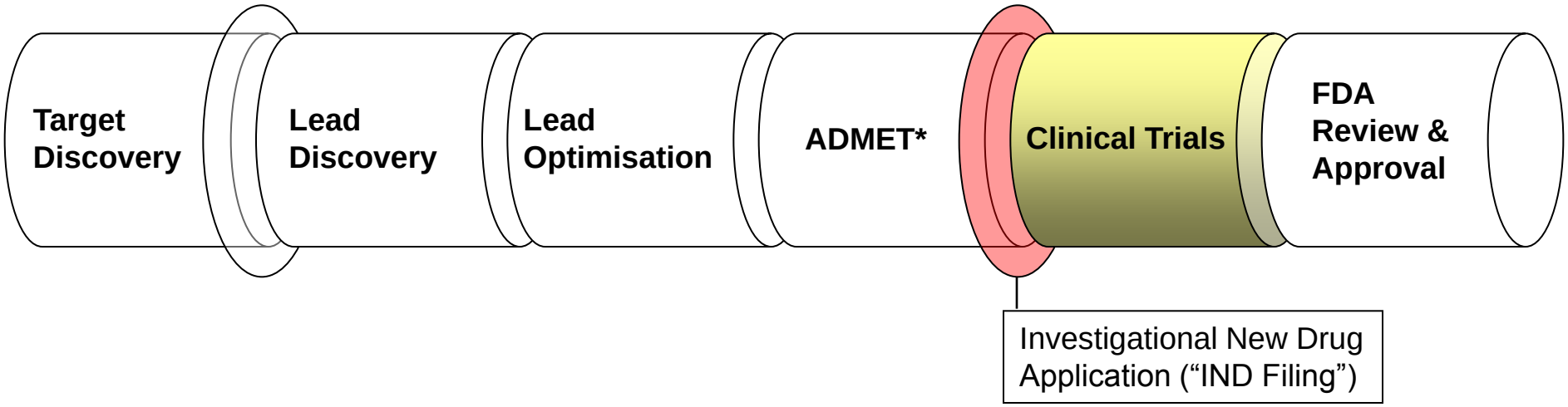


Figure 2 | **Potential flowchart for assessing whether a small-molecule new drug candidate is developable.** CYP450, cytochrome protein 450; hERG, human ether-a-go-go; MDCK, Madin–Darby canine kidney; MTD, maximum tolerated dose.

Clinical Development: IND Filing





IND = Application to begin testing of a new drug in humans.

Current Federal law requires that a drug be the subject of an approved marketing application before it is transported or distributed across state lines. Because a sponsor will probably want to ship the investigational drug to clinical investigators in many states, it must seek an exemption from that legal requirement. The IND is the means through which the sponsor technically obtains this exemption from the FDA.

Clinical Development: IND Filing

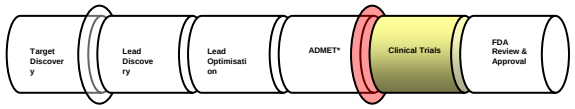
The IND application must contain information in three broad areas:

Animal Pharmacology and Toxicology Studies - Preclinical data to permit an assessment as to whether the product is reasonably safe for initial testing in humans. Also included are any previous experience with the drug in humans (often foreign use).

Manufacturing Information - Information pertaining to the composition, manufacturer, stability, and controls used for manufacturing the drug substance and the drug product.

Clinical Protocols and Investigator Information - Detailed protocols for proposed clinical studies to assess whether the initial-phase trials will expose subjects to unnecessary risks. Also, information on the qualifications of clinical investigators--professionals (generally physicians) who oversee the administration of the experimental compound--to assess whether they are qualified to fulfill their clinical trial duties. Finally, commitments to obtain informed consent from the research subjects, to obtain review of the study by an institutional review board (IRB), and to adhere to the investigational new drug regulations.

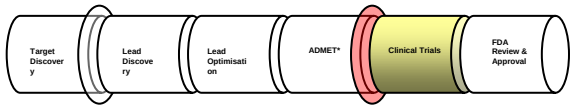
Clinical Trials - Historical



Historical Drug Trials:

- 1909: Paul Ehrlich - Salvarsan
- 1929: Alexander Fleming - Penicillin
- 1935: Gerhard Domagk - Sulfonamide
- 1944: Waksman – Streptomycin

Clinical Trial Designs & Terminology



- **Randomized/blinded trial**
- **Randomized/double blinded trial**
- **Non-randomized concurrent controlled trial**
- **Placebo trial**
- **…**

Randomized: Schemes used to assign participant to one group
example: every 3rd partcicipant gets higher dose

Nonrandomized: All with disease = cases; others = control

Blinded: Participants do not know if in experimental or control group

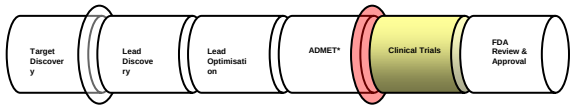
Double Blinded: Participants AND staff do not know group assignment

Placebo: Inactive pill w/ no therapeutic value (rare in trials of antibiotics)

Clinical Trial Protocols

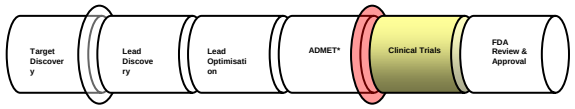
- Investigating two or more conditions: several treatment groups
  - example: drug vs. placebo; low dose vs. high dose
- Specific inclusion/exclusion criteria
- Sample size & power calculations: probability that a clinical trial will have a significant (positive) result, that is, have a *p-value* of less than the specified significance level (usually 5-10%) .
- Endpoint definition
  - example: clearance of infection

Clinical Trial Phases - Summary



	# Subjetcs	Length	Purpose	% Drugs Successfully Tested
Phase I	20 – 100	Several months – 2 years	Mainly Safety; pharmacokinetics	70%
Phase II	Up to several 100	Several months- 2 years	Short term safety; mainly effectiveness	33%
Phase III	100s – several 1000	1-4 years	Safety, dosage & effectiveness	25-30%

Clinical Trial Phases

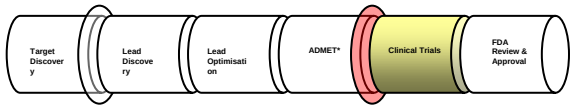


Phase I:
- small group of **healthy volunteers**
- evaluate drug safety
- determine a safe dosage range
- identify side effects
- collect data for pharmacokinetic analysis

Phase II:
- larger group of **people with disease**
- evaluate drug effectiveness
- further evaluate drug safety
- further evaluate dosage

Phase III:
- large groups of **people with disease**
- confirm drug's effectiveness
- monitor side effects
- compare drug to commonly used treatments
- monitor interactions with other drugs
- multicenter trial – many hospitals

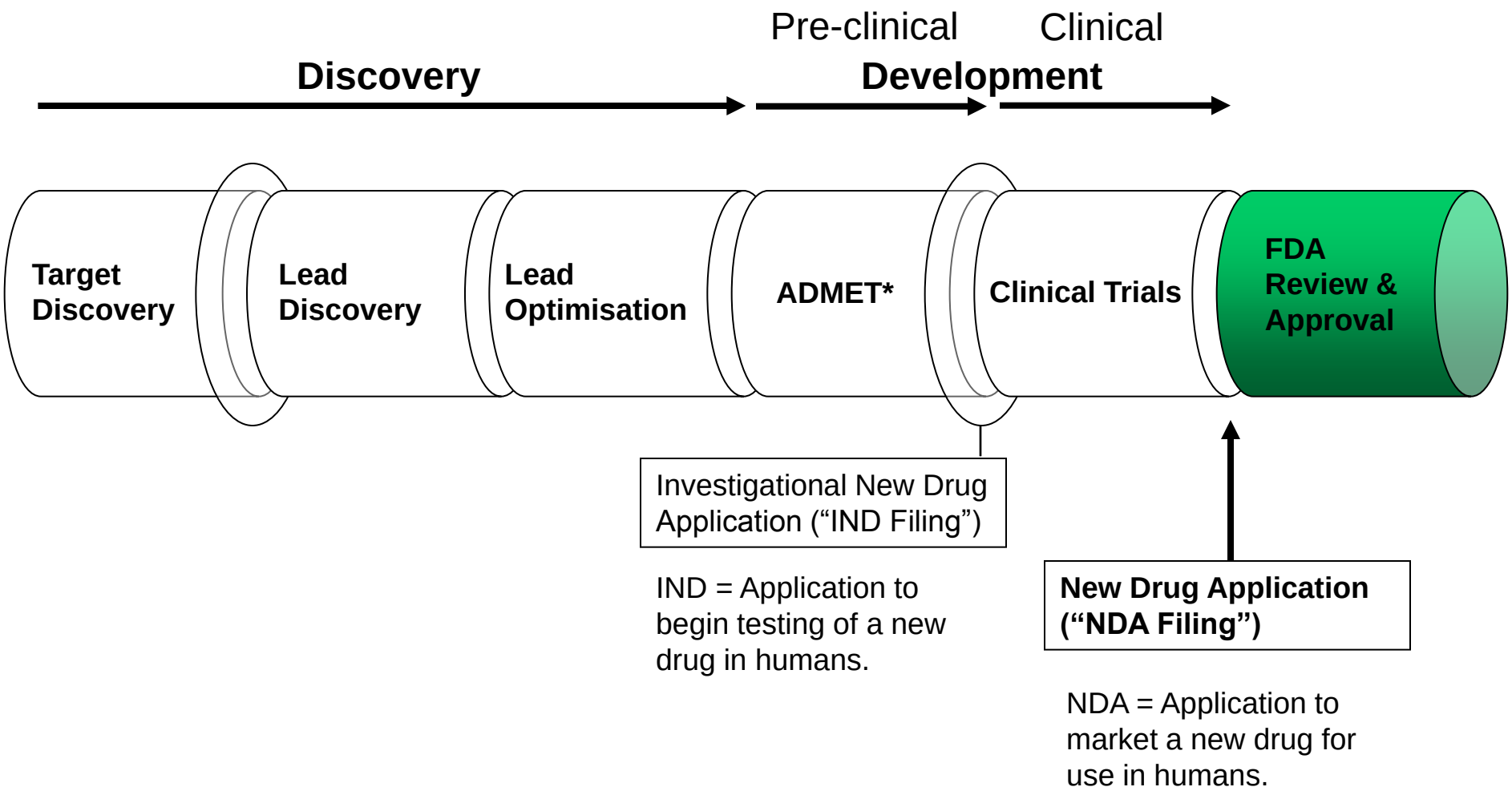
Clinical Trial Phases



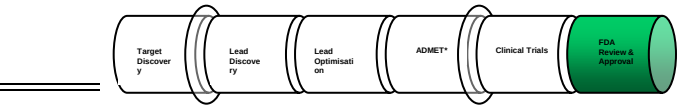
Phase IV:

- after the drug has been marketed
- information about drug effect in various populations
- side effects associated with long-term use.
- new indications: potential to extend patent protection

New Drug Application (NDA), FDA Review and Approval



NDA Review


Target Discovery
Lead Discovery
Lead Optimisation
ADMET*
Clinical Trials
FDA Review & Approval


FOOD & DRUG ADMINISTRATION
J. Harris
"RILOVONIN? I DON'T LIKE THE SOUND OF IT — LET'S REJECT IT."

NDA Review

Center for Drug Evaluation and Research (CDER) of the FDA

Medical

Biopharmaceutical

Pharmacology

Statistical

Chemistry

Microbiology

Advisory Committee Meeting

Meetings with Sponsor

Reviews Complete and Acceptable?

No

Additional Info or Revisions Requested or Submitted (Amendment)

Sponsor Revises

Yes

Yes

Yes

Labeling Review Acceptable? (1)

No

Inspection of Sites Acceptable? (2)

No

Pending Satisfactory Results

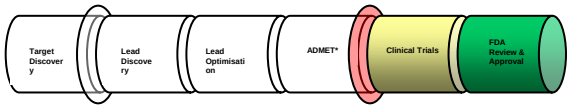
NDA Action

(1) Labeling in this context means offical instructions for use

(2) Manufacturing sites and sites where significant clinical trials are performed

Recommendation

Clinical Development & Approval Times



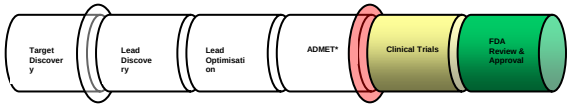
Mean Clinical Development and Approval Times by Therapeutic Category, 1990-1999

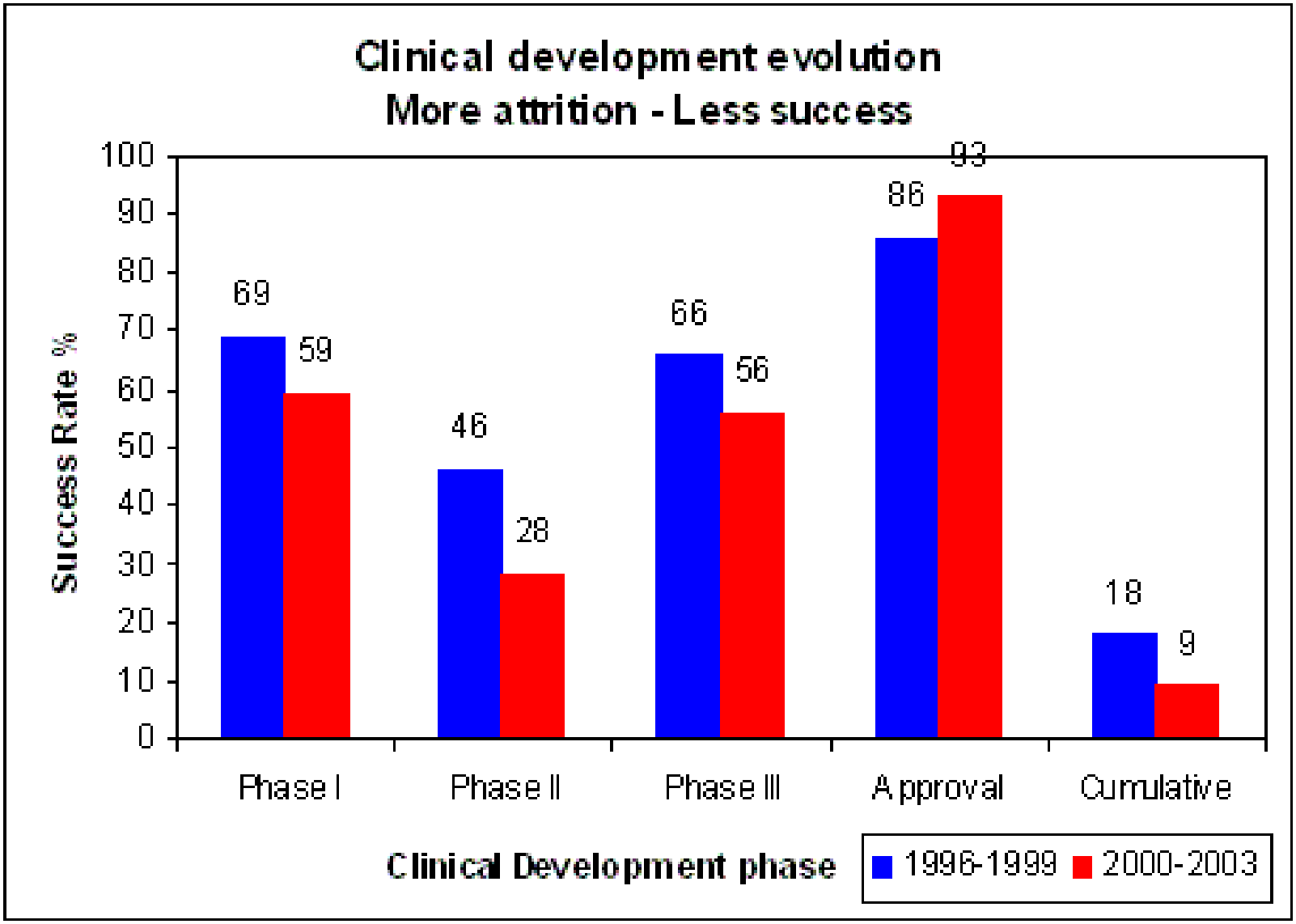
	1st in Humans to NDA	Approval Phase	
CNS	9.3	2.1	11.4
Analgesic/Anesthetic	9.4	2	11.4
Cardiovascular	8.8	2.3	11.1
All	8.6	1.8	10.4
Antiinfective	5.9	1.4	7.3

Years

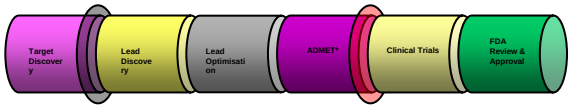
Source: DiMasi, "New Drug Development in the United States: 1963-1999" 2000

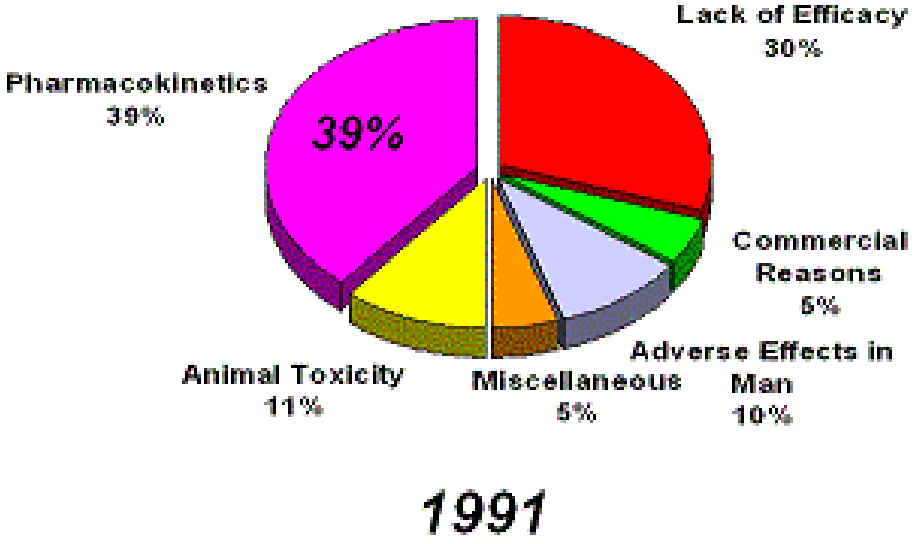
Clinical Development: Overall Success

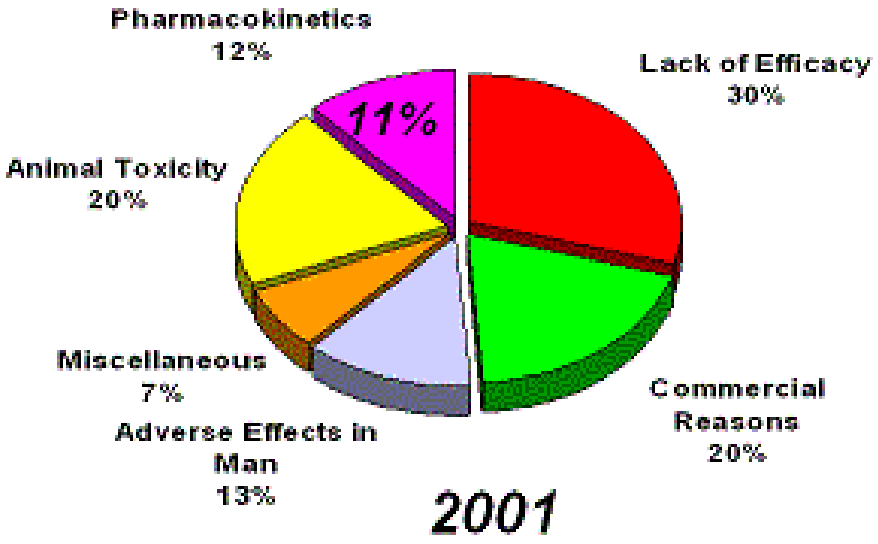




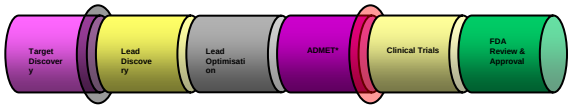
Why Drugs Fail

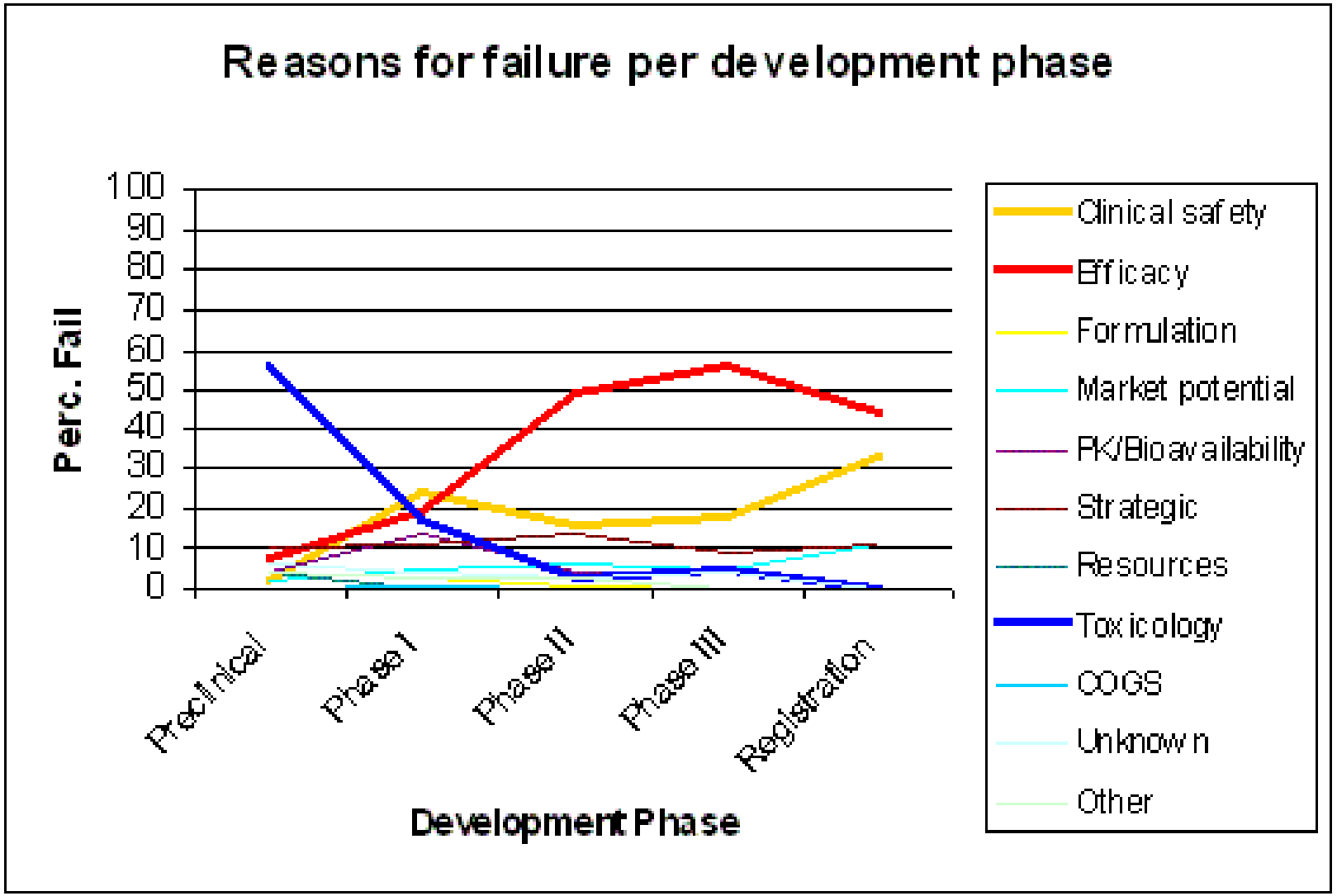




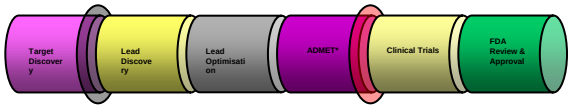


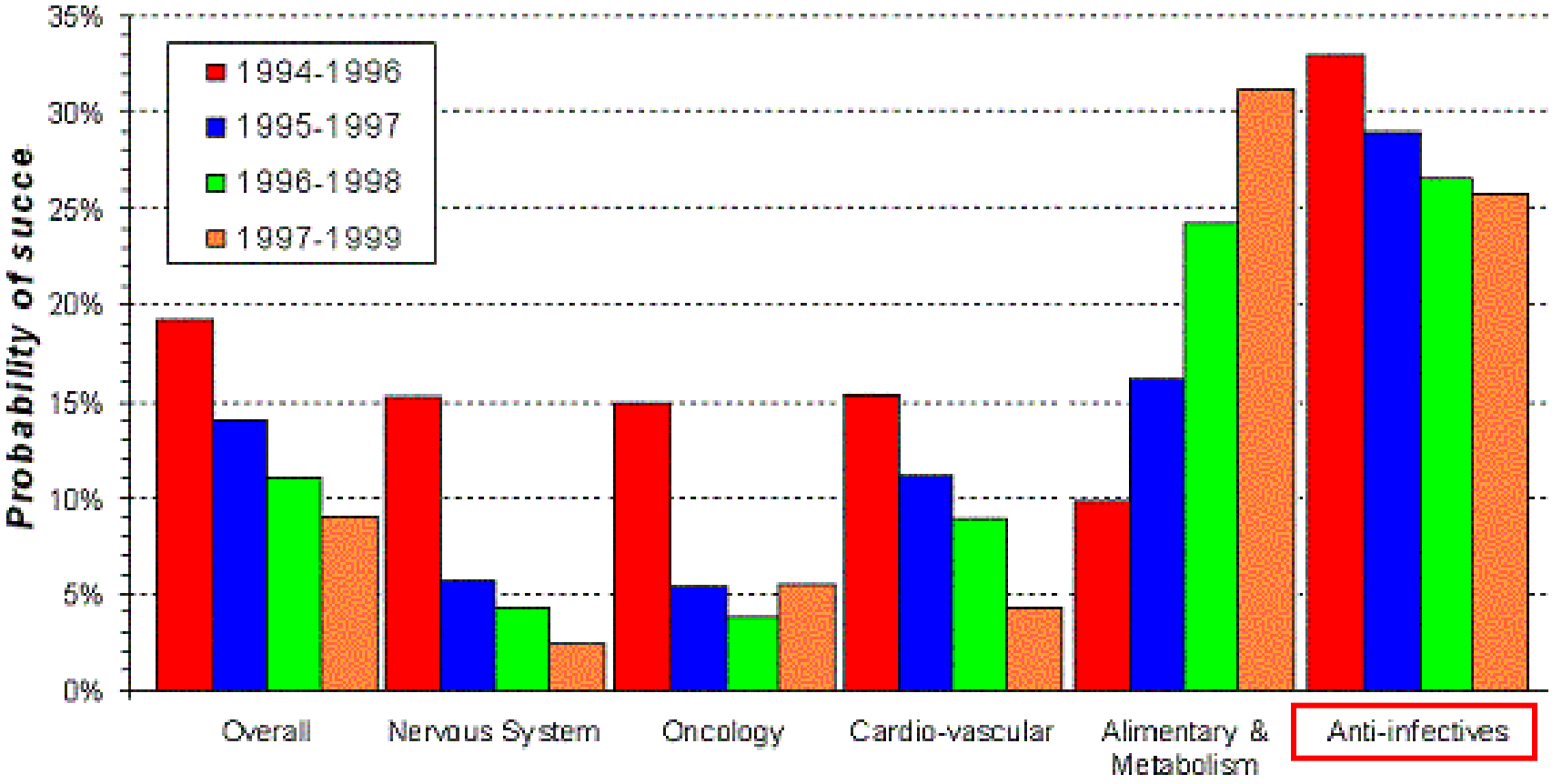
Why Drugs Fail





Antibiotics Are Relatively Successful Candidates





Trends in probability of success from 'first human dose' to market by therapeutic area