Some general aspects of pharmacology

- Context of cancer and anti-infective chemotherapy
  - selective targeting
  - resistance
- Drug toxicity (harmful effects of drugs)

Stephan Kellenberger
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1. Context of cancer- and anti-infective chemotherapy

Relevance

- Infectious diseases cause ~10 million (of 55 million) deaths per year (WHO data of the year 2000)
  - pneumonia 3.5 million
  - HIV/AIDS 2.6 million
  - diarrheal diseases 2.2 million
  - tuberculosis 2.6 million

- malignant neoplasms (cancers) 7 million

→ pharmacological strategies: targeting of selective differences between the microbe or cancer cell and the normal host cell

→ preventive efforts:
  - (to prevent infections:)
  - (to treat a cancer early in its development:)

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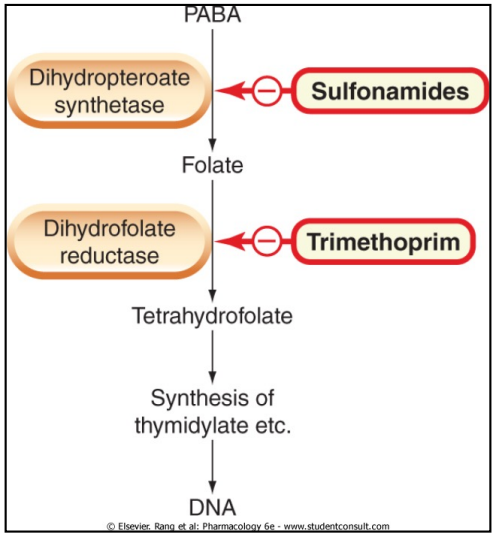
Mechanisms of selective targeting

Type of targeting	mechanisms	Example
Unique drug targets	drug targets genetic or biochemical pathway that is unique	- bacterial cell wall synthesis inhibitor - **inhibitor of folate synthesis (sulfonamides)**
Selective inhibition of similar targets	drug targets protein isoform that is unique to pathogen	- **dihydrofolate reductase inhibitor** - bacterial protein synthesis - tyrosine kinase inhibitors that target preferentially mutant kinases
Common targets	drug targets metabolic requirement that is specific to pathogen	- cancer cells require continued cell division --> drugs targeting processes involved in DNA synthesis, mitosis, cell cycle progression may kill cancer cells preferentially. ex. 5-Fluorouracil

differences in therapeutic index



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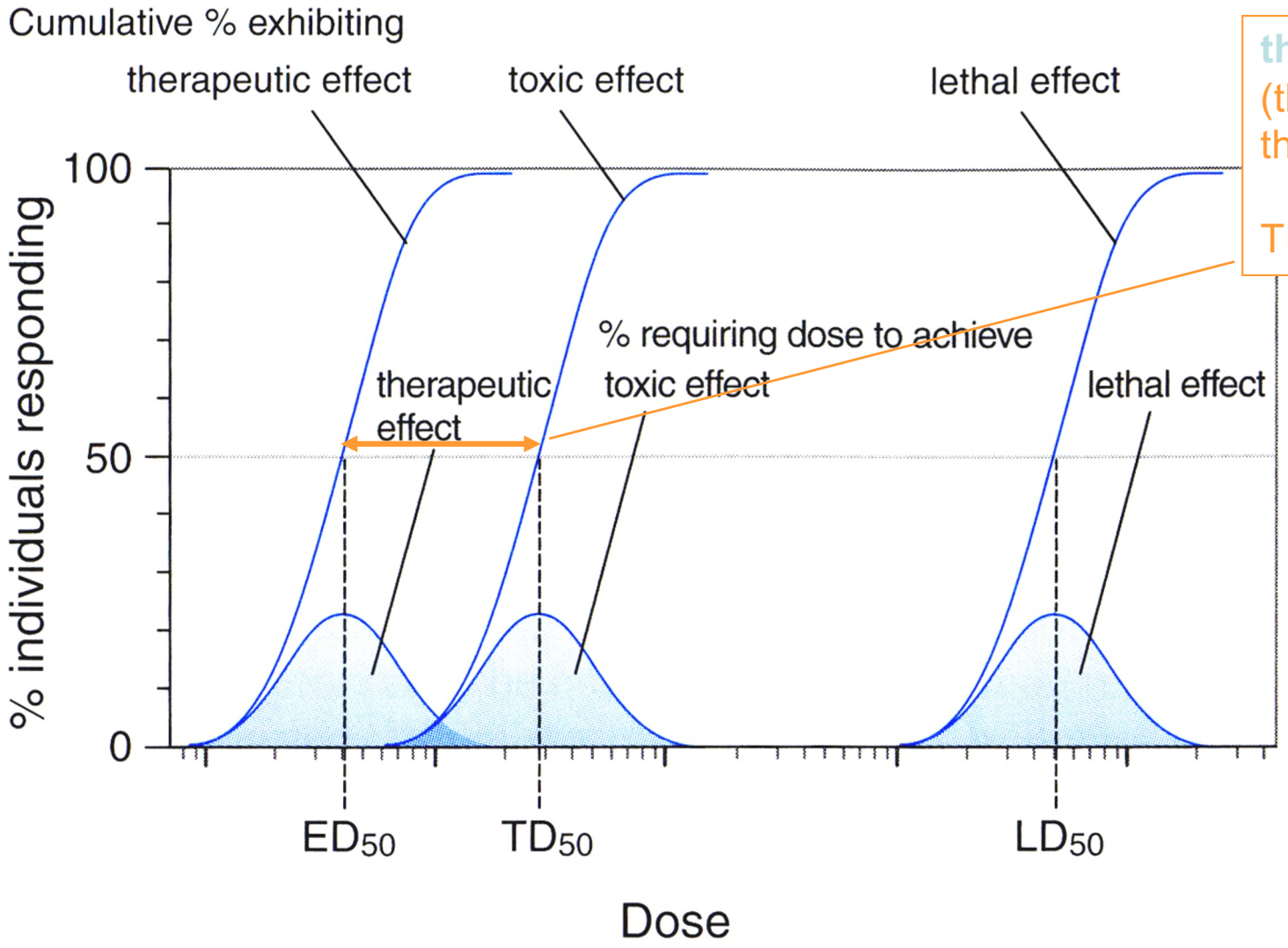

therapeutic index, TI
(therapeutic ratio, “marge thérapeutique”)

$TI = TD_{50}/ED_{50}$

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development of resistance

- resistance to anti-infective drugs is growing, examples:
  - Malaria, Tuberculosis, hospital-acquired infections
- pathogens and cancer cells are primed to evolve rapidly in response to adaptive pressure

  → high cell number, rapid growth rate, high mutation rate in pathogens or cancer cells

  → resistance through mutations

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Infections

“Les antibiotiques n’arrivent plus à sauver plusieurs centaines de malades par an en Suisse.

50% La moitié de la volaille vendue en Suisse contient des germes résistant aux antibiotiques.

“Selon l’OMS, l’évolution des résistances est l’une des plus grandes menaces pour la santé mondiale.

La quarantaine se poursuit à l’Hopital de la Broye

BACTÉRIE | Plus d’admission ni d’opération au service de chirurgie: les mesures exceptionnelles instaurées jeudi dernier à l’hôpital sont prolongées, alors que 25 patients porteurs d’un germe de type VRE ont été identifiés.

© Photo Jean-Paul Guinnard | En état d’alerte depuis jeudi dernier, le service de chirurgie est

EAUX USÉES Jeudi 22 mars 2012

Des bactéries résistantes aux antibiotiques dans le Léman

› ATS


(Keystone)

01 avril 2012 3581 vidéos Le site de l’émission

L’OMS sonne l’alerte : les bactéries multi résistantes aux antibiotiques prolifèrent. En cause, l’abus d’antibiotiques dans l’élevage. La médecine moderne est menacée.

L'OMS tire la sonnette d'alarme : le nombre de bactéries résistantes aux antibiotiques augmente dangereusement, et met en péril la médecine moderne. En cause : l'administration...

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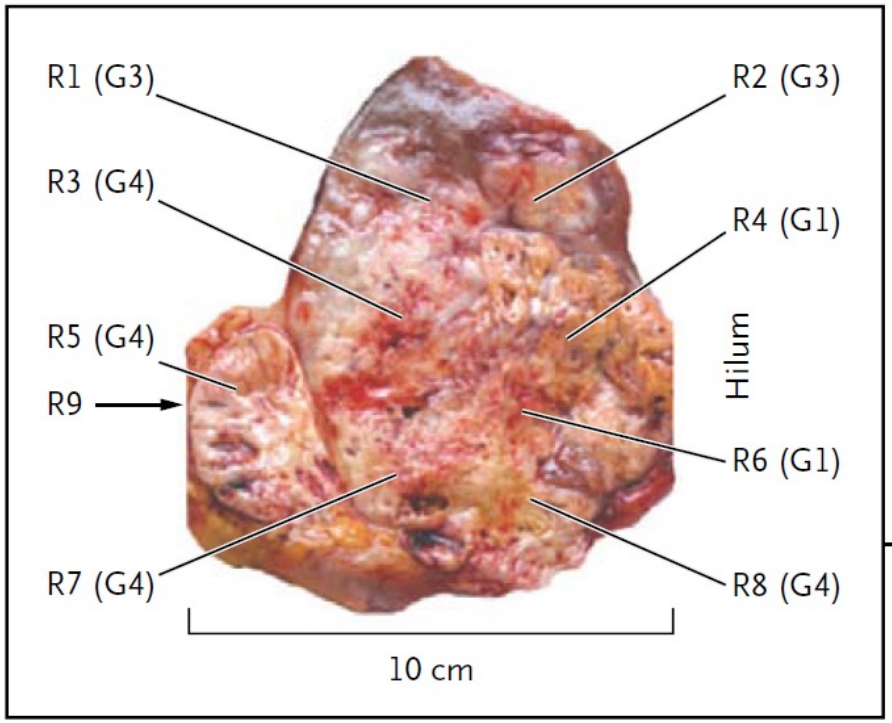
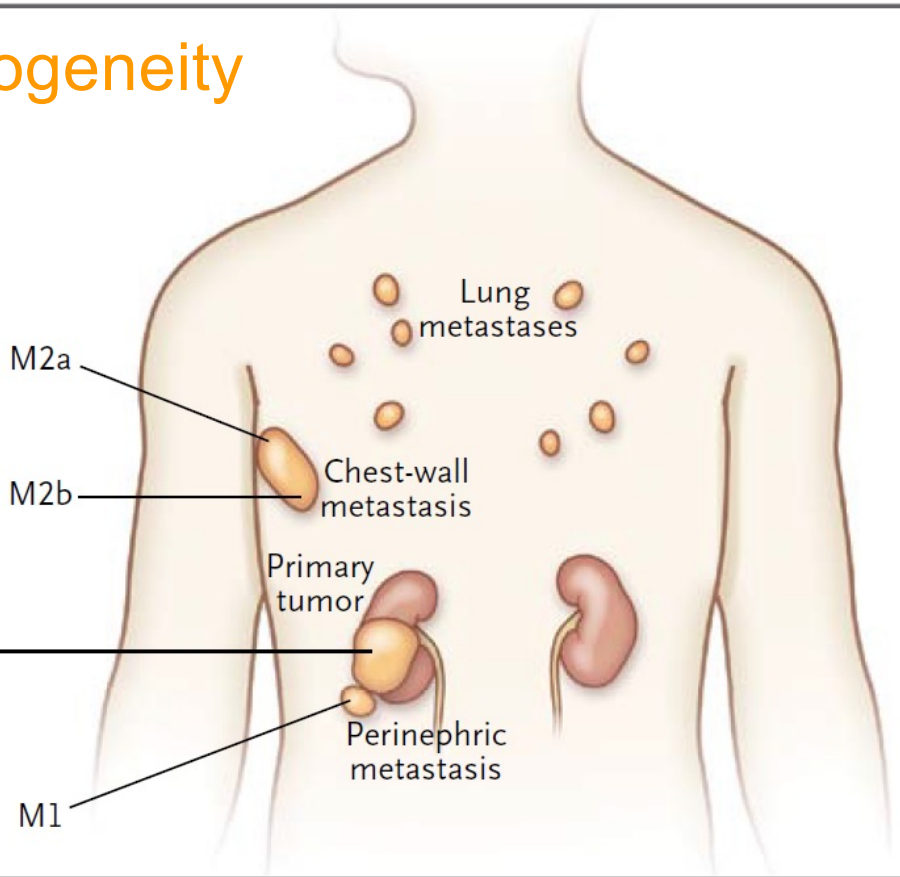
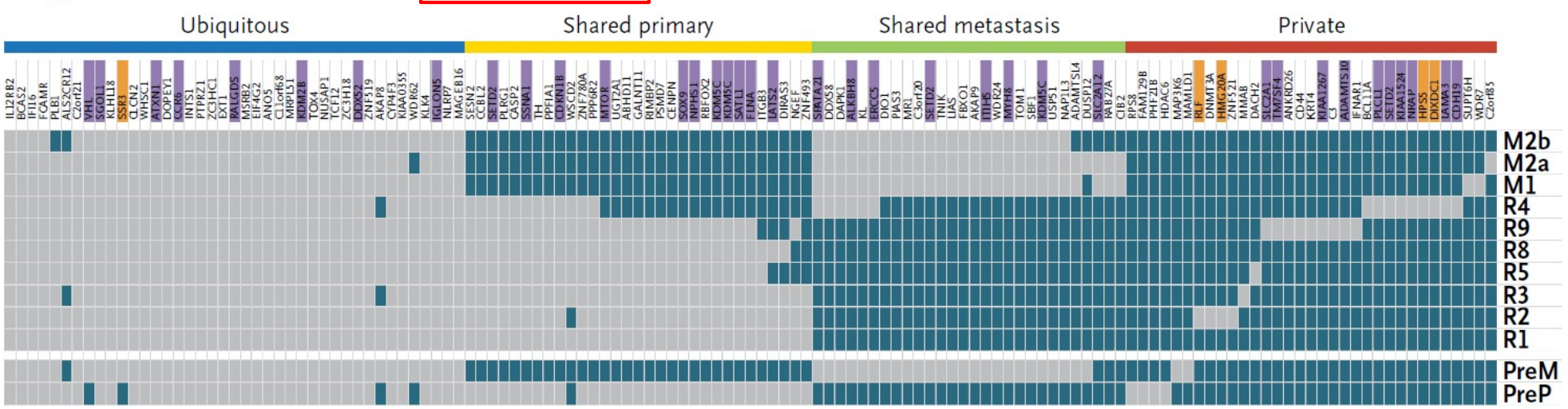
Genetic intratumor heterogeneity







Gerlinger M, p883

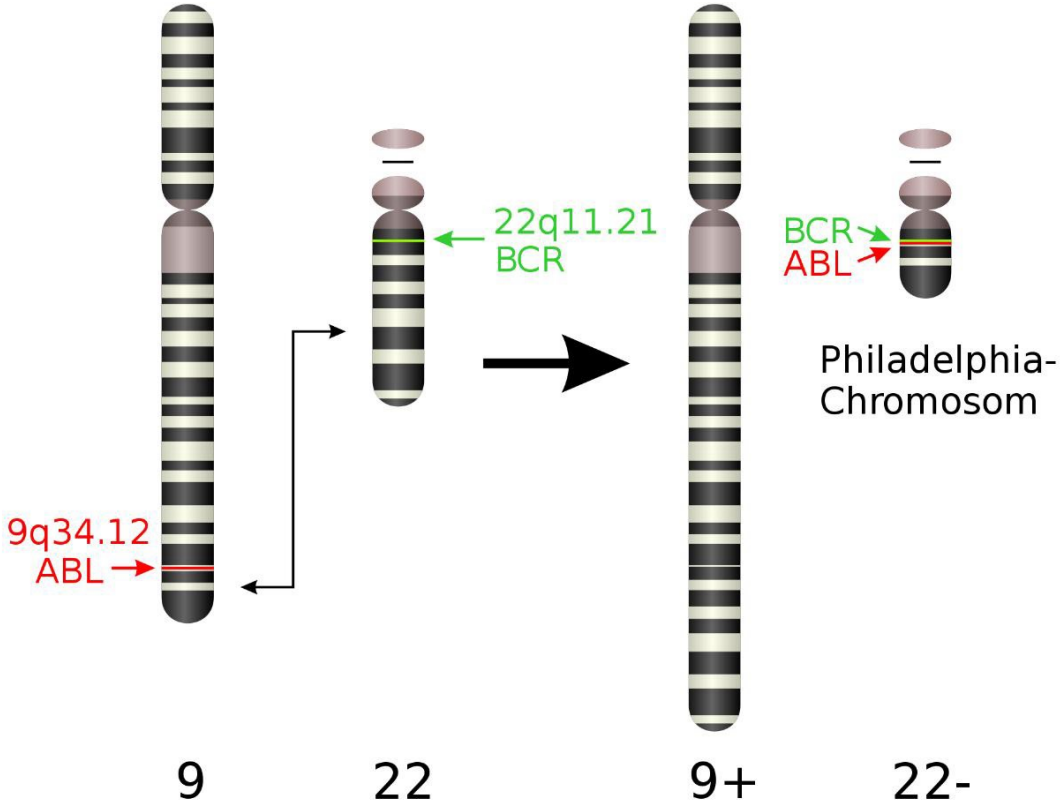
Figure 2 (facing page). Genetic Intratumor Heterogeneity and Phylogeny in Patient 1.

Panel A shows sites of core biopsies and regions harvested from nephrectomy and metastasectomy specimens. G indicates tumor grade. Panel B shows the regional distribution of 101 nonsynonymous point mutations and 32 indels in seven primary-tumor regions of the nephrectomy specimen (R1 through R5 and R8 through R9), in the perinephric fat of the nephrectomy specimen (M1), and in two regions of the excised chest-wall metastasis (M2a and M2b), as detected by exome sequencing (including the *VHL* mutation detected by Sanger sequencing). Regions R6 and R7 were excluded from analyses since only one nonsynonymous variant passed filtering. The heat map indicates the presence of a mutation (gray) or its absence (dark blue) in each region. The color bars above the heat map indicate classification of mutations according to whether they are ubiquitous, shared by primary-tumor regions, shared by metastatic sites, or unique to the region (private). Among the gene names, purple indicates that the mutation was validated, and orange indicates that the validation of the mutation failed. Because of limited DNA availability, only six mutations were validated in pretreatment samples of the primary tumor (PreP) and chest-wall metastases (PreM) (in *VHL, MTOR, SOX9, ALKBH8, SETD2,* and *KDM5C* splice sites). Panel C shows phylogenetic relationships of the tumor regions. R4a and R4b are the subclones detected in R4. A question mark indicates that the detected *SETD2* splice-site mutation probably resides in R4a, whereas R4b most likely shares the *SETD2* frameshift mutation also found in other primary-tumor regions. Branch lengths are proportional to the number of nonsynonymous mutations separating the branching points. Potential driver mutations were acquired by the indicated genes in the branch (arrows). Panel D shows regional ploidy profiling analysis. All other primary-tumor regions were diploid (not shown). DI denotes DNA index of the aneuploid peak, indicating the DNA content as compared with a diploid genome.

Chronic myeloid leukemia

15% of leukemias

Fusion of Abelson murine leukemia (ABL) gene (9) with breakpoint cluster region (BCR) gene (22) → deregulated, constitutively active tyrosine kinase



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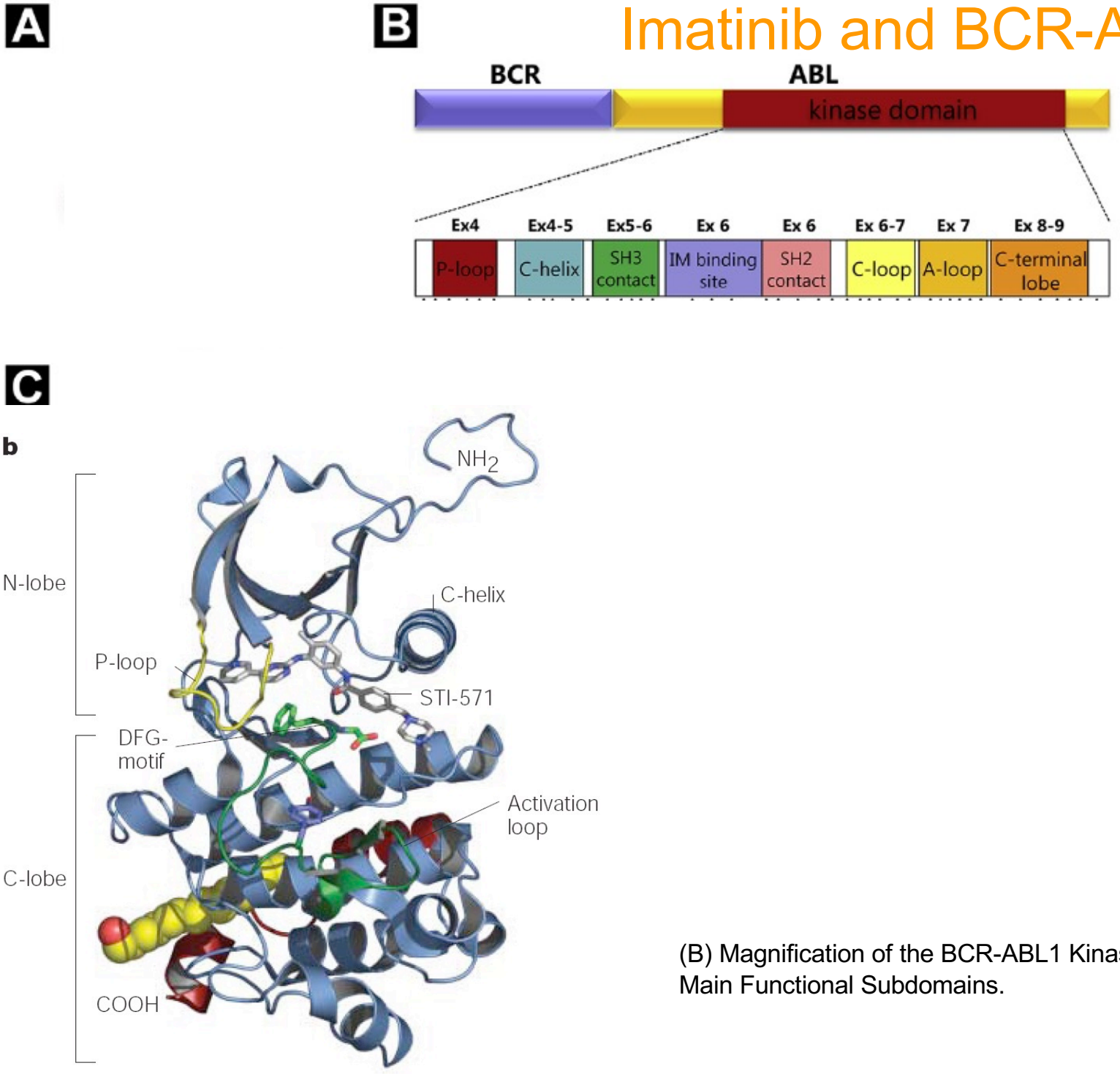
Imatinib and BCR-ABL



(B) Magnification of the BCR-ABL1 Kinase Domain, Showing the Main Functional Subdomains.

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Tyrosine kinase inhibitors for chronic myelogenous leukemia

Cancer type	Tyrosine kinase target	First-generation inhibitor	Second-generation inhibitor	Third-generation inhibitor
Chronic myelogenous leukemia	BCR-ABL	Imatinib	Dasatinib (2006) Nilotinib (2007) Bosutinib (2012)	Ponatinib (2012) Olverembatinib (approved in China in 2021) *Asciminib (2021)*

→ Second generation inhibitors are active against most mutant forms, except however the T315I mutation

→ 3rd generation inhibitors are also active against the T315I mutant
→ Asciminib binds to a different site (not the ATP binding site)

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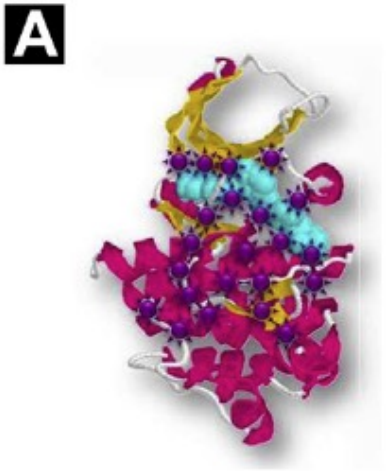
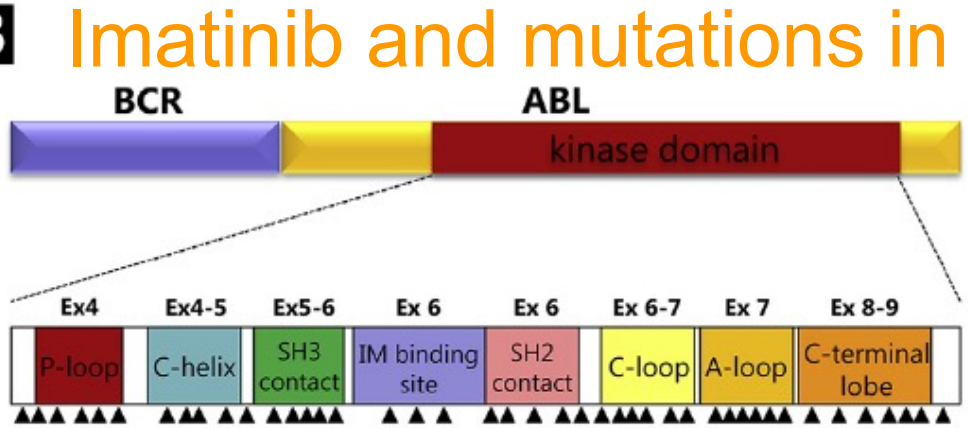
Imatinib and mutations in BCR-ABL





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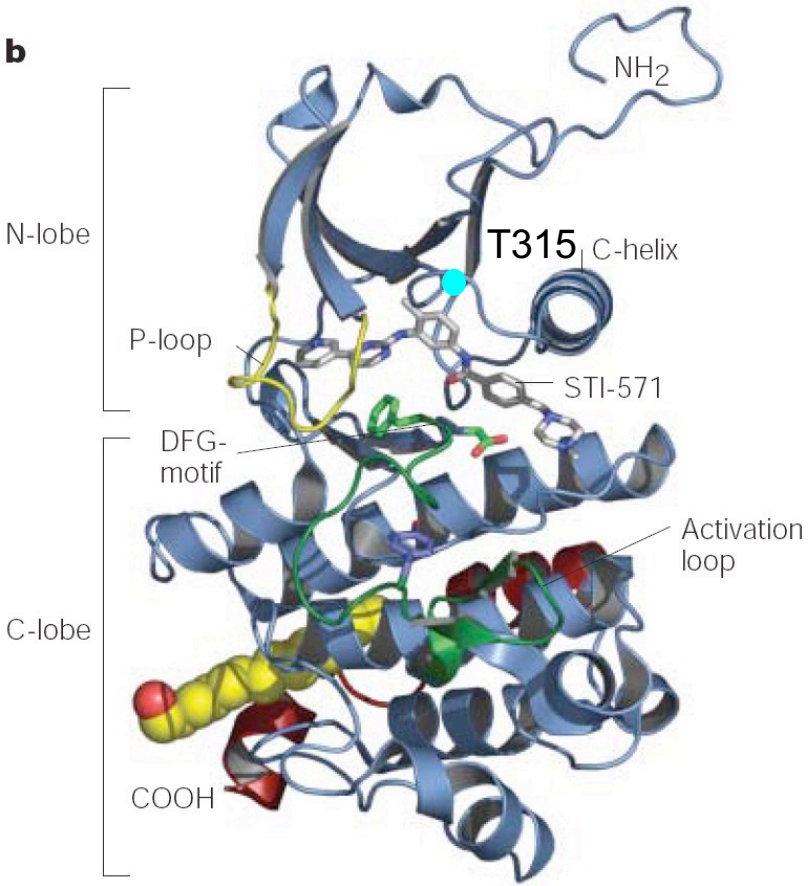

	Second generation			Third generation
	nilotinib	dasatinib	bosutinib	ponatinib
A397P S417F/Y I418S/V S438C E453G/K E459K/V P480L F486S	Y253F/H E255K/V **T315I** F359V/I/C	V299L **T315I** T315A F317L/V/I/C	E255K V299L **T315I** ?	?

pace Fill Representation; Cyan) Bound to ABL1 (Cartoon Representation; a-
genta, b-Sheets in Yellow, Turns in Pale Blue, All Other Residues in White).
ional Distribution of All Imatinib-Resistant Mutations (Purple) Is Shown. (B)
of the BCR-ABL1 Kinase Domain, Showing the Main Functional Subdomains.
s Indicate Imatinib-Resistant Mutations. (C) Summary of the Mutations
e Insensitive to Imatinib, Dasatinib, Nilotinib, and Bosutinib. The The T315I,
nt to First- and Second-Generation TKIs, Is Highlighted in Bold

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Table 3. *BCR-ABL* mutations in patients after imatinib treatment classified in terms of sensitivity to dasatinib or nilotinib according to available clinical and preclinical data

Mutation	% of patients with mutation after imatinib	Mutation class for therapeutic decision	
		Nilotinib	Dasatinib
T315I	14	D	D
M351T	12	A	A
G250E	12	A	A
F359V	9	C	A
M244V	9	A	A
Y253H	8	C	A
E255K	7	C	B
H396R	7	A	A
F317L	6	A	C
E355G	4	A	A
Q252H	4	B	B
E255V	4	C	B
E459K	4	A	A
F486S	3	A	A
L248V	3	A	A
D276G	3	A	A
E279K	3	A	A
Y253F	2	B	A
F359C	2	C	A
F359I	2	B	A
V299L	Rare	A	C

This table is adapted from a similar table listing mutations detected across all phases of CML or Ph+ ALL after imatinib therapy at a single institution.[83] Commonly detected mutations after second-line therapy include V299L, T315I/A and F317I/L for dasatinib and Y253F/H, E255K/V, T315I and F359C/V for nilotinib. A, sensitive to inhibitor; B, clinical evidence suggestive of reduced sensitivity, but presence of mutation should have no impact on clinical decisions; C, compelling clinical evidence to recommend an alternative inhibitor; D, no role for second-generation TKI therapy.

Cell Cycle 10:2, 250-260; January 15, 2011; © 2011 Landes Bioscience

Impact of *BCR-ABL* mutations on patients with chronic myeloid leukemia

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Ponatinib was the first and for a long time the only marketed kinase inhibitor that inhibits BCR-ABL containing the mutation T315I (10-15% of all patients)

- Approved by FDA in December 2012
- Suspended from market on October 2013 due to "increased frequency of blood clots and narrowing of blood vessels"
- Marketing allowed again, on a narrower patient population on December 20, 2013

Nat Biotech 32, 9 (2014)

Newer drugs that inhibit BCR-ABL containing the mutation T315I:
Olverembatinib (approved in China; current clinical studies in USA) and
Asciminib (approved in 2021 by FDA for Chronic myeloid leukemia)

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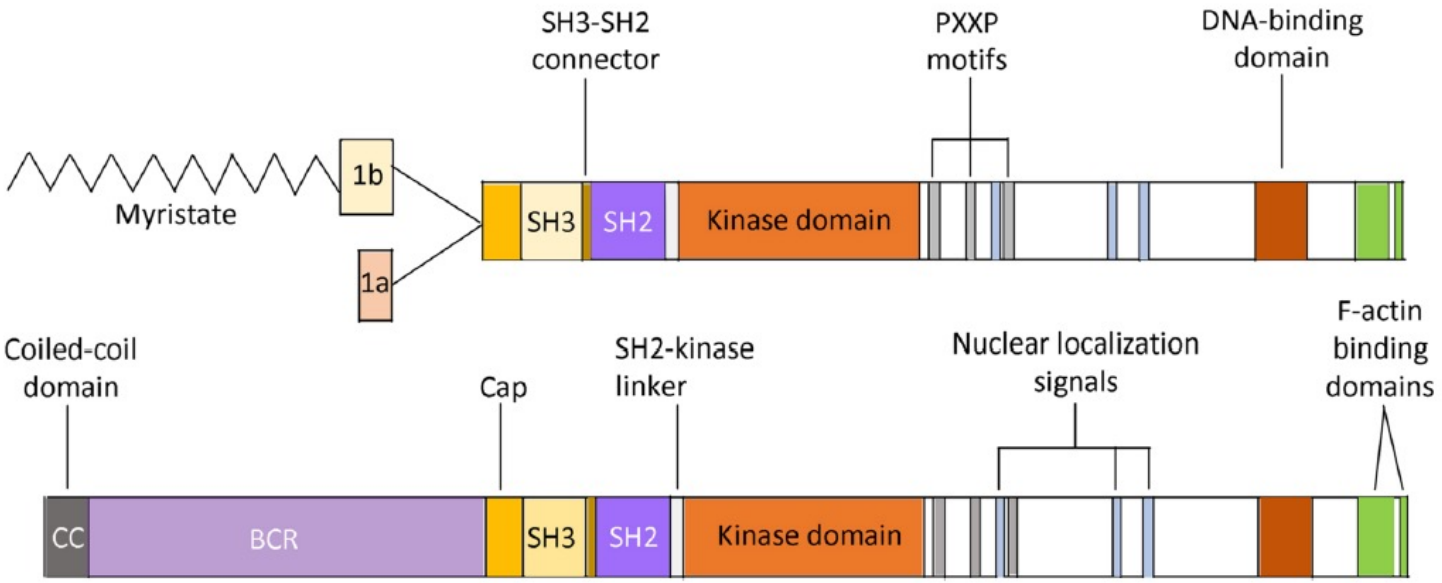
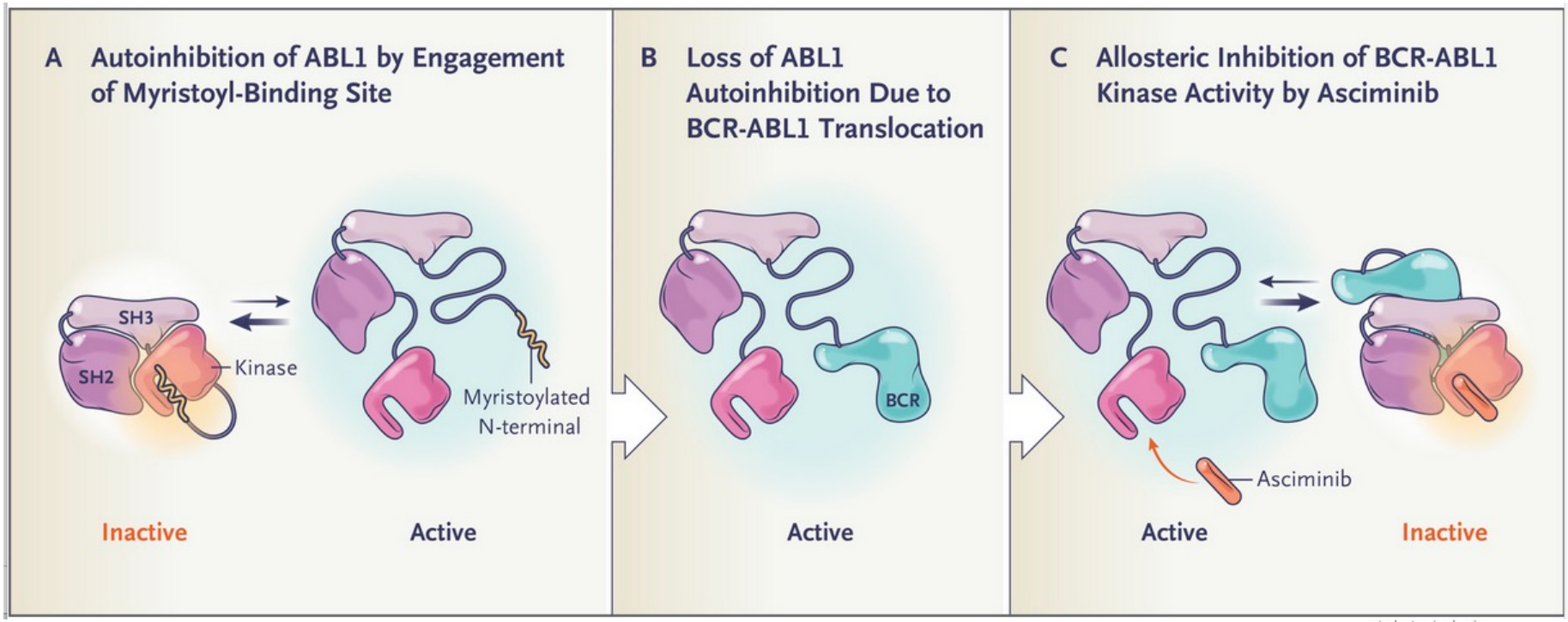


Fig. 1. Diagrammatic representation of the Abl-1a/Abl-1b, and BCR-Abl structures. The figure is not drawn to scale. Adapted from Ref. [29]



BCR-ABL inhibition by Asciminib

mechanisms of resistance

Mechanism	Example antimicrobial	example antineoplastic
Reduced intracellular concentration of the drug		
Inactivate drug	Inactivation of β-lactam antibiotics *(=penicillins, cephalosporines)* by β-lactamase	Inactivation of antimetabolites by deaminase
Prevent uptake of drug	prevention of aminoglycoside entry by altered porins	decreased methotrexate entry by decreased expression of reduced folate carrier
promote efflux of drug	efflux of multiple drugs by MDR membrane efflux pump	efflux of multiple drugs by MDR membrane efflux pump
Altered drug target	Expression of altered peptidoglycan that no longer binds vancomycin *(an antibiotic*)	Expression of mutant DHFR that no longer binds methotrexate
Insensitivity to apoptosis	*(not applicable)*	loss of active p53 *(p53 can induce apoptosis)*
Bypass metabolic requirement for target	inhibition of thymidylate synthase bypassed by exogenous thymidine	Loss of estrogen receptor-dependent growth results in tamoxifen resistance (tamoxifen is an estrogen antagonist)

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2. Harmful effects of drugs

- Adverse effects related to the main pharmacological action of the drug (ex. Postural hypotension with α1-adrenoceptor antagonists, sedation with anxiolytics, etc.)
  - Either reversible or not easily (drug dependence by opiate analgesics)
  - Can be serious (e.g. bleeding caused by anti-coagulants)

- Adverse effects unrelated to the main pharmacological action of the drug
  - May be predictable if the drug is taken in an excessive dose:
    - Hepatotoxicity of paracetamol
    - Ototoxicity of aminoglycoside antibiotics
  - A predictable subsidiary pharmacological effect can have serious implications for rare susceptible individuals
    - Drugs that affect the QT interval of the cardiac action potential
    - Anaphylactic shock that can be induced by penicillines

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Contexts of drug toxicity

- Drug overdose: in most cases accidental (dosing errors)
- Drug-Drug interactions (many people take multiple drugs at a time)
  - Pharmacokinetic drug-drug interactions (one drug changes the absorption, distribution, metabolism or excretion of another drug
    - Induction or inhibition of hepatic P450 enzymes *(see pharmacokinetics class)*
    - Drugs affecting multi-drug transporter (MDR)
    - Binding to plasma proteins
  - Pharmacodynamic drug-drug interactions (one drug changes the response to another drug)
    - Two drugs activate complementary pathways (e.g. antithrombotic drugs)
- Drug-Herb (or nutrition) interactions
  - Ex: grapefruit juice, ethanol (→ pharmacokinetics)
    - Tyramine-rich food (cheese, wine) with MAO inhibitors (pharmacodynamics, at adrenergic neuron)

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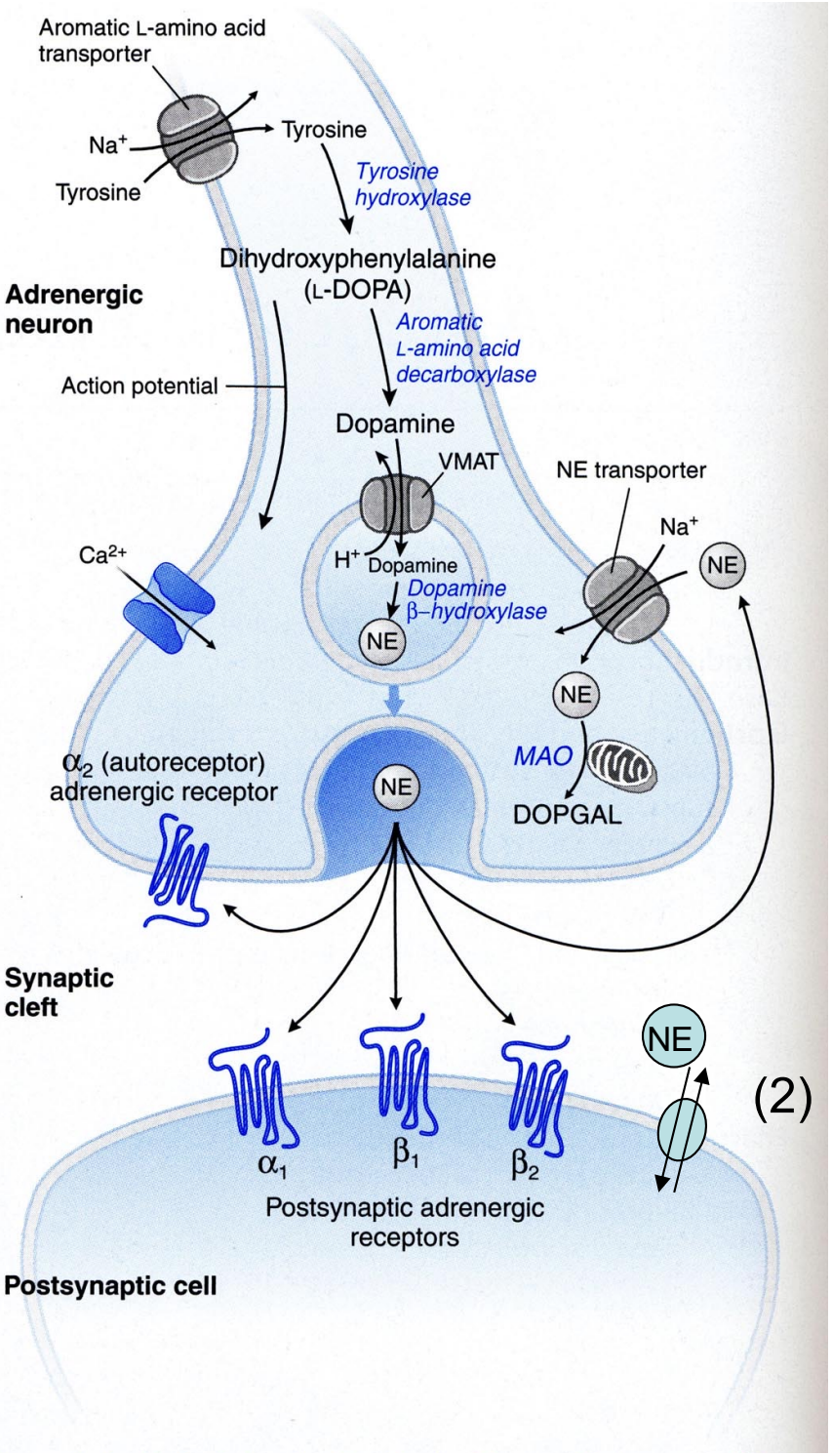

Drugs acting on noradrenergic nerve terminals

Drugs that affect noradrenaline synthesis:

- α-**methyltyrosine**: blocks tyrosine hydroxylase; not used clinically

- **Methyldopa** gives rise to false transmitter (methylnoradrenaline), which is a potent α_2 agonist, thus causing powerful presynaptic inhibitory feedback (also central actions). Rarely used as antihypertensive agent.

Drugs that affect transmitter transport:

- **Reserpine** blocks carrier-mediated noradrenaline accumulation in vesicles, thus depleting noradrenaline stores and blocking transmission. Effective in hypertension but may cause severe depression. Clinically obsolete.
- Indirectly acting sympathomimetic amines (e.g. **amphetamine**, **ephedrine**, **tyramine**) are accumulated by uptake 1 and displace noradrenaline from vesicles, allowing it to escape. Effect is much enhanced by monoamine oxidase (MAO) inhibition, which can lead to severe hypertension following ingestion of tyramine-rich foods (cheese, wine) by patients treated with MAO inhibitors.
- Indirectly acting sympathomimetic agents are central nervous system stimulants. **Methylphenidate** and **atomoxetine** are used to treat attention deficit-hyperactivity disorder.
- Drugs that inhibit uptake 1 include **cocaine** and **tricyclic antidepressant drugs**. Sympathetic effects are enhanced by such drugs.

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Toxicology studies during drug development

Toxicities that are analyzed very early in drug development:

- Mutagenesis (→ carcinogenesis)
- Teratogenesis (inducing malformations in fetus)
- Effects on QT interval in cardiac rhythm

Preclinical and clinical studies:

- Acute and chronic toxicology

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Teratogenesis and drug-induced fetal damage

- Teratogenesis = production of gross structural malformations during fetal development

The nature of drug effects on fetal development

Stage	Gestation period in humans	Main cellular process(es)	Affected by
Blastocyst formation	0-16 days	Cell division	Cytotoxic drugs, ?alcohol
Organogenesis	17-60 days approximately	Division Migration Differentiation Death	Teratogens Teratogens Teratogens Teratogens
Histogenesis and functional maturation	60 days to term	As above	Miscellaneous drugs (e.g. alcohol, nicotine, antithyroid drugs, steroids)

- Human teratogens:
  - Thalidomide
  - Cytotoxic drugs (alkylating agents)
  - Retinoids (Vitamin A derivatives)
  - Heavy metals
  - Some antiepileptic drugs (phenytoin, valproate, carbamazepine)
- Mechanisms of action are not understood, DNA damage is a factor

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Question

Which of the following types of toxicity cannot be accepted, and consequently, if it is found with a drug candidate during development, the drug development will be stopped?

A. A substance is carcinogenic
B. A substance displays hepatotoxicity
C. A substance is teratogenic

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Cardiac Rhythm: the cardiac action potential and the ECG recording

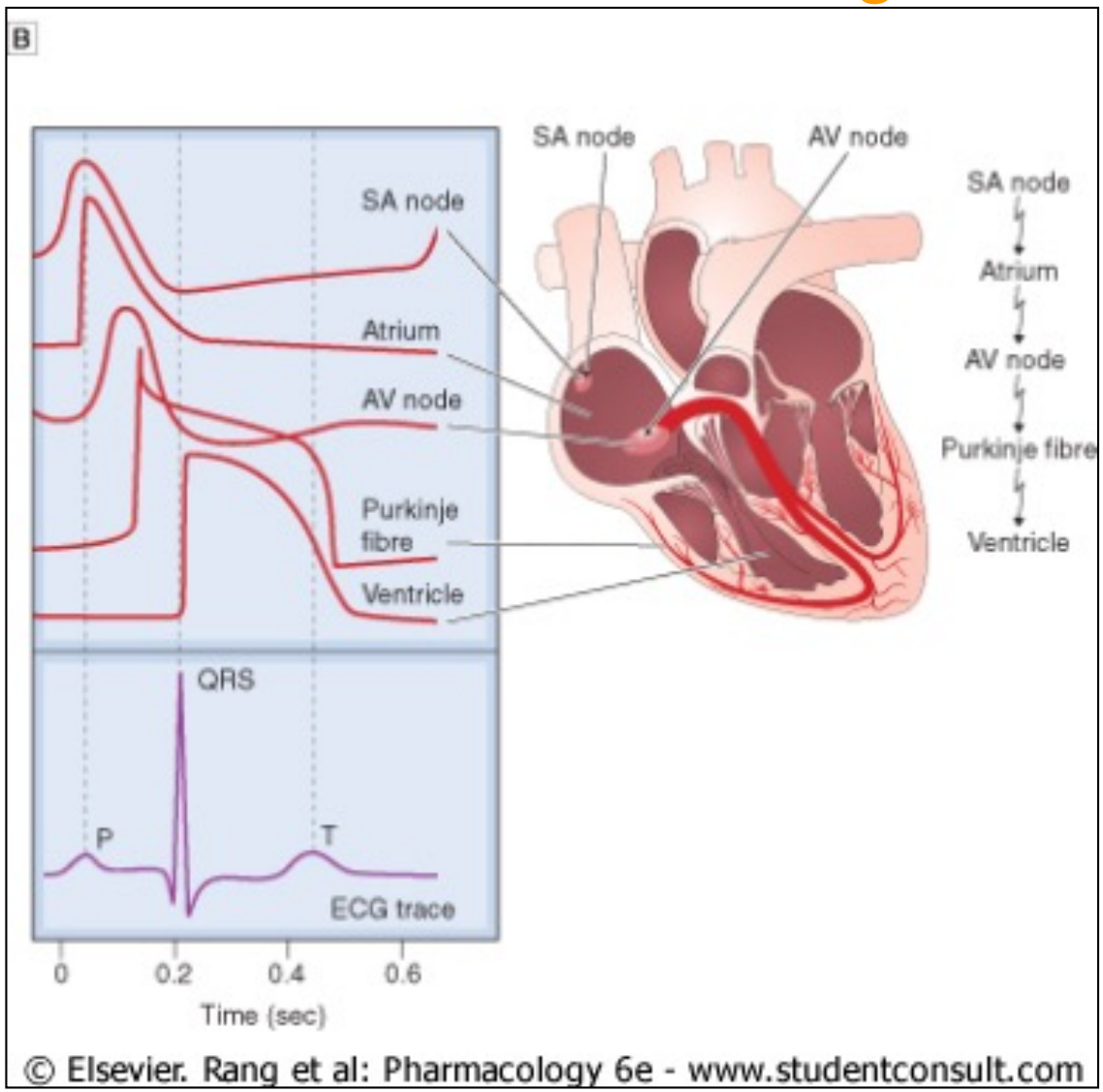
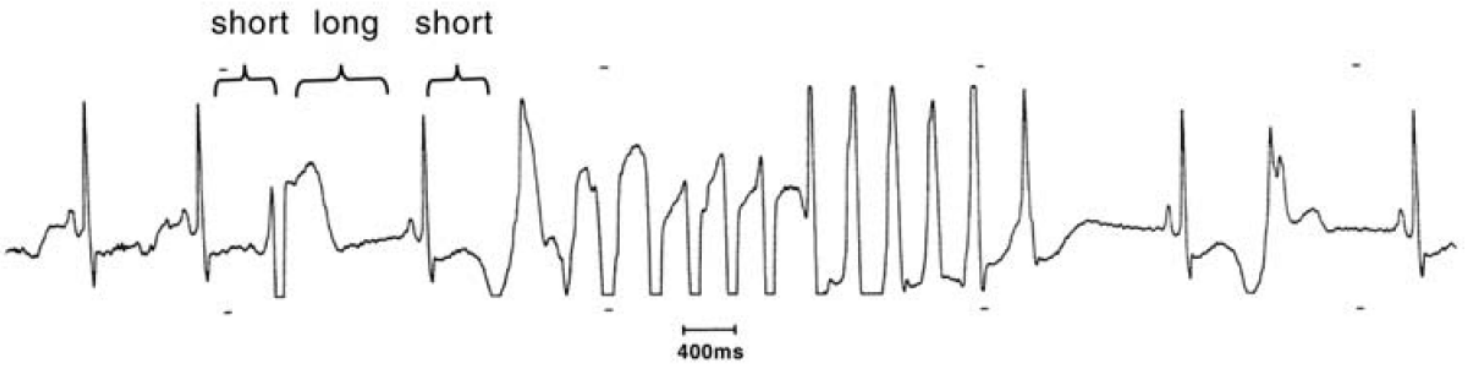


Figure 18-1 The cardiac action potential. Conduction of the impulse through the heart, with the corresponding electrocardiogram (ECG) trace. Note that the longest delay occurs at the atrioventricular (AV) node, where the action potential has a characteristically slow waveform. SA, sinoatrial. (Adapted from: (A) Noble D 1975 The initiation of the heartbeat. Oxford University Press, Oxford.)

Risk of prolonged QT interval

- The prolonged ventricular action potential increases the risk of ventricular arrhythmias, especially a form of ventricular tachycardia called “torsades de pointes” (TdP)



Characteristic short-long-short sequence preceding a short run of TdP in a patient on high-dose procainamide and amiodarone. Adapted with permission from Trohman and Sahu.[21]

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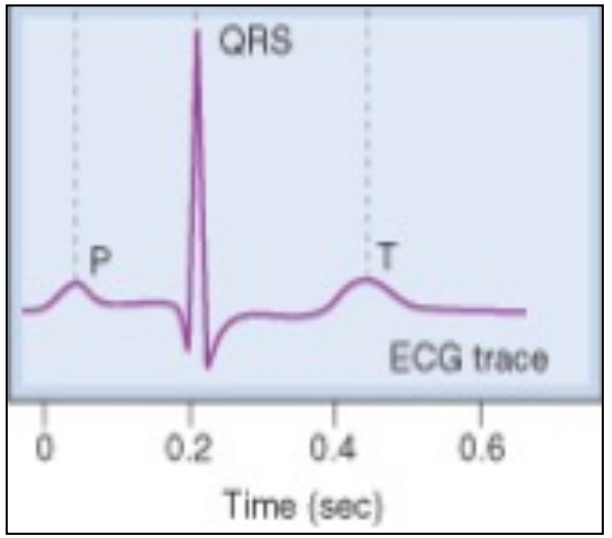
The QT interval can be increased

- By mutations in cardiac ion channels
- By drugs



Risk factors

- Most patients with a prolonged QT interval never develop TdP (risk ~1:100'000). The risk of torsades starts to grow when long QT is combined with other risk factors:
  - bradycardia, especially with occasional "premature" beats
  - hypokalemia
  - hypomagnesemia
  - "stimulant" conditions such as exercise, emotion, or use of drugs like dopamine, epinephrine
  - the risk is higher for females

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drug-induced long QT: relevance for drug development

- although this adverse drug reaction is generally a very rare event, it is an important safety issue
- any new drug needs to be tested for an inhibition of hERG (a K channel) and for an effect on the QT duration
- these tests are carried out early in the development
- QT duration is a “surrogate marker”
- pharmacosurveillance (ADR reporting systems)

- (Toxicology studies: in pre-clinical development, in phase II clinical studies)

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Toxicity and other testing during drug development

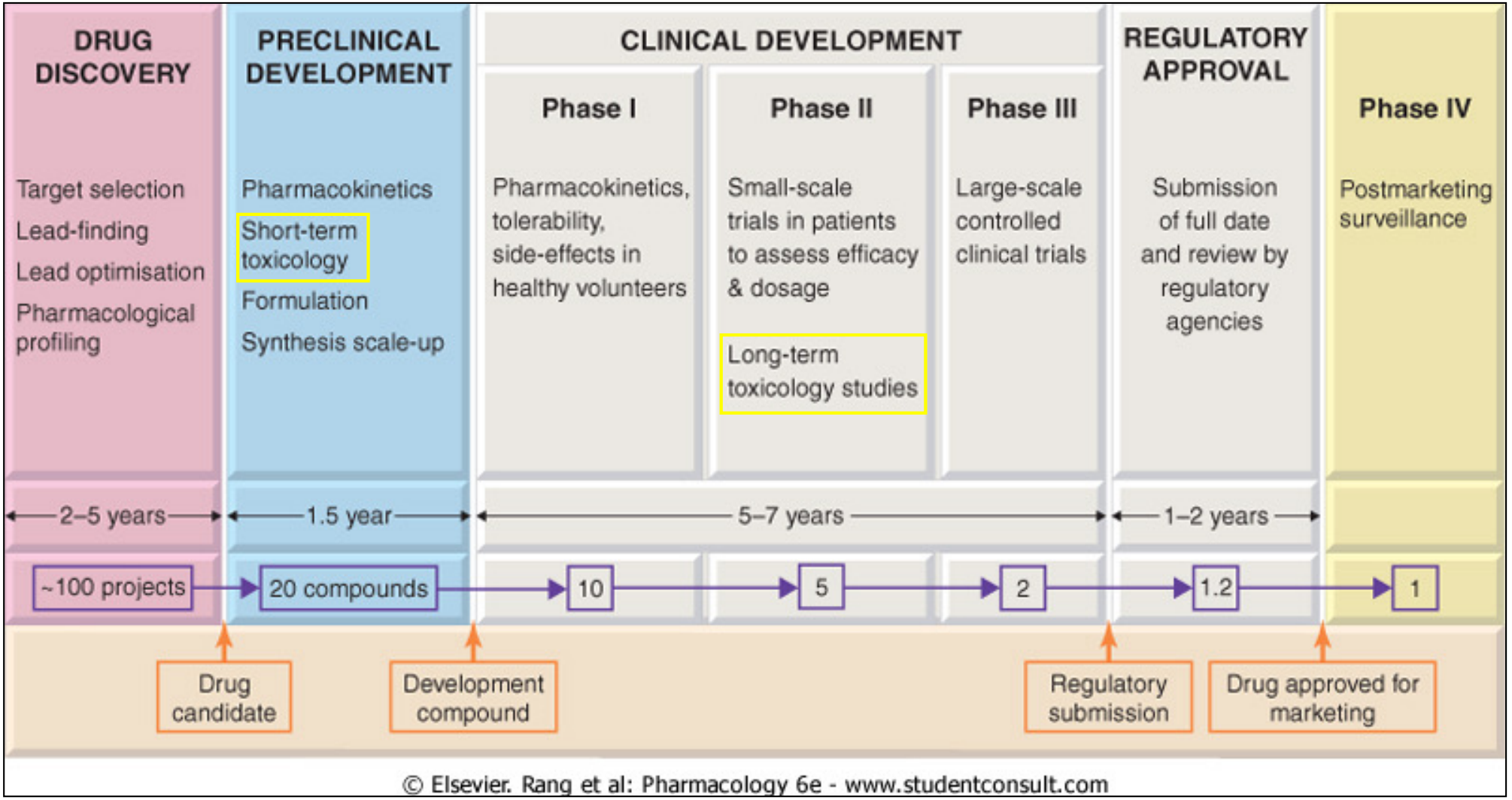

Figure 56-1 The stages of development of a 'typical' new drug, i.e. a synthetic compound being developed for systemic use. Only the main activities undertaken at each stage are shown, and the details vary greatly according to the kind of drug being developed.

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Situation

Bessie B. is a 78-year old woman who is brought to her family physician, Dr. Joy, by her daughter for an evaluation of excessive sleeping. Her family has noted her to be progressively confused and slow over the past week, falling asleep during dinner the past two evenings. This morning, she did not get up at her usual time. (etc.)

Mrs. B. is treated for hypertension, stable coronary artery disease, a history of supraventricular tachycardia, and anxiety. Her medication includes aspirin, alprazolam (a benzodiazepine, used to treat the anxiety), diltiazem (a Calcium channel blocker [treatment of the coronary heart disease]), hydrochlorothiazide (a diuretic used to treat the hypertension) and lovastatin (used to lower the cholesterol). She was recently started on an antibiotic treatment with clarithromycin for bronchitis.

Q1: Which of Mrs. B.'s medications might account for her drowsiness?

Q2: The doctor is concerned that Mrs. B. might have a drug-drug interaction. Which of the drugs might have induced this interaction?

Q3: Macrolide antibiotics (as clarithromycin) inhibit certain drug metabolizing enzymes. How can this affect other drugs?

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