THERAPEUTIC DRUG MONITORING II.

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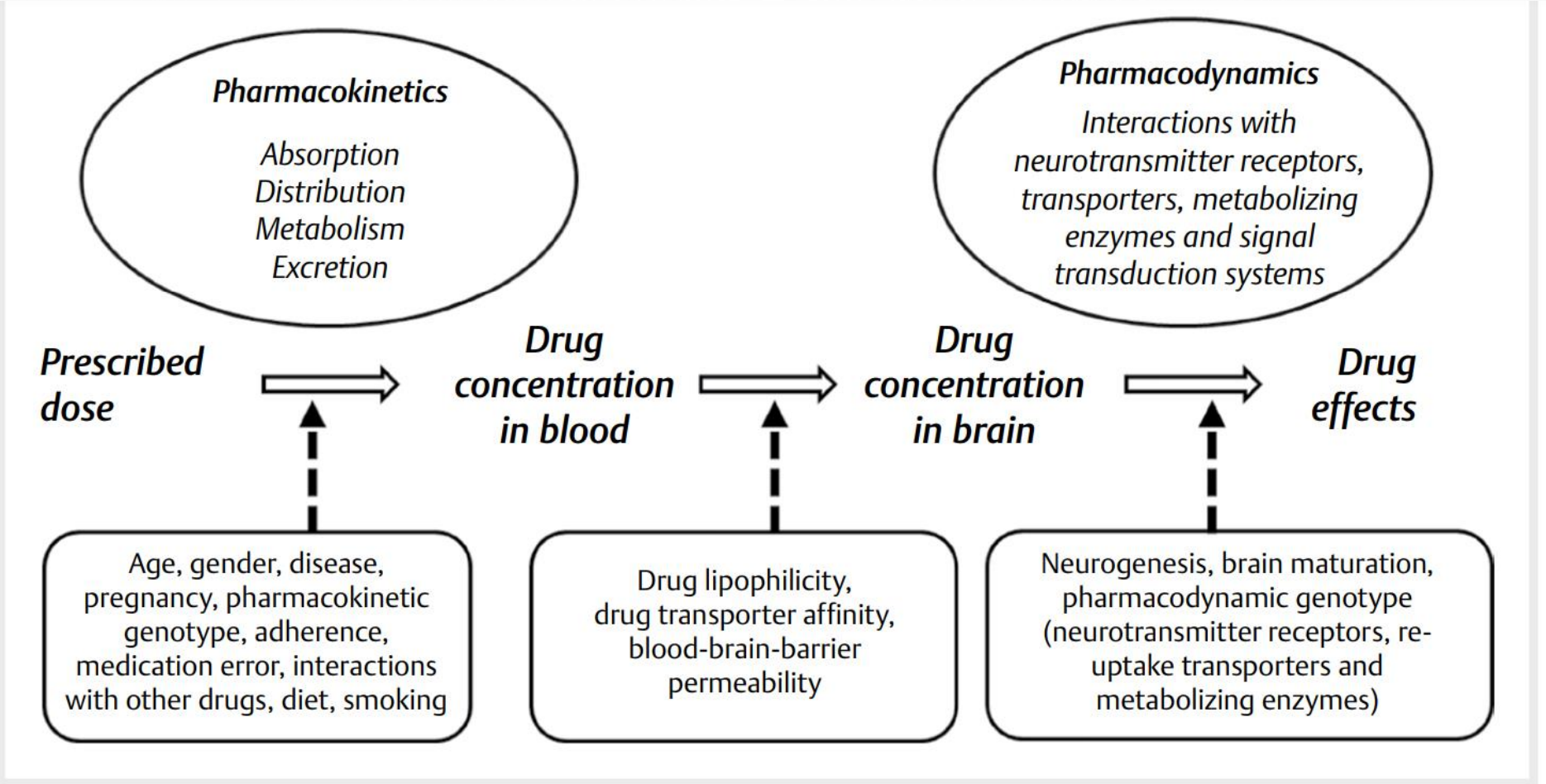
DRUGS ADMINISTERED ALONG WITH THERAPEUTIC DRUG MONITORING

Publication	Drugs. drug groups
Hiemke C et al. Pharmacopsychiatry 2018;51:9.	antipsychotics, antidepressants, anticonvulstants, anxiolytics, antiparkinson drugs
Jacob S. Nair AB. Drugs R D 2016;16:303.	Anticonvulsants
Patsalos PN et al. Epilepsia 2008;49:1239.	Anticonvulsants
Aonuma K et al. Circ J 2017;81:581.	Cardiovascular drugs
Roberts JA et al. Brit J Clin Pharmacol 2011;73:27.	Antibiotics
Back D et al. Ther Drug Monit 2006;28:468.	Antivirals
Ashbee HR et al. J Antimicrob Ther 2014;69:1162.	Antifungals
Freudenberger K et al. Trends Anal Chem 2016;79:257.	Immunosuppressants
Mitrev N et al. Aliment Pharmacol Ther 2017;46:1037.	infliximab. adalimumab
Choong E et al. Ther Drug Monit 2018;40:84.	busulfan
Paci A et al. Eur J Cancer 2014;50:2010.. Widmer N et al. Eur J Cancer 2014;50:2020.	Oncology drugs
Abdul-Aziz MH et al. Intensive Care Med 2020;46:1127.	Antibiotic TDM in intensive care position paper

DRUGS FOR CENTRAL NERVOUS SYSTEM DISORDERS



► **Fig. 1** From prescribed dose to drug effects modulated by multiple factors leading to marked pharmacokinetic and pharmacodynamic variability.

DRUGS FOR CENTRAL NERVOUS SYSTEM DISORDERS

- DRUG GROUPS:
  - ANTIDEPRESSANTS
  - ANTIPSYCHOTICS
  - MOOD STABILIZERS
  - ANTICONVULSANTS
  - ANXIOLYTICS AND DRUGS FOR SLEEP DISORDERS
  - ANTIDEMENTIA DRUGS
  - DRUGS OF SUBSTANCE USE
  - DRUGS OF ATTENTION DEFICIT AND HYPERACTIVITY DISORDER
  - ANTIPARKINSON DRUGS
- RECOMMENDATIONS ARE ON A SCALE OF 1 THROUGH 4 (1: STRONGEST)

DRUGS FOR CENTRAL NERVOUS SYSTEM DISORDERS

- FAST AND EFFICIENT ABSORPTION FROM GASTROINTESTINAL TRACT, PEAK CONCENTRATION IS ATTAINED IN 1-6 HOURS
- BIOAVAILABILITY: 5-100%
- RAPID DISTRIBUTION BETWEEN CENTRAL COMPARTMENT AND CENTRAL NERVOUS SYSTEM
- LARGE APPARENT VOLUME OF DISTRIBUTION
- METABOLISM COMMONLY TAKES PLACE (CYP450, UDP GLUCURONOSYL TRANSFERASES)
- ELIMINATION HALF LIFE: 12-36 H
- LINEAR PHARMACOKINETICS

SPECIAL ASPECTS OF MONITORING ANTIPSYCHOTIC TREATMENT

- SEVERAL PATIENTS UNDERGO POLYTHERAPY
- DEPOT ANTIPSYCHOTICS (LONG-ACTING INJECTABLES, LAI)
  - FLUPHENAZINE, FLUPENTHIXOL, HALOPERIDOL, ZUCLOPENTHIXOL
  - ARIPIPRAZOLE (MONOHYDRATE, LAUROXIL), PALIPERIDONE PALMITATE, OLANZAPINE PAMOATE, RISPERIDONE MICROSPHERES
    - GIVEN EVERY 2-12 WEEKS

ADVERSE EFFECTS OF ANTIPSYCHOTICS

- TARDIVE DYSKINESIA
- AKATHISIA
- NEUROLEPTICS-INDUCED DEFICIT SYNDROME (NIDS)
- AGRANULOCYTOSIS
- EFFECTS ON NEWBORNS: THIRD-TRIMESTER EFFECTS AND LACTATION

ADVERSE EFFECTS OF ANTICONVULSANTS

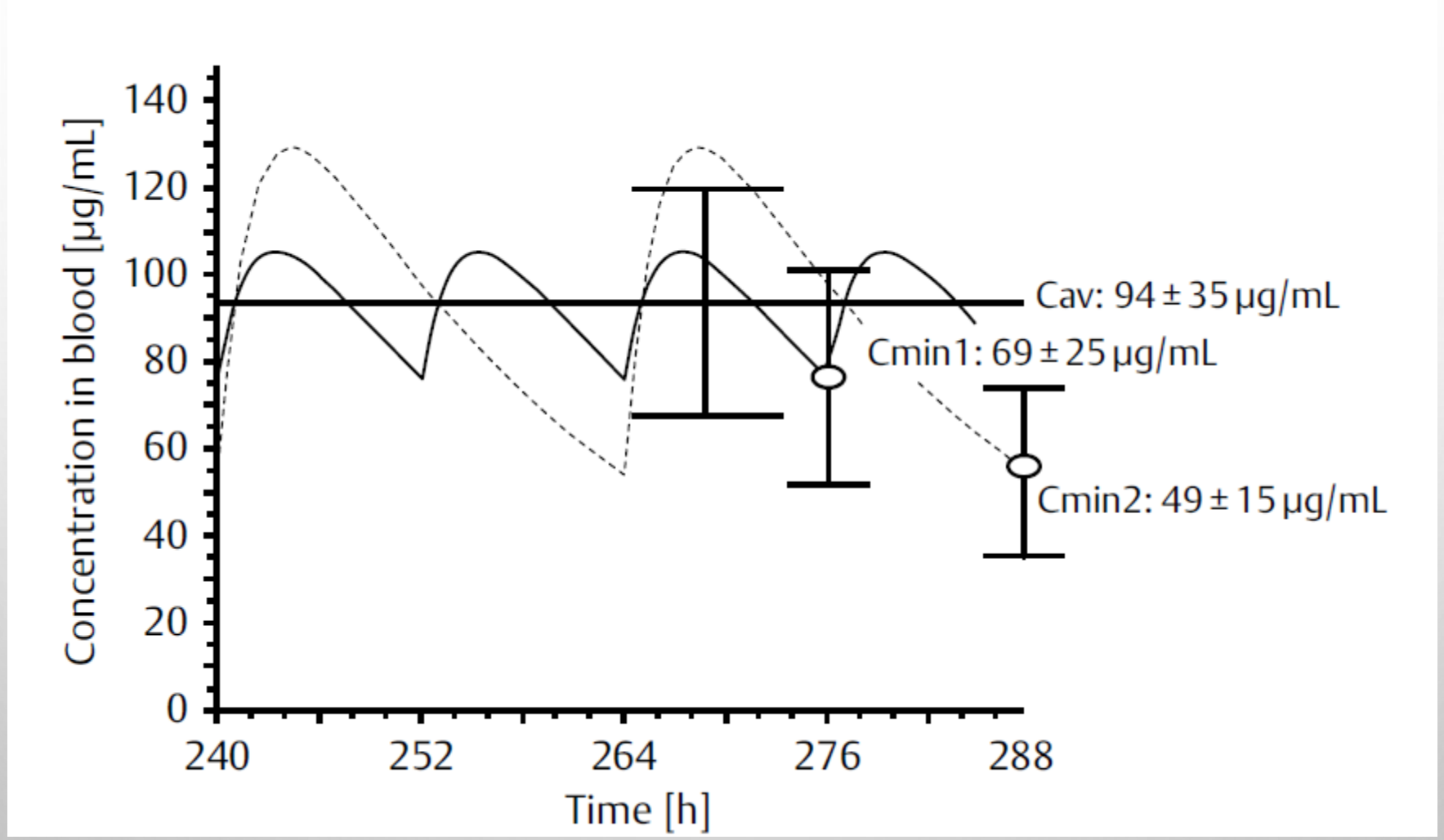
- NEUROLOGICAL EFFECTS
  - SEDATION
  - MOVEMENT COORDINATION DISORDERS
  - DIPLOPIA
  - MOOD INSTABILITY
  - SEXUAL DISORDERS
- CHRONIC ADVERSE EFFECTS
  - BODY MASS CHANGES
- EFFECTS ON FETUS AND THE NEWBORN
  - CONGENITAL MALFORMATIONS
  - VALPROATE HAS AN OUTSTANDING RISK POTENTIAL

AGNP GUIDELINE:
DOSE-DEPENDENT REFERENCE RANGES

$$c_{av} = \frac{D_m}{d_i} \times \frac{F}{Cl}$$

C_{av}: mean steady-state concentration (ng/mL)
D_m: daily maintenance dose (ng)
d_i: dosing interval (min)
CL: clearance (ml/min)
F: bioavailability (unitless)

Limitation: when elimination half life is relatively short and the dosing interval is longer than the elimination half life, the equation will not predict trough concentrations efficiently



Hiemke C et al. Pharmacopsychiatry 2018;51:9.

AGNP GUIDELINE:
DOSE-DEPENDENT REFERENCE RANGES

$$c_t = \frac{D_m}{d_i} \times \frac{F}{Cl} \times \left(\frac{k_e \times d_i}{1 - e^{-k_e d_i}} \right) \times e^{-k_e t}$$

$$k_e = \frac{ln2}{t_{1/2}}$$

C_t: concentration at any t time (ng/mL)
D_m: daily maintenance dose (ng)
D_i: dosing interval (min)
F/Cl: reciprocal apparent clearance (min/mL)
k_e: elimination rate constant (min)
t: time from intake to sampling

Limitation: assumes single compartment pharmacokinetics, does not take repeated dose intake into account

AGNP GUIDELINE:
DOSE-DEPENDENT REFERENCE RANGES

$$c_{min} = \frac{D_m}{24} \times \underbrace{\frac{F}{Cl} \times \frac{k_e \times 24}{1 - e^{-k_e \times 24}} \times e^{-k_e t}}_{\text{„DRC-factor”}}$$

C_{min}: estimated trough concentration (ng/mL)
D_m: daily maintenance dose (ng)
F/Cl: reciprocal apparent clearance (min/mL)
k_e: elimination rate constant (min)
t: time from intake to sampling

Limitation: assumes single compartment pharmacokinetics and once-daily dosing, does not take repeated dose intake into account

AGNP GUIDELINE: DOSE-DEPENDENT REFERENCE RANGES

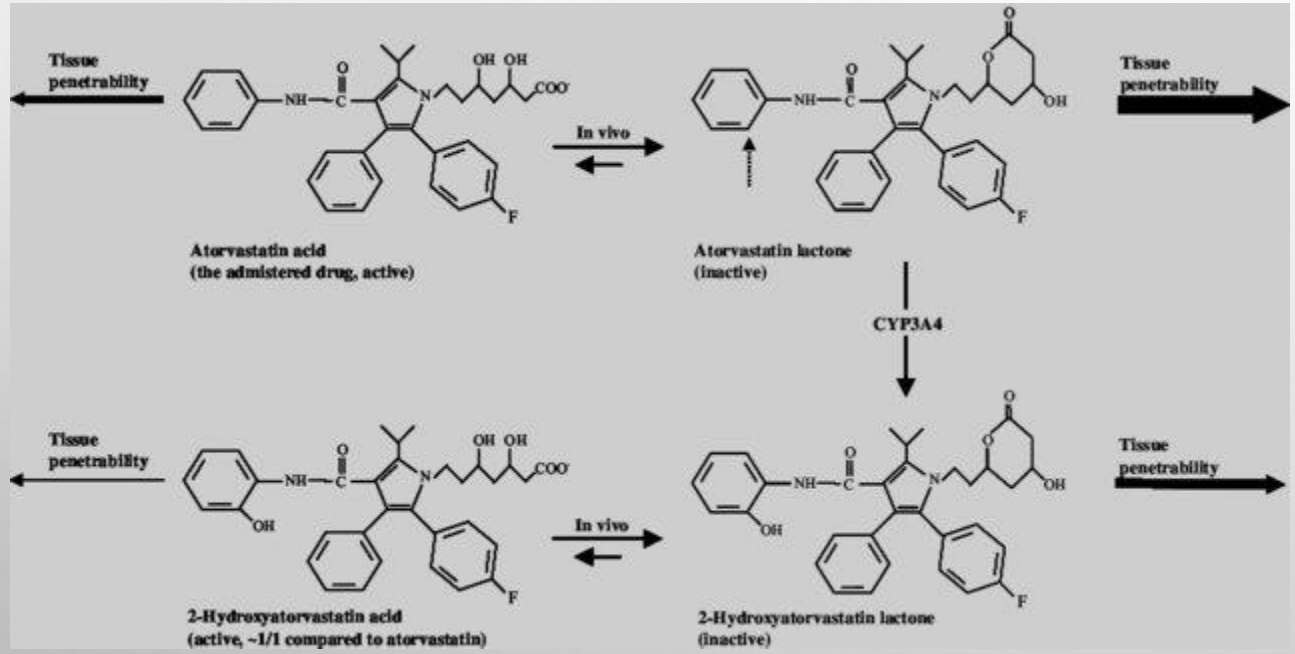
Definition

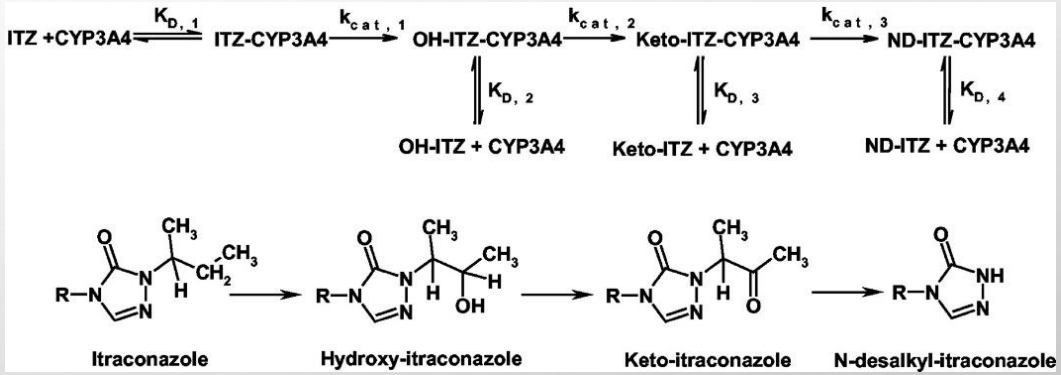
The "dose-related reference range" reported in the present guidelines is defined as the mean–SD to mean + SD range of the trough concentration of a drug under steady-state conditions. The mean ± SD includes 68 % of a population of "normal" patients who ingested their medication, are aged 18–65 years, with a body weight of 70 kg and without pharmacokinetically relevant comorbidity, comedication or genetic abnormalities in drug metabolism. Dose-related reference ranges are obtained by multiplying DRC factors low and high of ▶ **Table 5** by the daily dose.

Drugs and metabolites	CL/F ± SD	F	t1/2	Δt	DRC factors			Comments	References
	[mL/min]	[%]	[h]	[h]	mean	low	high		
Vilazodone	415 ± 129	70	32	24	1.28	0.88	1.67	CL affected by CYP3A4	[127–128]
Vortioxetine	550 ± 83	80	66	24	1.11	0.94	1.28	CL affected by CYP2D6	[47, 548, 834]
Antipsychotic drugs									
Amisulpride	586 ± 174	50	16	24	0.67	0.47	0.87	CL not affected by CYP enzymes, renal excretion	[138, 827, 829, 1018]
Aripiprazole dehydroaripiprazole active moiety	53 ± 16 132 ± 49	90	70 94	24	11.72 4.82 16.54	8.15 3.04 11.19	15.29 6.60 21.89	CL affected by CYP2D6 and CYP3A4.	[626, 751–752]
Asenapine	2,761 ± 1,783	35	24	12	0.25	0.09	0.41	CL similar in patients elder or younger than 65 years	[197, 312]
Benperidol	1,266 ± 513	50	6	12	0.40	0.24	0.56		[1068]
Brexpiprazole	23 ± 13	95	91	24	27.4	11.6	43.2	CL affected by CYP2D6	[443]
Bromperidol	1,598 ± 607	30	20	12	0.42	0.26	0.58		[690, 1155]
Cariprazine N-desmethylcariprazine N-didesmethylcariprazine	279 ± 67 869 ± 178 125 ± 32	100	44 37 446	24	2.05 0.63 5.45	1.56 0.50 4.06	2.54 0.76 6.85	CL affected by CYP3A4	[840]
Chlorpromazine	623 ± 203	30	30	24	0.83	0.56	1.10		[1338]
Chlorprothixene	2,507 ± 478	50	10	12	0.25	0.20	0.30		[62, 970]
Clozapine N-desmethylclozapine	637 ± 367 667 ± 283	50	12 8	12 24 12 24	1.01 0.50 0.87 0.31	0.43 0.21 0.50 0.18	1.59 0.79 1.25 0.44	CL may be enhanced in smokers due to induction of CYP1A2 and decreased during inflammation. CL/F is twofold higher in Asian than Caucasian patients, for clozapine, t1/2 is prolonged to 30 h in intoxicated patients.	[133, 216, 240, 290, 575, 703, 1075]
Flupentixol	2,148 ± 814	50	35	12	0.32	0.20	0.44	CL associated with CYP2D6	[2, 595, 914]
Fluphenazine	9,990 ± 2,820	35	16	12	0.07	0.05	0.09	CL affected by CYP2D6	[944]
Fluspirilene	No data								
Haloperidol	826 ± 203	60	18	12	0.81	0.61	1.01	CL affected by CYP2D6	[205, 381, 914, 941]
Iloperidone	1,258 ± 425	100	18	12	0.53	0.35	0.71	CL affected by CYP2D6	[175, 1031]
Levomepromazine	2,630 ± 1,580	50	28	12	0.26	0.10	0.42		[255, 779]
Levosulpiride	425 ± 140	30	8	12	1.35	0.90	1.79	CL affected by P-gp (ABCB1)	[214, 1331]
Loxapine	807 ± 138	30	7	4 24	1.51 0.21	1.26 0.17	1.78 0.25	Aerosol application	[1126, 1164]
Lurasidone	3,902 ± 702	20	18	24	0.11	0.09	0.13	CL affected by food intake (fat content)	[225, 358, 951]
Melperone	2,555 ± 476	60	5	12	0.18	0.14	0.21		[135]

SIMULTANEOUS MEASUREMENT OF ANTIPSYCHOTICS AND THEIR PHARMACOLOGICALLY ACTIVE METABOLITES

- PHARMACOLOGICALLY ACTIVE METABOLITES ARE FREQUENTLY IMPORTANT IN EXERTING THE CLINICAL EFFECTS OF A DRUG
- ACTIVE METABOLITES CAN EVEN DOMINATE CLINICAL EFFECTS
- SEVERAL HUNDRED DRUGS ARE METABOLIZED TO ACTIVE PRODUCTS





Molden E. Heart Drug 2004;4:55.

Isoherranen N. Drug Metab Dispos 2004;32:1121.

SIMULTANEOUS MEASUREMENT OF ANTIPSYCHOTICS AND THEIR PHARMACOLOGICALLY ACTIVE METABOLITES

- THE SIMULTANEOUS MEASUREMENT OF THE DRUG AND ITS PHARMACOLOGICALLY ACTIVE METABOLITE IS RECOMMENDED:
  - AMITRIPTYLINE + NORTRIPTYLINE (THERAPEUTIC RANGE, TR: 80-200 NG/ML)
  - BUPROPION + HYDROXYBUPROPION (TR: 850-1500 NG/ML, 550-1500 NG/ML WHEN TREATING SLEEP DISORDERS)
  - CLOMIPRAMINE + N-DESMETHYLCLOMIPRAMINE (TR: 230-450 NG/ML)
  - DOXEPIN + N-DESMETHYLDOXEPIN (TR: 50-150 NG/ML)
  - FLUOXETINE + N-DESMETHYLFLUOXETINE (TR: 120-500 NG/ML)
  - IMIPRAMINE + DESIPRAMINE (TR: 175-300 NG/ML)
  - VENLAFAXINE + O-DESMETHYLVENLAFAXINE (TR: 100-400 NG/ML)
  - ARIPIPRAZOLE + DEHYDROARIPIPRAZOLE (TR: 150-500 NG/ML)
  - QUETIAPINE (TR: 100-500 NG/ML) + N-DESALKYLQUETIAPINE (TR: 100-250 NG/ML)

SIMULTANEOUS MEASUREMENT OF ANTIPSYCHOTICS AND THEIR PHARMACOLOGICALLY ACTIVE METABOLITES

- THE SIMULTANEOUS MEASUREMENT OF THE DRUG AND ITS PHARMACOLOGICALLY ACTIVE METABOLITE IS RECOMMENDED:
  - RISPERIDONE + 9-HYDROXYRISPERIDONE (TR: 20-60 NG/ML)
  - CLOBAZAM (TR: 30-300 NG/ML) + N-DESMETHYL CLOBAZAM (TR: 300-3000 NG/ML)
  - METHSUXIMIDE + N-DESMETHYL METHSUXIMIDE (TR: 10-40 NG/ML)
  - OXCARBAZEPINE + 10-HYDROXYCARBAZEPINE (TR: 10-35 NG/ML)
  - DIAZEPAM + N-DESMETHYLDIAZEPAM (TR: 100-2500 NG/ML)
  - BUSPIRONE + 6-HYDROXYBUSPIRONE + 1-(PYRIMIDINYL)-PIPERAZINE (TR: 1-4 NG/ML)
  - FLURAZEPAM (TR: 0-4 NG/ML 1-3 HOURS AFTER ADMINISTRATION) + N-1-DESALKYLFLURAZEPAM (TR: 10-22 NG/ML 1-3 HOURS AFTER ADMINISTRATION, 75-165 NG/ML 10 HOURS AFTER ADMINISTRATION)
  - MEDAZEPAM + DESMETHYLMEDAZEPAM + TEMAZEPAM + OXAZEPAM (TR: 200-2500 NG/ML)
  - DISULFIRAM (TR: 50-400 NG/ML) + DIETHYLTHIOMETHYL-CARBAMATE-METHYLESTER (TR: 270-10 NG/ML)

AGNP guideline:
C/D ratio

- C: TROUGH CONCENTRATION
- D: DOSE

- HIGH C/D RATIO: SLOW METABOLISM
- LOW C/D RATIO: RAPID METABOLISM

- C/D RATIOS CAN PROVIDE FURTHER INFORMATION ON THE PATIENT OR ON DRUG PK WHEN EVALUATION IS BASED ON POP PK OR ON LONGITUDINAL MONITORING
- CAN BE A TOOL FOR IDENTIFYING DRUG INTERACTIONS

AGNP guideline
Metabolite-to-parent compound ratio, MPR

Drug	Active metabolite	MPR
Aripiprazole	Dehydro aripiprazole	0.3-0.5
Escitalopram	N-desmethyl escitalopram	0.3-1.0
Fluvoxamin	Fluvoxamin acid	0-1.2
Clozapin	N-desmethyl clozapine	0.45-0.79
Quetiapine	N-desalkyl quetiapine	0.54-3.10
Mianserine	N-desmethyl mianserine	0.5-0.8
Mirtazapine	N-desmethyl mirtazapine	0.5-1.2
Olanzapine	N-desmethyl olanzapine	0.1-0.3
Sertraline	N-desmethyl sertraline	1.7-3.4
Risperidone	Paliperidone	3.6-22.7
Venlafaxin	O-desmethyl venlafaxine	2.7-7.7

LABORATORY TESTING OF ANTIPSYCHOTICS

- SAMPLE: SERUM (NATIVE BLOOD) OR PLASMA (ANTICOAGULANT: EDTA)
- METHOD: LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETER

Per os administered, direct-acting anticoagulants

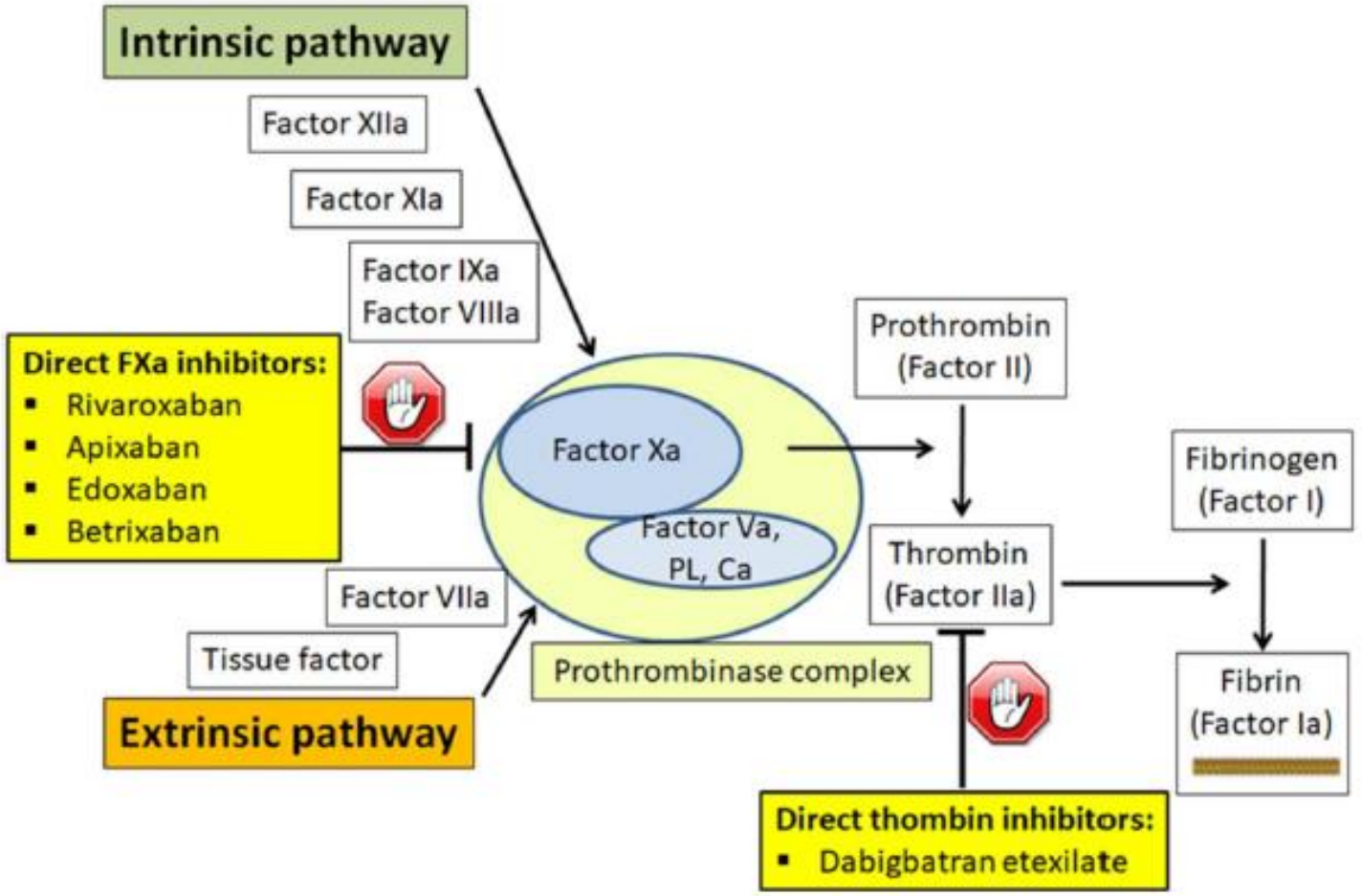


FIGURE 1. Coagulation cascade and targets of DOACs: The extrinsic and intrinsic pathways of the coagulation cascade converge at the prothrombinase complex constituted by factor Xa, factor Va, phospholipids (PLs), and calcium (Ca). Direct FXa inhibitors block activated factor X (factor Xa), thereby preventing consecutively thrombin and fibrin formation. The direct thrombin inhibitor dabigatran is administered as its prodrug dabigatran etexilate and blocks thrombin and consequently fibrin formation.

Wieland E. Shipkova M. Ther Drug Monit 2019;41:180-91.

Per os administered, direct-acting anticoagulants: pharmacodynamic and pharmacokinetic properties

Property	Apixaban	Dabigatran	Edoxaban	Rivaroxaban
Target protein	Factor Xa	Factor IIa	Factor Xa	Factor Xa
Dosing regime	b.i.d.	b.i.d.	q.d.	q.d.
Time to reach peak concentration	3-4 hours	1.5-3 hours	1-2 hours	2-4 hours
Elimination half-life	10-14 hours	12-14 hours	9-11 hours	5-9 hours
Bioavailability	50%	3-7%	60%	80-100%
Protein binding	85%	35%	40-60%	90-95%
Renally eliminated fraction	25%	80%	50%	40%
Interactions during metabolism	CYP3A4, p-glycoprotein	P-glycoprotein	P-glycoprotein	CYP3A4, p-glycoprotein

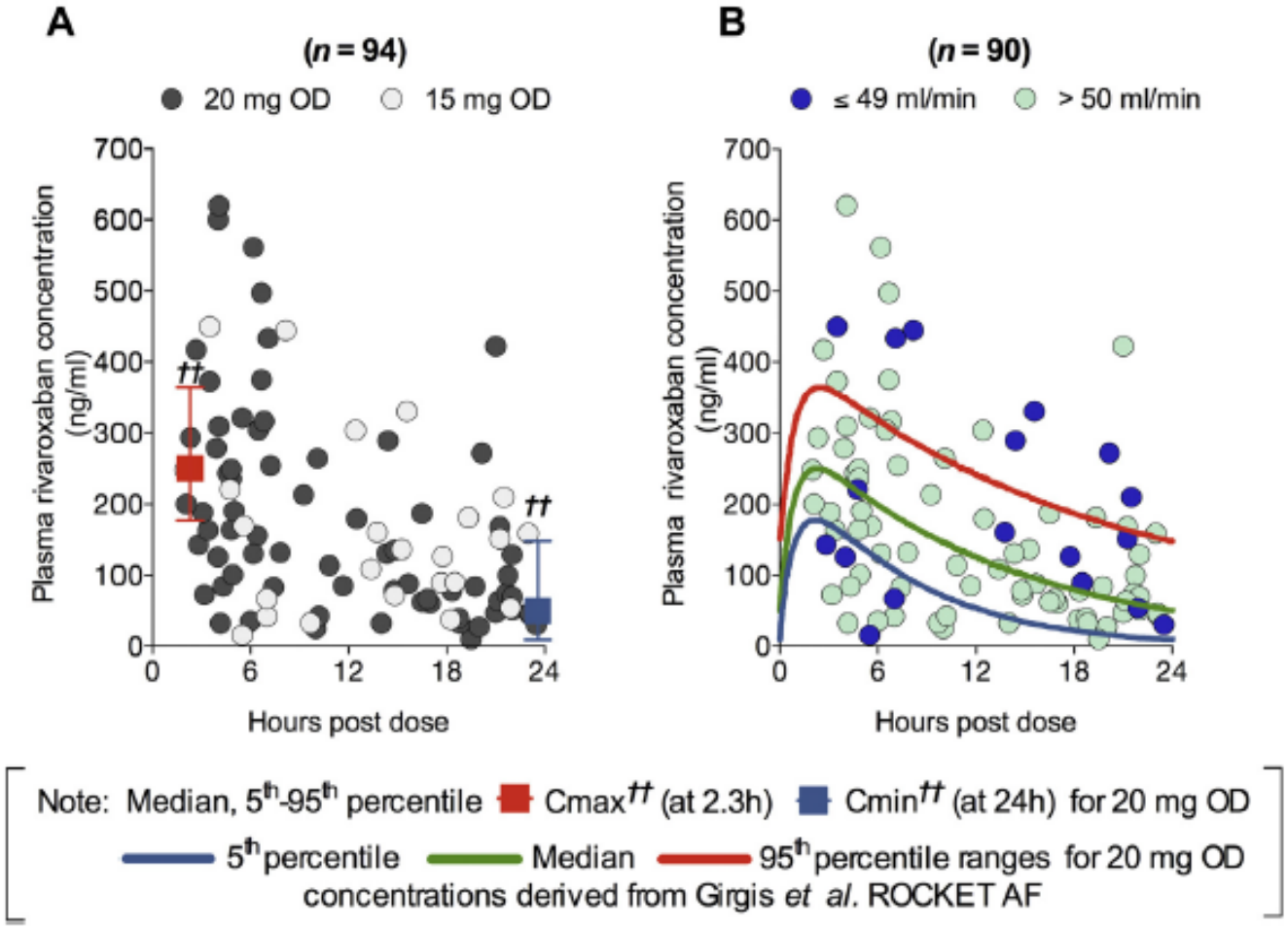


Figure 2. Observed plasma rivaroxaban concentration in relation to (**A**) dose and criteria required for appropriate dosing, (**B**) estimated creatinine clearance, superimposed onto clinical trial pharmacokinetic population data for patients with atrial fibrillation (AF) (See Methods section). Estimated creatinine clearance calculated using Cockcroft-Gault's formula; patients missing serum creatinine data were excluded (**B**, n = 4). C_{max}, maximum concentration; C_{min}, minimum concentration; OD, once daily.

Gulilat M et al. Canadian J Cardiol 2017;33:1036-43.

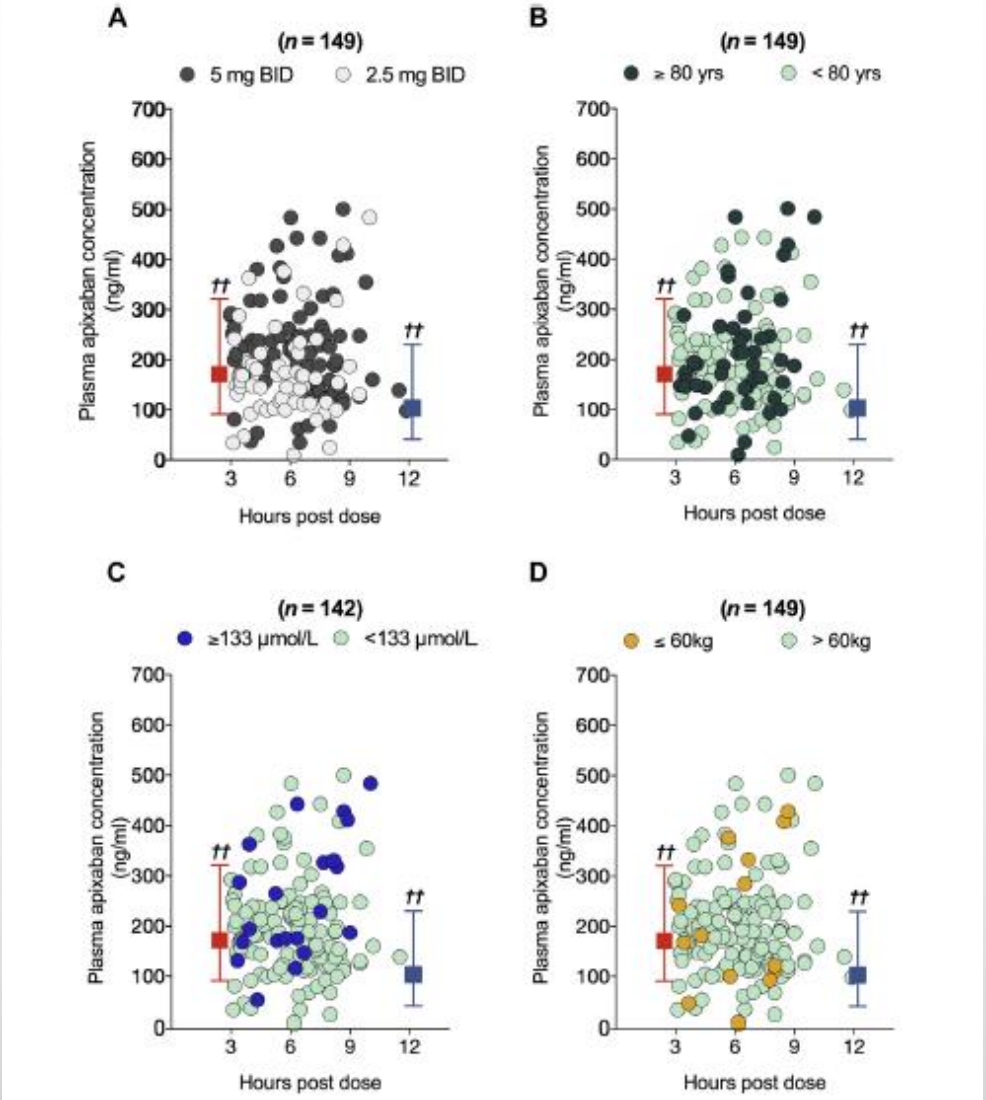


Figure 3. (**A**) Observed plasma apixaban concentration in relation to dose, and criteria required for appropriate dosing: (**B**) age, (**C**) serum creatinine, and (**D**) weight. Measured concentration compared with predicted maximum (C_{max}) and minimum (C_{min}) plasma concentrations values for 5-mg, twice-daily dose, as noted in the apixaban product monograph for patients with atrial fibrillation (AF). (**C**) Patients missing serum creatinine data were excluded (n = 7). BID, twice daily.

Gulilat M et al. Canadian J Cardiol 2017;33:1036-43.

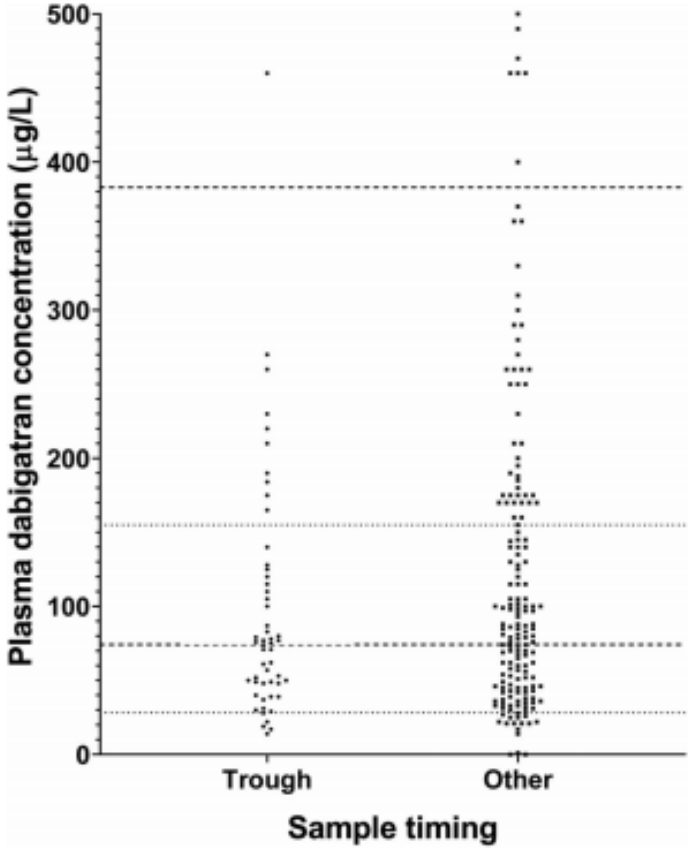


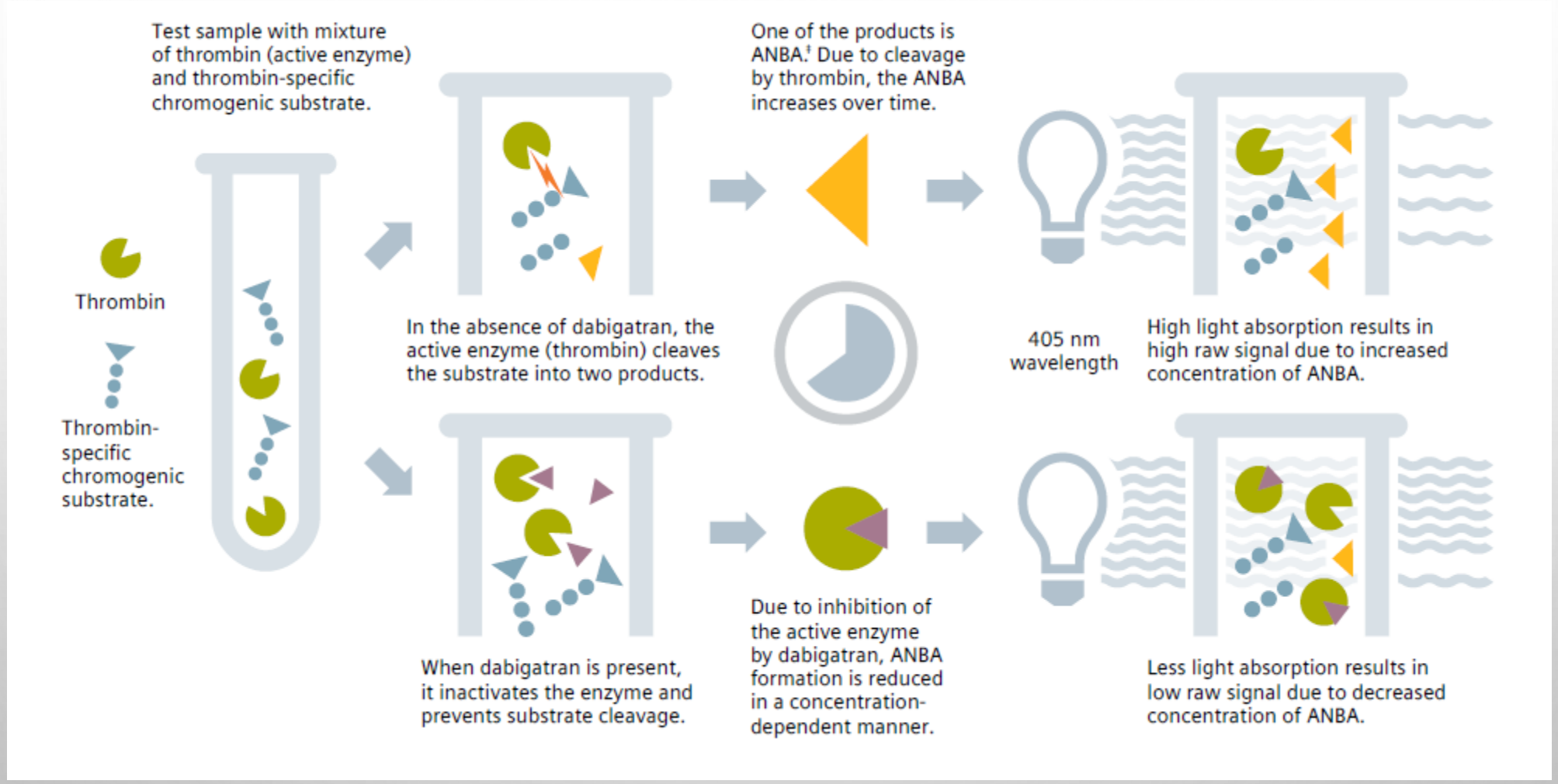
FIGURE 1. Scatter plot of dabigatran concentrations stratified into (A) "trough" with administration of last dose recorded in the in-patient electronic chart as >10 hours before the sample, and (B) "Other," comprising all other samples, such as those where the last dose was received in the community, and nontrough samples. Horizontal lines are the 10th and 90th centiles from the RE-LY trial.[3] Dotted lines are 10th to 90th centiles of the trough concentrations for the 110 mg twice daily dose (28.2 and 155 mcg/L). Dashed lines are 10th to 90th centiles of peak concentrations for the 150 mg twice daily dose (74.3 and 383 mcg/L). Two values in the "other" group are not shown (630 and 1060 mcg/L).

Chin PKL et al. Ther Drug Monit 2020;42:468-72.

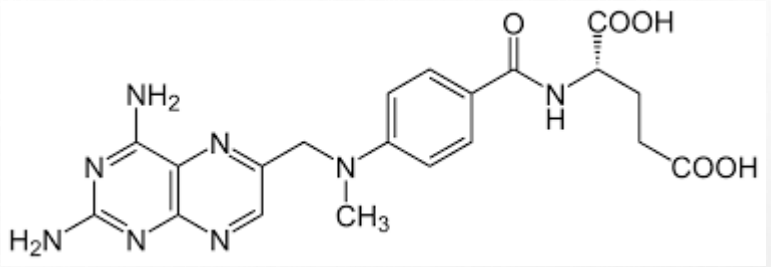
LABORATORY TESTING OF DIRECT-ACTING ANTICOAGULANTS

- SAMPLE: SERUM (NATIVE BLOOD) OR PLASMA (ANTICOAGULANT: EDTA)
- SERUM APIXABAN AND RIVAROXABAN CONCENTRATIONS ARE TYPICALLY HIGHER BY 15-20% AND BY 35-40% THAN CITRATE PLASMA CONCENTRATIONS, RESPECTIVELY
- SERUM APIXABAN AND RIVAROXABAN CONCENTRATIONS ARE TYPICALLY HIGHER BY 5% AND BY 15% THAN EDTA PLASMA CONCENTRATIONS, RESPECTIVELY
- ANALYTICAL METHODS: LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY, ACTIVITY TESTS

Aakeroy R et al. Ther Drug Monit 2022;44:578.


Test sample with mixture of thrombin (active enzyme) and thrombin-specific chromogenic substrate.
Thrombin
Thrombin-specific chromogenic substrate.
In the absence of dabigatran, the active enzyme (thrombin) cleaves the substrate into two products.
One of the products is ANBA.† Due to cleavage by thrombin, the ANBA increases over time.
405 nm wavelength
High light absorption results in high raw signal due to increased concentration of ANBA.
When dabigatran is present, it inactivates the enzyme and prevents substrate cleavage.
Due to inhibition of the active enzyme by dabigatran, ANBA formation is reduced in a concentration-dependent manner.
Less light absorption results in low raw signal due to decreased concentration of ANBA.

METHOTREXATE



- „S-PHASE-SPECIFIC" CYTOTOXIC AGENT
- INHIBITS ENZYMES OF THE FOLIC ACID CYCLE
- LOW-DOSE THERAPY: <50 MG/M^2 (URINARY BLADDER, BREAST CANCER, DESMOID TUMORS, LGL-LEUKEMIA, ALL, ACUTE PROMYELOCYTE LEUKEMIA, MYCOSIS FUNGOIDES, IMMUNE MEDIATED DISORDERS)
- MID-DOSE THERAPY: 50-500 MG/M^2 (MALIGN GESTATIONAL TROPHOBLAST DISEASE)
- HIGH-DOSE (HDMTX) THERAPY: >500 MG/M^2 (CNS PROPHYLAXIS IN LEUKEMIA AND HIGH-RISK LYMPHOMA, LEPTOMENINGEAL METASTASIS, CNS LYMPHOMA, OSTEOSARCOMA)

METHOTREXATE: ADVERSE EFFECTS

- LIVER FAILURE
- RENAL FAILURE
- HEMATOLOGICAL TOXICITY
- LUNG FAILURE
- NEUROTOXICITY (ENCEPHALOPATHIA)
- SKIN DISORDERS

METHOTREXATE: LEUCOVORIN RESCUE

- FOLINIC ACID (5-FORMYLTETRAHYDROFOLIC ACID) – L-LEUCOVORIN (LEVOLEUCOVORIN) IS THE ACTIVE FORM
- LEUCOVORIN PREPARATIONS: TABLET (5-25 MG), INJECTION (50-500 MG)
- INDICATIONS: METHOTREXATE OVERDOSE AND HDMTX THERAPY
- DAILY MONITORING OF METHOTREXATE CONCENTRATIONS IS REQUIRED:
  - NORMAL MTX ELIMINATION: <10 MCMOL/L @ 24 H, <1 MCMOL/L @ 48 H, <0.2 MCMOL/L @ 72 H
  - DELAYED LATE MTX ELIMINATION: >0.2 MCMOL/L @ 72 H, >0.05 MCMOL/L @ 96 H
  - DELAYED EARLY MTX ELIMINATION: >50 MCMOL/L @ 24 H, >30 MCMOL/L @ 36 H, > 5 MCMOL/L @ 48 H, OR >100% SERUM CREATININE ELEVATION @ 24 H COMPARED TO INITIAL VALUE

METHOTREXATE MONITORING

- HOMOGENOUS ENZYME IMMUNOASSAY (CE-IVD REAGENTS, AUTOMATED PLATFORM)
- LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LAB-DEVELOPED TESTS)
- TEST SAMPLE: SERUM (NATIVE BLOOD)

BUSULFAN

- CHRONIC MYELOGENOUS LEUKEMIA, CNS TUMORS, AUTOLOGOUS OR ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
- PEDIATRICS: CONDITIONING FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION
- MECHANISM OF EFFECT: ALKYLATING AGENT, MYELOSUPPRESSIVE AND IMMUNOSUPRESSIVE EFFECTS
- FORMULATION: IV. INFUSION
- COMBINATION WITH FLUDARABIN (ONLY ADULTS)

BUSULFAN

- SUBTHERAPEUTIC DOSES RESULT IN GRAFT REJECTION AND RELAPSE
- ADVERSE EFFECTS:
  - VENOOCCLUSIVE DISEASE (C_{MAX} CORRELATES WITH RISK)
  - INFECTIONS (40% OF ADULTS, FEBRILE NEUTROPENIA IS DEVELOPED BY 90% OF PEDIATRIC PATIENTS)
  - LIVER FAILURE
- TEST METHOD: LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

AUC calculated based on concentrations obtained after 1st dose

Steady-state AUC calculated using Bayesian modeling

Steady-state AUC calculated using Bayesian modeling and individualized treatment goals

Table 4. Achievement of AUC target range

	% Achievement of target AUC (% below; % above)	
	Conventional target range 900–1500 µmol min/L	*Local target range 980–1250 µmol min/L*
AUC_{dose1}		
Overall ($n = 142$)	67.6 (29.5, 2.9)	43.9 (43.2, 12.9)
< 9 kg ($n = 23$)	56.2 (43.8, 0)	34.8 (52.2, 13)
AUC_{16}[a]		
Overall ($n = 142$)	74.8 (18.0, 7.2)	43.9 (30.2, 25.9)
< 9 kg ($n = 23$)	69.6 (30.4, 0)	26.1 (47.8, 26.1)
$adjAUC_{16}$[b]		
Overall ($n = 142$)	90.8 (7.8, 1.4)	66.2 (16.9, 16.9)
< 9 kg ($n = 23$)	82.6 (17.4, 0)	56.4 (21.8, 21.8)

Abbreviation: AUC = area under the concentration-time curve. [a]Comparison of AUC_{16} and $adjAUC_{16}$ for conventional and local target range (overall and < 9 kg). [b]χ^2 Pearson's test: statistically significant ($P < 0.001$).

Philippe M et al. Bone Marrow Transplant 2016;51:72.

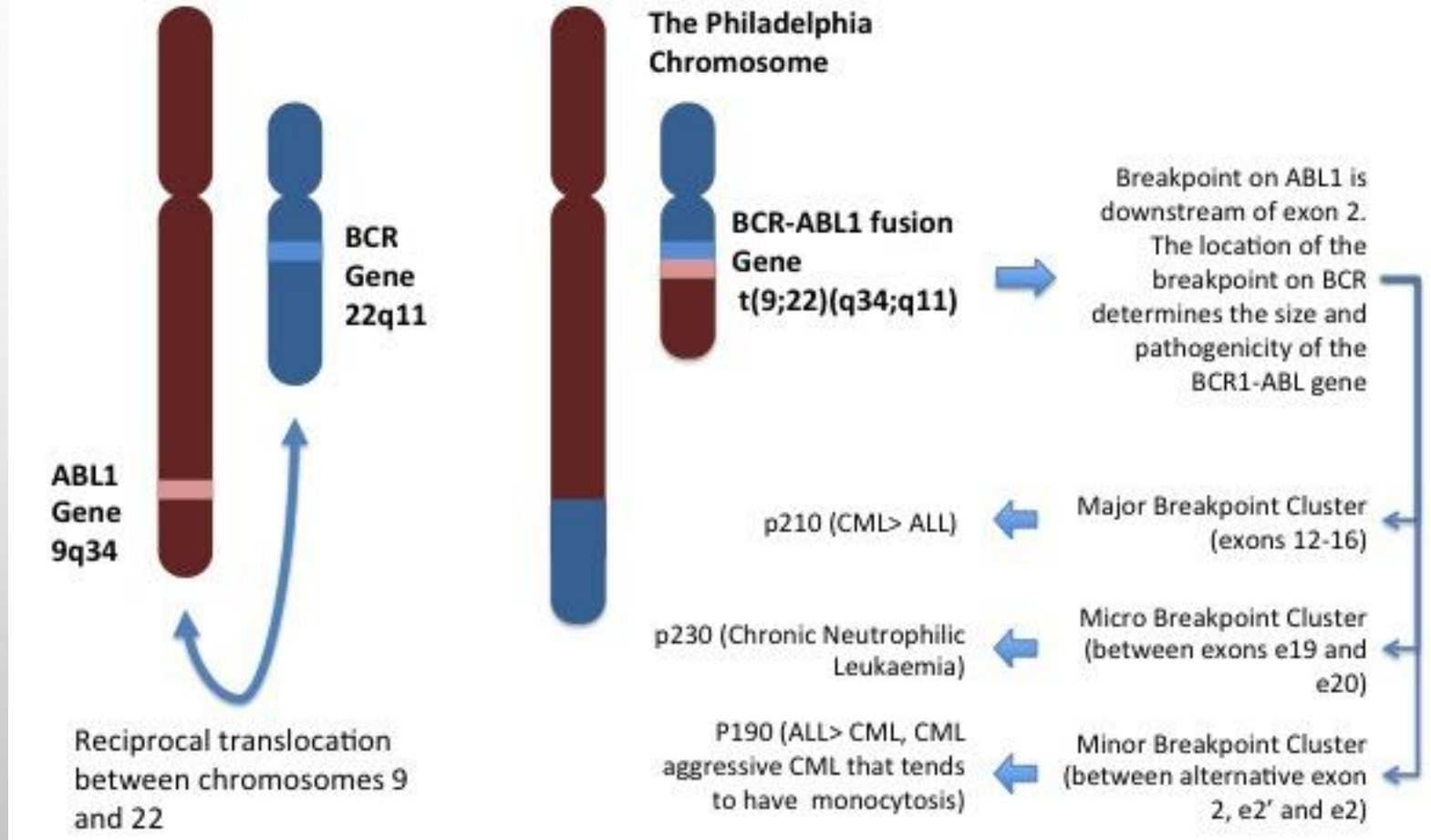
5-FLUORURACIL

- PRODRUG OF 5-FLUORODEOXYURIDINE MONOPHOSPHATE (ANTIMETABOLITE)
- INDICATIONS: GASTROINTESTINAL TUMORS, ADVANCED HEAD-NECK TUMOR, BREAST CANCER
- FORMULATIONS: IV. INFUSION, TOPICAL
- SATURABLE FIRST-PASS EFFECT (DIHYDROPIRIMYDINE DEHYDROGENASE)
- BONE MARROW TOXICITY
- AUC: 20-30 MG*H/L
- TEST SAMPLE: SERUM (NATIVE BLOOD)
- TEST METHOD: LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

TAMOXIFEN

- BREAST ESTROGEN RECEPTOR (ER) ANTAGONIST, ENDOMETRIAL ESTROGEN RECEPTOR AGONIST
- MORE OF A PRODRUG WITH ACTIVE FORMS SUCH AS 4-HYDROXYTAMOXIFEN AND N-DESMETHYL-4-HYDROXYTAMOXIFEN (ENDOXIFEN) EXERTING MOST OF THE CLINICAL EFFECT
- COMPLEX METABOLISM (CYP2D6, CYP3A4)
- INDICATION: ER-POSITIVE BREAST CANCER
- TDM: ENDOXIFEN, TROUGH TARGET >6 NG/ML
- TEST SAMPLE: SERUM, PLASMA
- TEST METHOD: LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

Molecular Biology of Chronic Myeloid Leukaemia



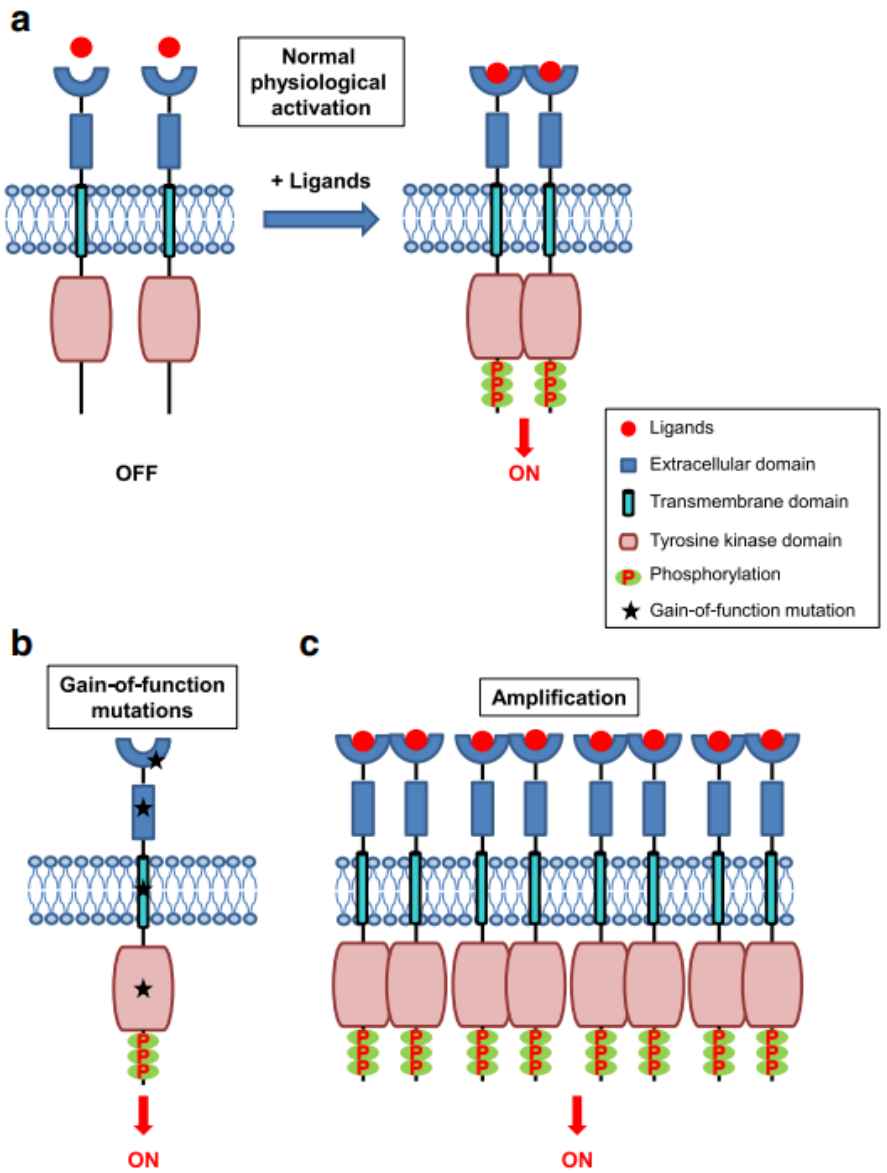
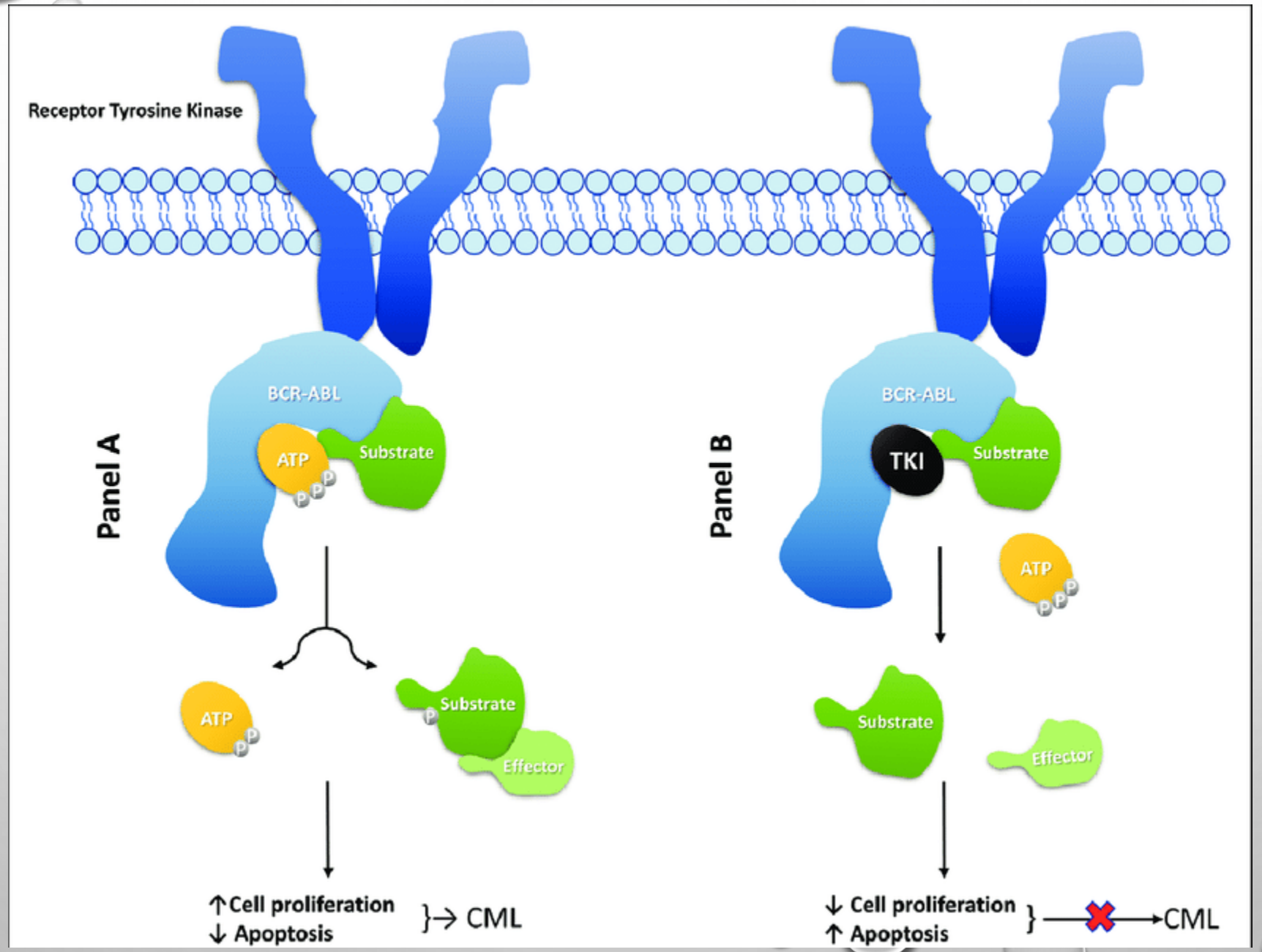


Fig. 1 Mechanisms of physiological and oncogenic RTK activation. **a** Schematic representation of RTK activation in normal physiology. RTKs are activated through formation of inter-molecular dimerization in the presence of ligands, resulting in kinase activation and phosphorylation of the receptor C-terminal tail. **b** Schematic representation of potential gain-of-function mutations in the various subdomains of an RTK. The mutations lead to constitutive activation of the RTK, typically in the absence of ligand. **c** Overexpression of RTKs – often as a result of genomic amplification of the RTK gene - leads to increased local concentration of receptors


Receptor Tyrosine Kinase
Panel A
BCR-ABL
ATP
Substrate
ATP
Substrate
Effector
↑Cell proliferation
↓ Apoptosis
}→ CML
Panel B
BCR-ABL
TKI
Substrate
ATP
Substrate
Effector
↓ Cell proliferation
↑ Apoptosis
} CML

TKI	Time to market	Development company	Target	Application of disease
Imatinib	2001	Novartis	Abl, PDGFR, SCFR	CML, GIST
Gefitinib	2003	AstraZeneca	EGFR	NSCLC
Nilotinib	2004	Novartis	Bcr-Abl, PDGFR	CML
Sorafenib	2005	Bayer	Raf, VEGFR, PDGER	Advanced RCC
Sunitinib	2006	Pfizer	PDGFR, VEGFR,	GIST, Advanced RCC
Dasatinib	2006	Bristol-Myers Squibb	Bcr-Abl, SRC, PDGFR	CML
Lapatinib	2007	GlaxoSmithKline	EGFR	Breast cancer
Pazopanib	2009	GlaxoSmithKline	VEGFR, PDGFR, FGFR	Advanced RCC,STS,NSCLC
Crizotinib	2011	Pfizer	ALK	NSCLC
Ruxolitinib	2011	Novartis	JAK1, JAK2	myelofibrosis
vandetanib	2011	AstraZeneca	VEGFR, EGFR	Advanced Thyroid cancer
Axitinib	2012	Pfizer	VEGFR	Advanced RCC
Bosutinib	2012	Wyeth	Abl, SRC	CML
Afatinib	2013	Boehringer Ingelheim	EGFR	NSCLC
Erlotinib	2013	Roche	EGFR	NSCLC
Ceritinib	2014	Novartis	ALK	NSCLC
Osimertinib	2015	AstraZeneca	EGFR	NSCLC
Lenvatinib	2015	Eisai	VEGFR	DTC
Alectinib	2015	Roche	ALK	NSCLC
Regorafenib	2017	Bayer	VEGFR, EGFR	HCC, CRC,GIST
Neratinib	2017	Puma	HER2	Breast cancer
Brigatinib	2017	Ariad	ALK	NSCLC

https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-018-0801-5/tables/1

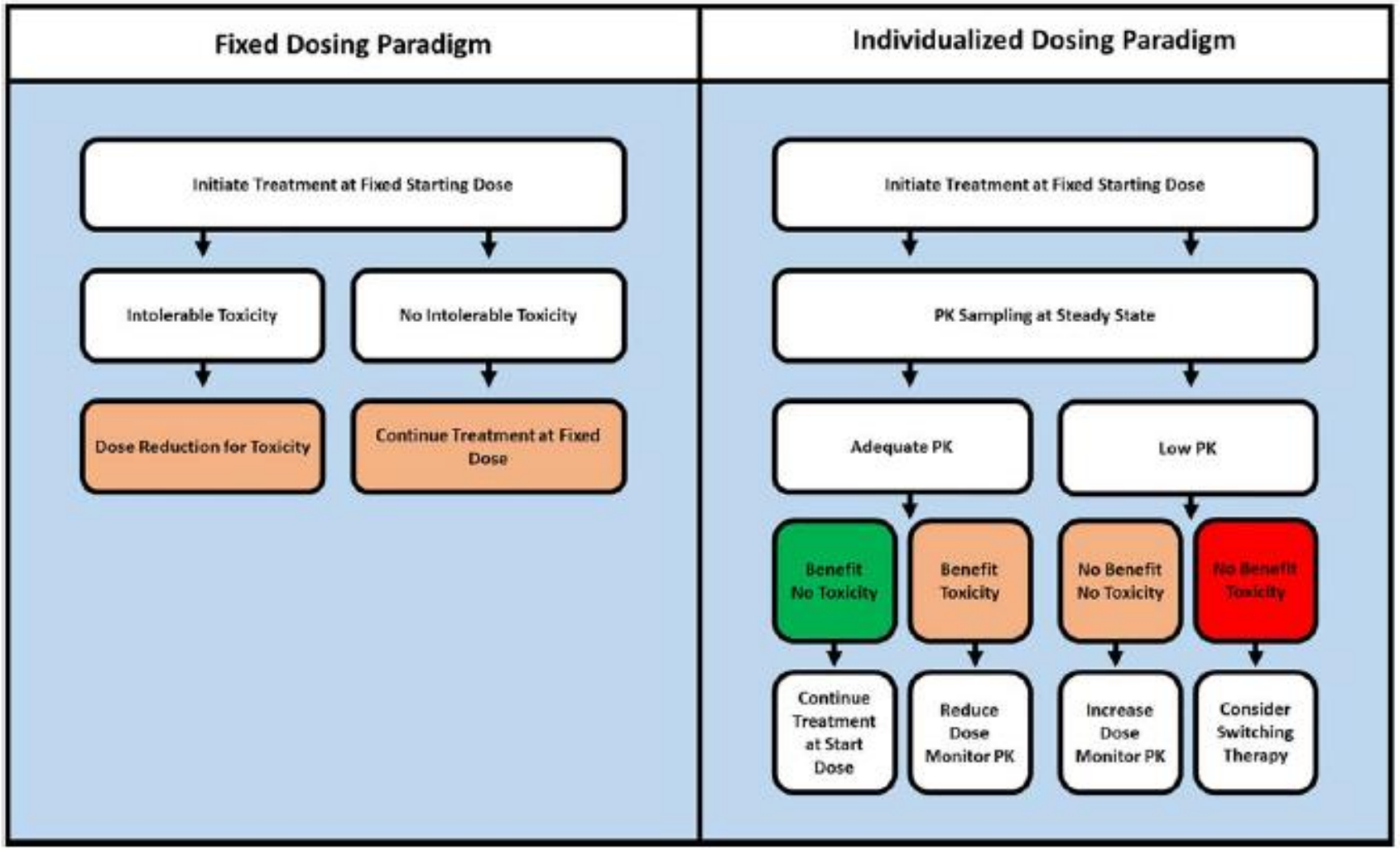


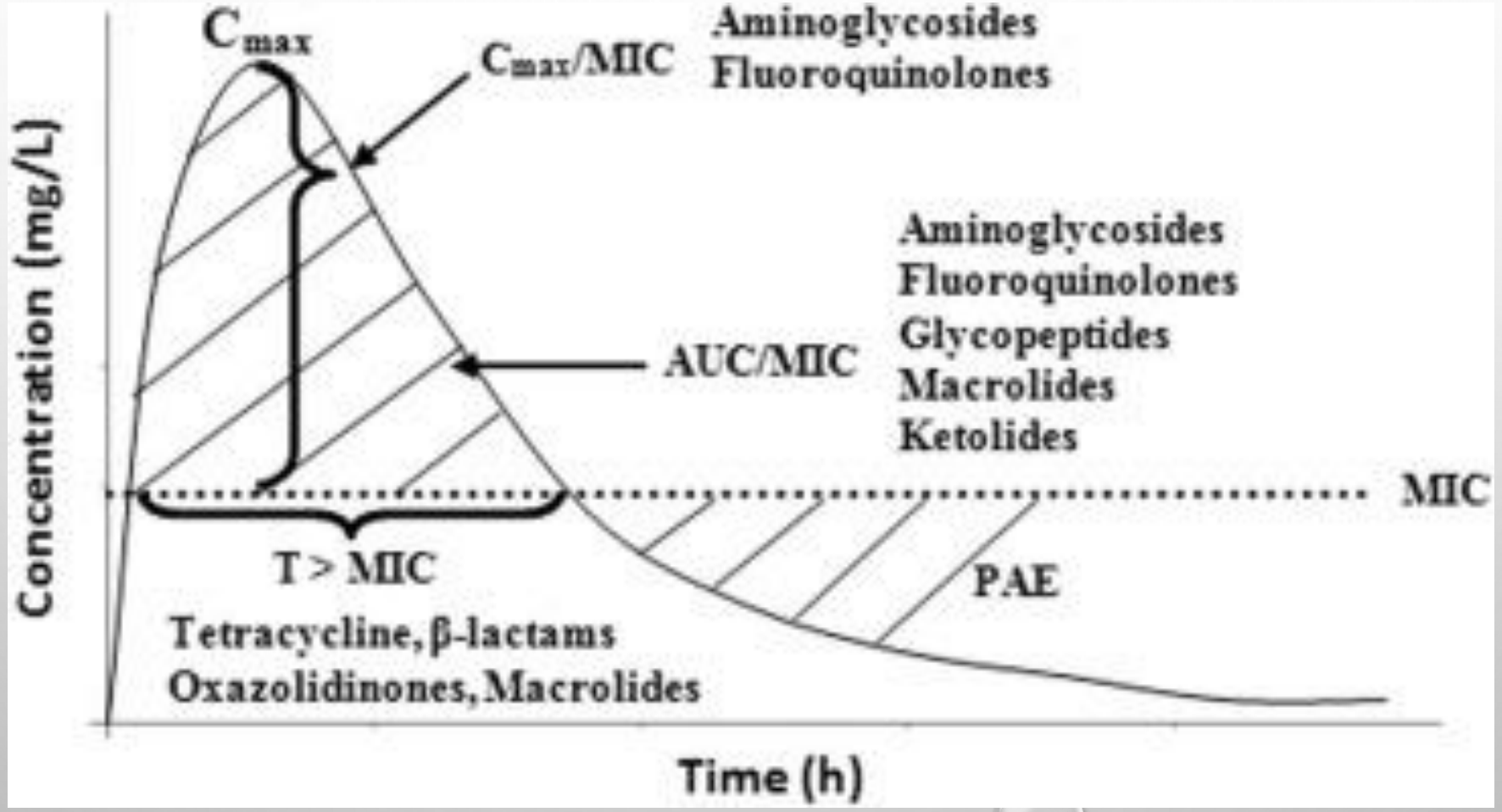
Figure 1 Current fixed dosing paradigm (left) vs. the proposed individualized or TDM dosing algorithm (right).

Verheijen RB et al. Clin Pharm Ther 2017;102:765-76.

DISTRIBUTION OF PER OS SMALL-MOLECULE ANTICANCER DRUGS WITH SPECIFIC PROTEIN TARGETS INTO PERIPHERAL FLUID SPACES

- POOR PENETRATION THROUGH BLOOD-BRAIN BARRIER
- LOW CONCENTRATIONS ATTAINED IN SALIVA
- ACCUMULATION IN PLEURAL FLUID, ASCITIC FLUID
- EXCRETED BY BREAST MILK
- PENETRATION THROUGH BLOOD-TESTIS BARRIER

THERAPEUTIC MONITORING OF ANTIBIOTICS



BASICS: MINIMAL INHIBITORY CONCENTRATIONS

- MINIMAL INHIBITORY CONCENTRATION (MIC): THE LOWEST CONCENTRATION OF AN ANTIBIOTIC WHICH VISIBLY INHIBITS THE GROWTH OF AN ISOLATED MICROORGANISM STRAIN
- EUCAST MIC: MIC VALUES PUBLISHED BY THE EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
- MIC VALUES ARE DETERMINED AS A SERIES OF 2^N CONCENTRATIONS
- MIC IS DETERMINED IN VITRO, AND IS THEREFORE PRIMARILY SUITABLE FOR IDENTIFYING ANTIBIOTIC RESISTANCE

MIC determination using E-test: https://www.youtube.com/watch?v=ATBoj5jWJhg

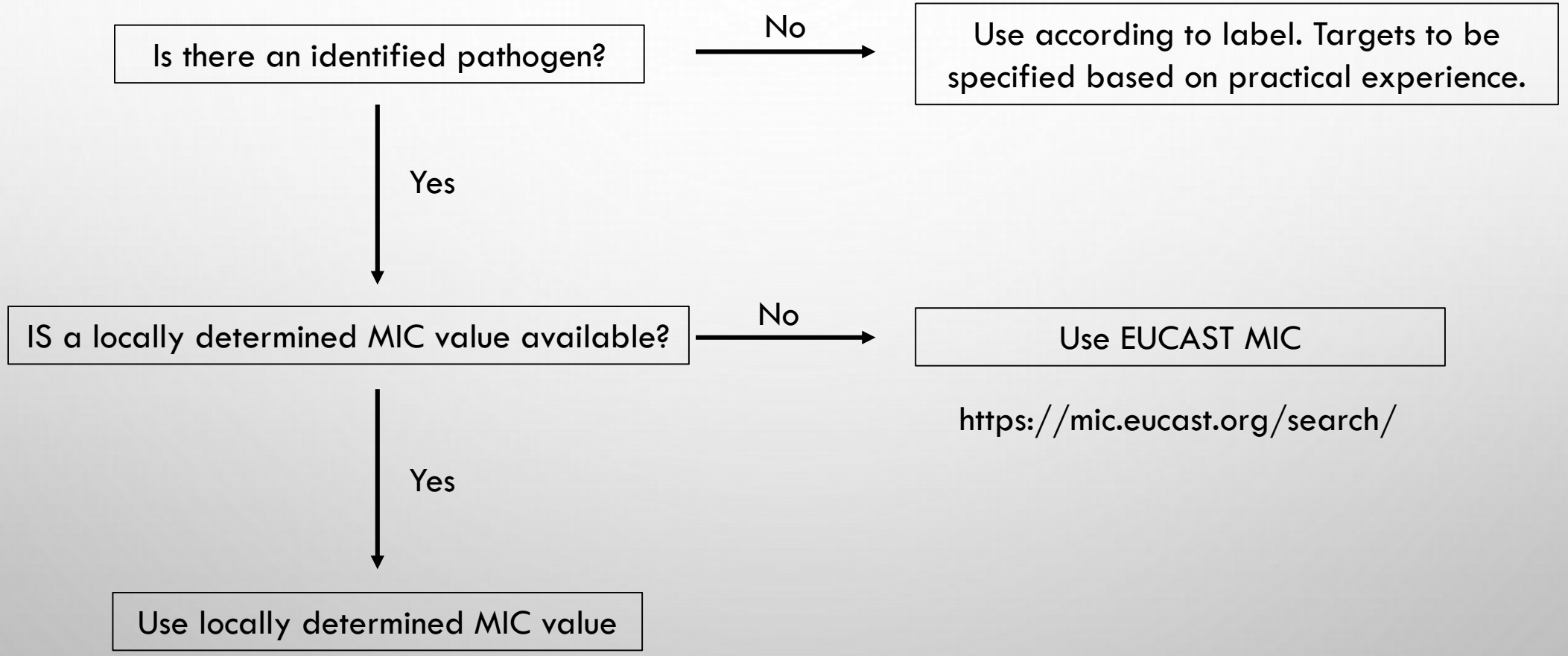
Susceptibility of Pseudomonas aeruginosa against various antibiotics (2022)

MIC EUCAST

Login

	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	Distributions	Observations	(T)ECOFF	Confidence interval
Meropenem	0	0	3	38	134	2610	6008	10843	11960	8798	5154	3763	3229	3934	385	441	31	13	0	55	57344	2	2 - 8
Moxifloxacin	0	0	0	1	1	2	6	46	230	680	481	333	264	208	180	182	443	8	0	8	5089	4	4 - 32
Netilmicin	0	0	0	0	0	2	4	8	53	175	235	192	74	36	9	1	7	42	25	5	863	8	4.000 - 32.000
Norfloxacin	0	0	0	0	0	0	6	49	139	38	17	8	0	0	0	1	0	0	0	2	258	ID	
Ofloxacin	0	0	0	0	3	6	26	98	586	561	275	180	133	48	145	102	0	0	0	8	2163	4	1 - 16
Piperacillin	0	0	0	0	0	0	9	4	45	340	1095	3429	1446	884	331	237	199	245	192	12	8456	16	
Piperacillin-tazobactam	0	0	5	1	0	4	23	37	453	886	3147	10479	5692	3595	1879	1506	3135	863	161	71	31866	16	
Sitafloxacin	0	0	1	0	7	39	162	64	26	20	14	12	5	2	0	1	0	0	0	5	353	0.5	0.125 - 1
Sparfloxacin	0	0	0	0	0	1	9	46	119	183	84	40	34	31	21	26	10	3	0	2	607	-	
Sulbactam	0	0	0	0	0	0	0	0	0	1	2	0	0	0	1	2	8	5	328	1	347	-	
Ticarcillin	0	0	0	0	0	0	1	1	2	247	71	119	386	3761	5303	1917	2106	2806	30	3	16750	-	
Ticarcillin-clavulanic acid	0	0	0	0	0	0	1	1	2	545	138	260	955	5251	10981	4742	4496	5206	12	4	32590	(16)	
Tigecycline	0	0	0	0	0	0	0	1	5	7	13	47	136	290	116	27	5	3	0	6	650	64	
Tobramycin	0	0	0	3	5	12	387	2712	10547	7139	2054	535	345	448	364	224	73	35	116	42	24999	2	1 - 4
Trimethoprim-sulfamethoxazole	0	0	0	0	2	4	3	1	5	15	40	124	142	66	21	106	0	0	0	4	529	(32)	8 - 64

ALGORITHM OF MIC DETERMINATION



PHARMACOKINETIC-PHARMACODYNAMIC (PK/PD) INDICES

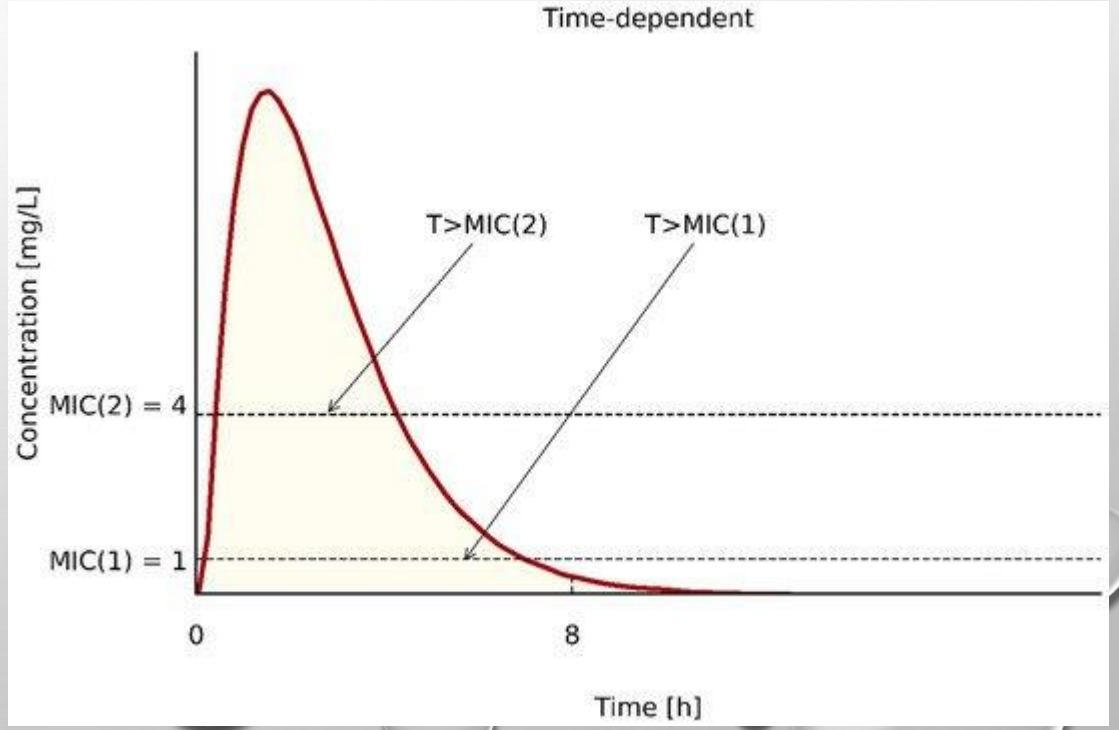
- TIME-DEPENDENT EFFICACY: T>MIC
- PEAK CONCENTRATION-DEPENDENT EFFICACY: C_{MAX}/MIC
- EXPOSURE-DEPENDENT EFFICACY: AUC_{0-24}/MIC
- TARGETS MUST BE ESTABLISHED FOR EVALUATING THE EFFICACY OF TREATMENT
- VANCOMYCIN TROUGH >15 MG/L: INCREASED RISK OF NEPHROTOXICITY
- AMINOGLYCOSIDE TROUGH 2 MG/L: INCREASED RISK OF NEPHRO- AND OTOTOXICITY
- DILEMMA: INCREASED DRUG EXPOSURE MAY BE BOTH THE CAUSE AND THE RESULT OF IMPAIRED RENAL FUNCTION

TIME DEPENDENT (T>MIC) ANTIBIOTICS

- GOAL: TO KEEP DRUG CONCENTRATION OVER THE TARGET FOR A FRACTION OF THE DOSING INTERVAL
- BETA-LACTAMS (PENICILLINS, CARBAPENEMS)

$$\%T_{C>MIC} = \ln\left[\frac{\text{Dose}}{Vd \times MIC}\right] \times \left[\frac{t_{1/2}}{0.693}\right] \times \left[\frac{100}{DI}\right] \quad (1)$$

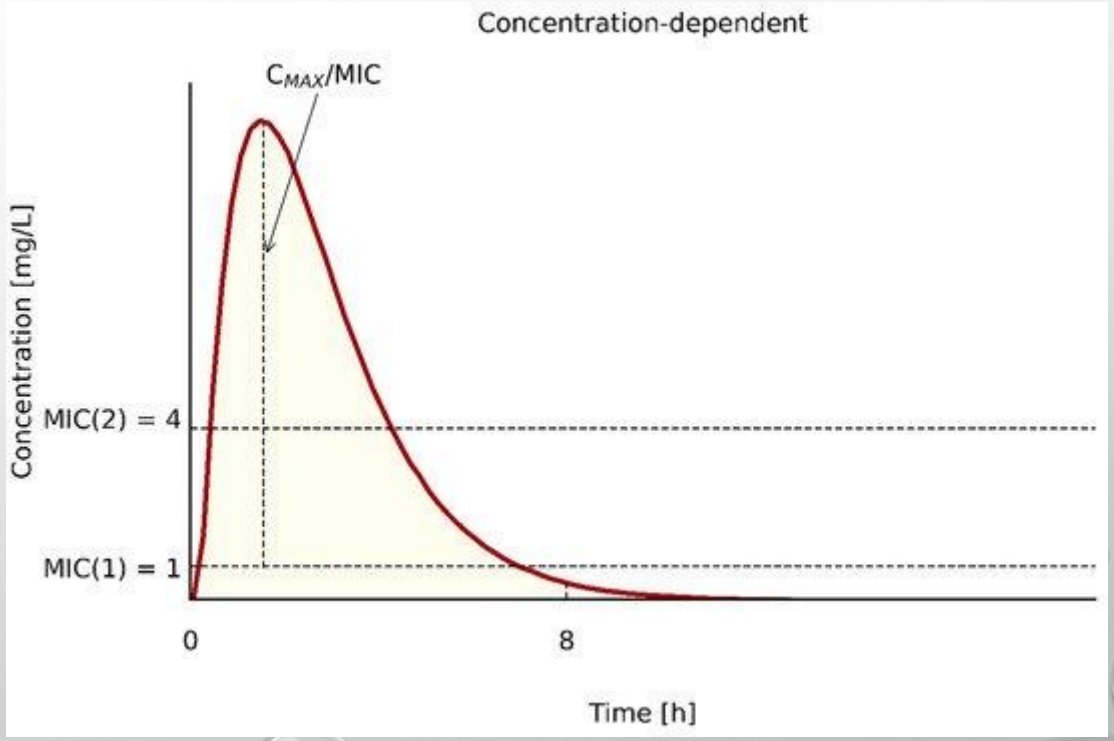
where: ln—natural logarithm, Vd—volume of distribution (L/kg), (kg), $t_{1/2}$—serum half-life (hours), elimination rate constant (h-), DI = dosing interval (hours).



Kowalksa-Krochmal B. Dudek-Wicher R. Pathogens 2021;10:165.

PEAK CONCENTRATION-DEPENDENT (C_{MAX}/MIC) ANTIBIOTICS

- GOAL: TO ATTAIN A DEFINED RATIO OF PEAK CONCENTRATION AND MIC
- AMINOGLYCOSIDES, FLUOROQUINOLONES



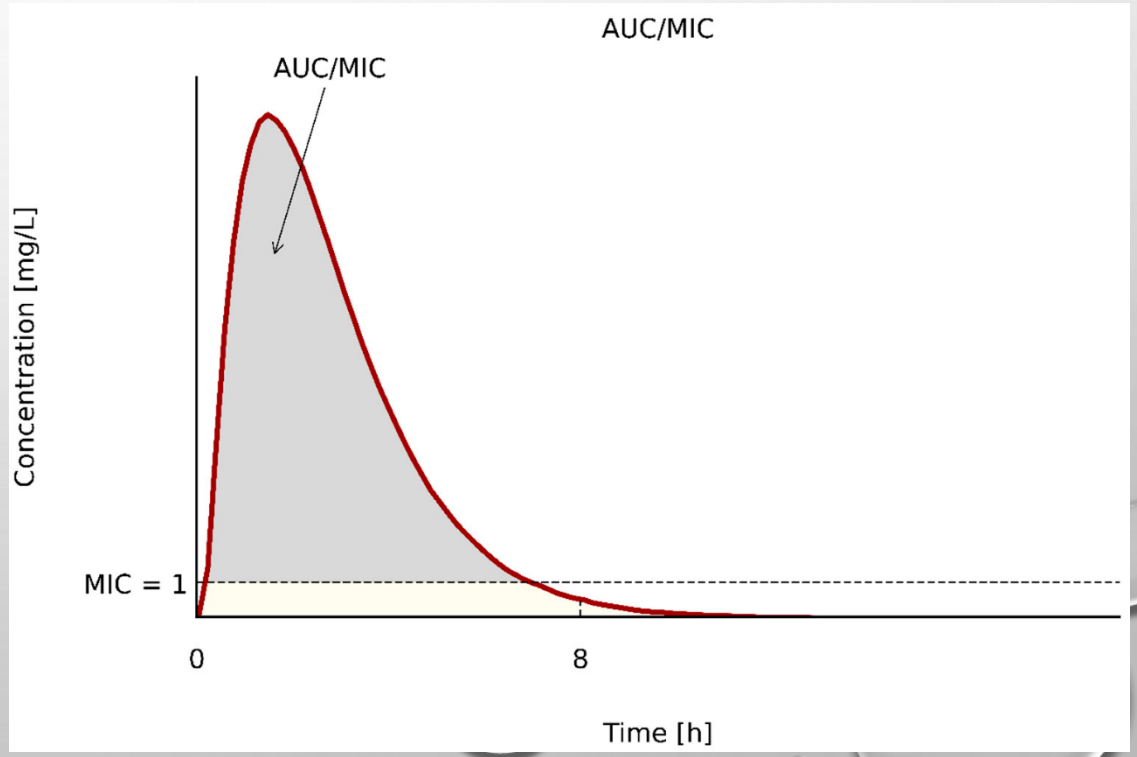
Kowalksa-Krochmal B. Dudek-Wicher R. Pathogens 2021;10:165.

AUC-DEPENDENT (AUC/MIC) ANTIBIOTICS

- GOAL: TO ATTAIN CONCENTRATIONS WHICH CORRESPOND TO A DEFINED RATIO OF THE AREA UNDER THE CONCENTRATION-TIME CURVE AND MIC OR ITS MULTIPLICATE
- GLYCOPEPTIDES, LINEZOLID

$$\frac{\mathrm{AUC}}{\mathrm{MIC}} = \ln\left[\frac{\mathrm{Dose}}{\mathrm{Vd} \times \mathrm{MIC}}\right] \times \left[\frac{t_{1/2}}{0.693}\right] \times \left[\frac{24}{\mathrm{DI}}\right] \quad (2)$$

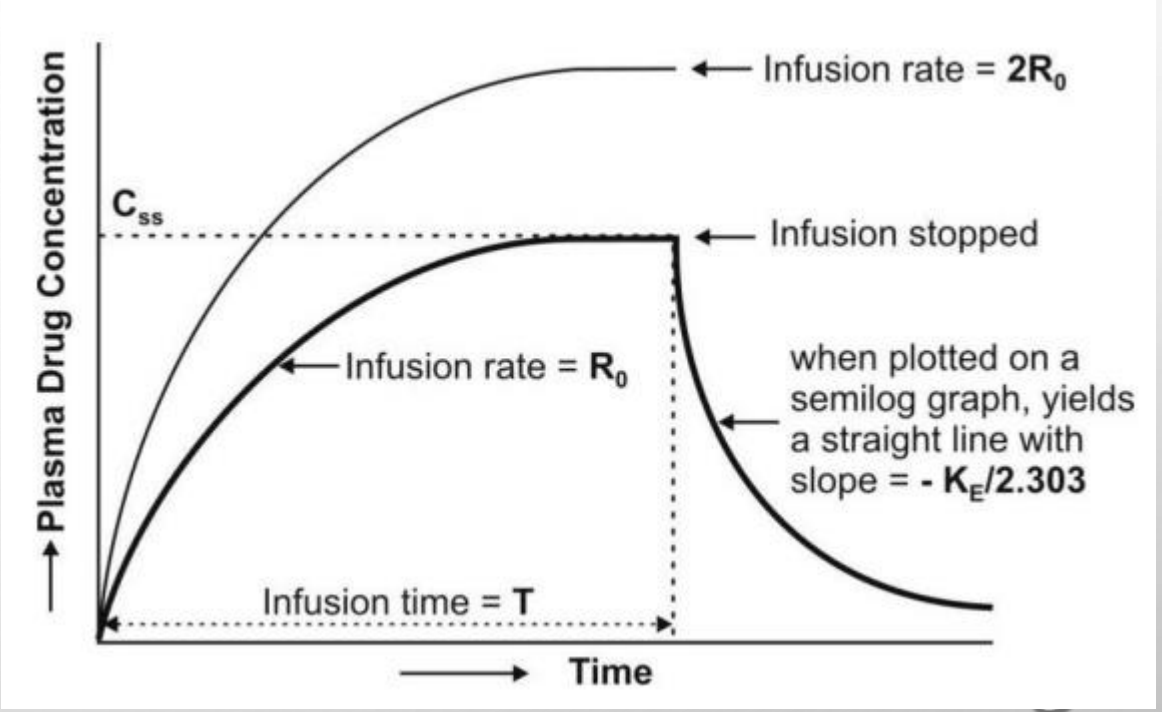
where: ln—natural logarithm, Vd—volume of distribution (L/kg), $t_{1/2}$—serum half-life (hours), DI—dosing interval (hours).



Kowalksa-Krochmal B. Dudek-Wicher R. Pathogens 2021;10:165.

ESTABLISHING PK/PD INDICES

- T>MIC: >2 MEASUREMENTS REQUIRED
  (EG. 1X AND 2X $T_{1/2}$. OR 2X AND 4X $T_{1/2}$)
- C_{MAX}/MIC: 10-15 MIN AFTER ENDING INFUSION
- AUC/MIC:
  - (A) AT LEAST 3 MEASUREMENTS REQUIRED
    - BEFORE STARTING INFUSION
    - WITHIN 15 MIN OF ENDING INFUSION
    - BEFORE STARTING NEXT INFUSION
  - (B) WHEN USING A CALCULATOR APPLICATION, AT RECOMMENDED SAMPLING TIMES



METHODS FOR TESTING ANTIBIOTICS

- IMMUNOASSAY
  - AMINOGLYCOSIDES: GENTAMICIN, TOBRAMYCIN
  - GLYCOPEPTIDES: TEICOPLANIN, VANCOMYCIN
- HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV/VIS DETECTION
  - BETA-LACTAMS: AMPICILLIN, CEFEPIME, CEFTAZIDIME, LINEZOLID, MEROPENEM, PIPERACILLIN
  - BETA-LACTAMASE INHIBITORS: SULBACTAM, TAZOBACTAM
- LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY
  - AMINOGLYCOSIDES
  - GLYCOPEPTIDES
  - BETA-LACTAMS
  - FLUOROQUINOLONES

Table 4. Type of PK/PD ratio and target value for clinical efficacy.

PK/PD Type	Antibiotics	PK/PD Index	References
T > MIC	penicillins	≥50	[58,60,64,69]
	cephalosporins	≥50–70	[58,60,64,69]
	carbapenems	≥40	[58,60,64,69]
	for patients with immunosupresion	100	[60,65,66]
Cmax/MIC	aminoglycosides	>8	[58,64]
	fluoroquinolones	>8	[58,64]
	polymyxins	∫Cmax/MIC ≥ 6	[73]
	metronidazole	NK	
AUC/MIC	aminoglycosides	>70 [47]; ≥156 [41]	[58,64]
	ciprofloxacin	AUC/MIC > 125; ∫AUC/MIC > 88	[60]
	levofloxacin	AUC/MIC > 34; ∫AUC/MIC > 24	[60]
	vancomycin	AUC/MIC > 400; ∫AUC/MIC > 200	[60]
	daptomycin	AUC/MIC 388–537 [47]; AUC/MIC ≥ 666 [57]	[64,74]
	oksazolidinones	>80	[58,64,74]
	polymyxins	total AUC/MIC > 50; ∫AUC/MIC > 25	[64,73]
	fosfomycin	>8.6	[64]
	tygecycline	skin and skin structure infections AUC/MIC ≥ 17.9	[76]
		Intra-abdominal infections AUC/MIC ≥ 6.96	[76]
		hospital acquired pneumonia AUC/MIC ≥ 4.5	[76]

Kowalksa-Krochmal B. Dudek-Wicher R. Pathogens 2021;10:165.

MONITORING FREE DRUG CONCENTRATIONS

- EXPERIMENTAL TECHNIQUES:
  - EQUILIBRIUM DIALYSIS
  - ULTRAFILTRATION

- ESTIMATES BASED ON LITERATURE DATA:

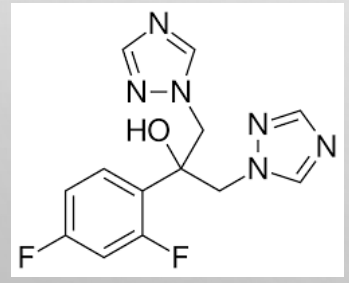
$$free\ drug\ concentration = measured\ concentration \times (1 - protein - bound\ fraction)$$

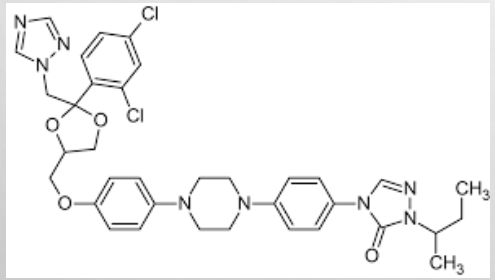
MONITORING TRIAZOLE SYSTEMIC ANTIFUNGALS

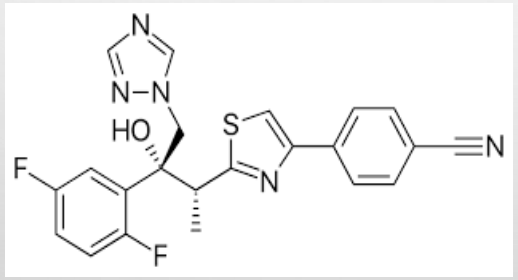
- TREATMENT OF SYSTEMIC ASPERGILLOSIS AND CANDIDIASIS
- SYSTEMIC FUNGAL INFECTIONS DEVELOP WITH ORGAN TRANSPLANT, COVID INFECTION, INTENSIVE CARE, OR OTHER IMMUNE DEFICIENT CONDITIONS (MORTALITY: 30-70%)
- **FLUCONAZOLE**: THE BENEFIT OF TDM IS NOT FULLY SUPPORTED. RECOMMENDED AUC_{0-24}/MIC>100
- **ITRACONAZOLE**: CARDIOVASCULAR ADVERSE EFFECTS, MONITORING IS INDICATED DUE TO THE FORMATION OF VARIOUS METABOLITES
- **ISAVUCONAZOLE**: OBESE PATIENTS, LIVER FAILURE, PEDIATRICS
- **POSACONAZOLE**: >0.7 MG/L TROUGH TARGET(PROPHYLAXIS). >1 MG/L IN FUNGAL INFECTIONS. CYP450 3A4 INHIBITOR
- **VORICONAZOLE**: TROUGH THERAPEUTIC RANGE: 1.0-5.5 MG/L. CYP2C19 SUBSTRATE

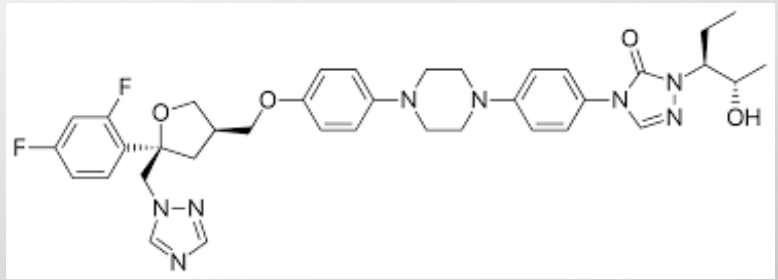
TESTING TRIAZOLE ANTIFUNGALS

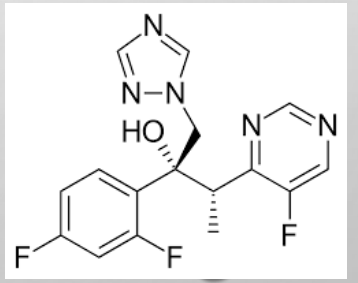
- TEST METHODS:
  - HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV/VIS OR FLUORESCENCE DETECTION
  - LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY
- TEST SAMPLE: SERUM, PLASMA


fluconazole


itraconazole


isavuconazole


posaconazole


voriconazole

MONITORING ANTIVIRAL DRUGS

- HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)
- <u>MONITORED DRUGS:</u>
  - **PROTEASE INHIBITORS:** INDINAVIR, RITONAVIR, SAQUINAVIR, NELFINAVIR, AMPRENAVIR, LOPINAVIR, ATAZANAVIR, DARUNAVIR, FOSAMPRENAVIR, TIRANAVIR
  - **NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS:** NEVIRAPIN, DELAVIRDIN, EFAVIRENZ, ETRAVIRIN, RILPIVIRIN
  - **INTEGRASE INHIBITORS:** DOLUTEGRAVIR, ELVITEGRAVIR, RALTEGRAVIR
- <u>INDICATIONS:</u>
  - MONITORING THERAPY ADHERENCE
  - DRUG INTERACTIONS (CYP3A4, 2B6, 2D6, 2C19)
  - PREGNANCY

MONITORING ANTIVIRAL DRUGS

Drug	Sampling Time	Therapeutic Ranges (ng/mL)
Tenofovir from TDF	Trough	40–180
Efavirenz	12-h after intake	1000–4000
Etravirine	Trough	>300
Nevirapine	Trough	3000–6000
Rilpivirine	Trough	>20
Amprenavir	Trough	>400
Atazanavir	Trough	150–800
Darunavir	Trough	>550
Indinavir	Trough	150–550
Lopinavir	Trough	1000–7000
Saquinavir	Trough	100–250
Tipranavir	Trough	>20,500
Dolutegravir	Trough	>64
Elvitegravir	Trough	>45
Raltegravir	Trough*	>40
Maraviroc	Trough	>50

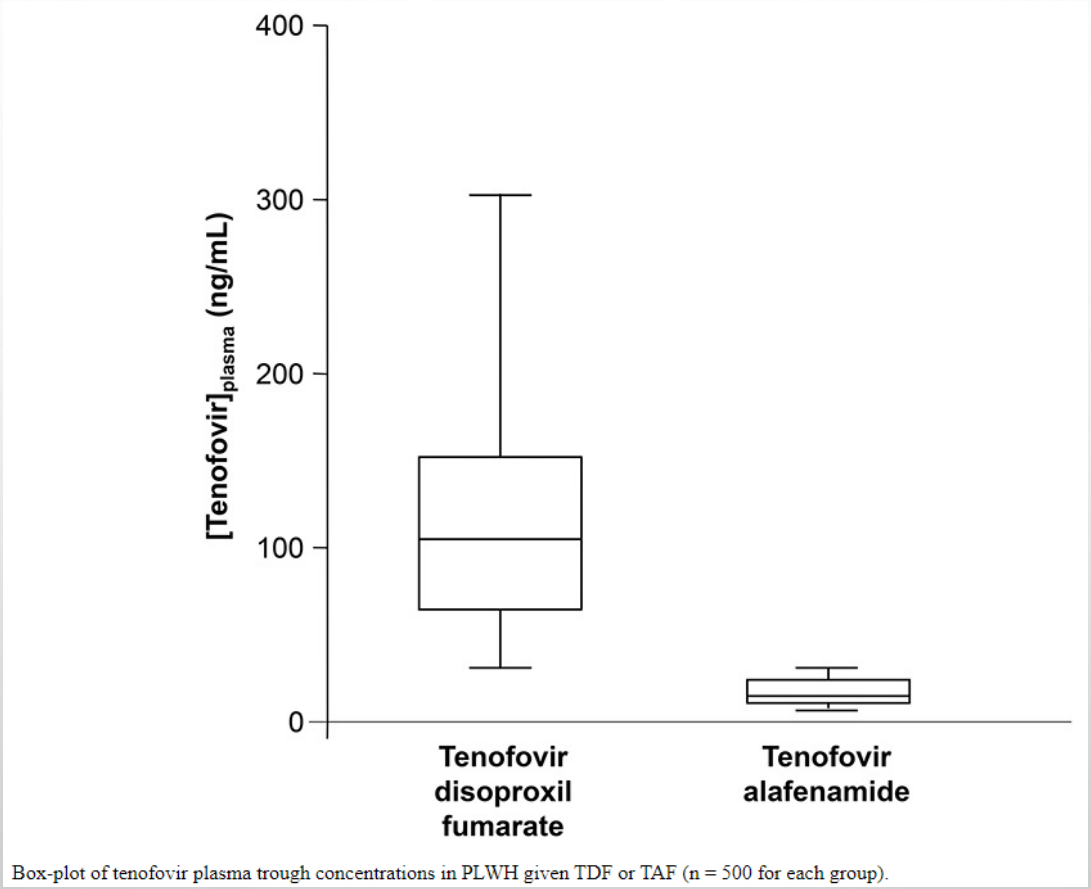
These ranges were retrieved from available literature (summarized in Ref. 29) for tenofovir, efavirenz, etravirine, nevirapine, amprenavir, atazanavir, indinapir, lopinavir, saquinavir, tipranavir, and maraviroc. For the other drugs, the lower therapeutic thresholds are protein-adjusted 90% ICs.

*Consider the assessment of the area under the curve given the poor predictive value of raltegravir trough concentrations.

→ Trough concentrations are correlated with the risk of developing renal tubule impairment

Cattaneo D. et al. Ther Drug Monit 2021;42:64.

MONITORING ANTIVIRAL DRUGS



Box-plot of tenofovir plasma trough concentrations in PLWH given TDF or TAF (n = 500 for each group).

Cattaneo D. et al. Ther Drug Monit 2021;42:64.

MONITORING GANCICLOVIR

- VALGANCICLOVIR (PRODRUG): A PER OS ANTIVIRAL
- ACTIVE FORM: GANCICLOVIR
- APPLICATION: CYTOMEGALOVIRUS INFECTION PROPHYLAXIS AFTER ORGAN TRANSPLANT
- TARGET CONCENTRATIONS:
  - PEAK: 3-12.5 MG/L
  - TROUGH: 1-3 MG/L

METHODS OF TESTING ANTIVIRALS

- LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

MONITORING IMMUNOSUPPRESSANTS

- INHIBIT T-CELL IMMUNE RESPONSE
- CALCINEURIN INHIBITORS
  - CYCLOSPORINE A
  - TACROLIMUS
- MAMMALIAN TARGET OF RAPAMYCIN (MTOR) INHIBITORS
  - EVEROLIMUS
  - SIROLIMUS (RAPAMYCIN)
- INOSINE-MONOPHOSPHATE DEHYDROGENASE INHIBITOR
  - MYCOPHENOLATE

APPLICATION OF IMMUNOSUPPRESSANTS

- CYCLOSPORINE A:
  - PROPHYLAXIS OF GRAFT REJECTION AFTER LIVER, KIDNEY AND HEART TRANSPLANT
  - RHEUMATOID ARTHRITIS, PSORIASIS
  - ORPHAN APPLICATIONS: LUNG TRANSPLANT, CROHN'S DISEASE
  - PER OS AND IV INJ. FORMULATIONS
- TACROLIMUS:
  - PROPHYLAXIS OF GRAFT REJECTION AFTER LIVER, KIDNEY AND HEART TRANSPLANT
  - PER OS, IV INF. AND IV INJ. FORMULATIONS

APPLICATION OF IMMUNOSUPPRESSANTS

- SIROLIMUS:
  - PROPHYLAXIS AFTER KIDNEY TRANSPLANT
  - LYMPHANGIOLEIOMYOMATOSIS
  - COADMINISTRATION WITH CYCLOSPORINE A FOR AT LEAST 2-4 MONTHS
  - PER OS AND IV INF. FORMULATIONS
- EVEROLIMUS:
  - PROPHYLAXIS OF GRAFT REJECTION AFTER KIDNEY, LIVER AND HEART TRANSPLANT
  - ADVANCED RENAL CELL CARCINOMA
  - SUBEPYNDEMAL GIANT CELL ASTROCYTOMA (SEGA)
  - PANCREAS NEUROENDOCRINE TUMORS
  - HER2-NEGATIVE BREAST CANCER (WITH EXAMESTANE)
  - PER OS FORMULATIONS

APPLICATION OF IMMUNOSUPPRESSANTS

- MYCOPHENOLATE (IN THE FORM OF MYCOPHENOLATE MOFETIL):
  - PROPHYLAXIS OF GRAFT REJECTION AFTER KIDNEY, LIVER AND HEART TRANSPLANT
  - COMBINATION WITH CYCLOSPORIN A AND CORTICOSTEROIDS
  - PER OS FORMULATONS ARE AVAILABLE

PHARMACOKINETIC PROPERTIES OF IMMUNOSUPPRESSANTS

	Cyclosporin A	Everolimus	Mycophenolate	Sirolimus	Tacrolimus
Molecular weight	1202	958	320	914	822
Bioavailability	Depends on formulation	15%	>70%	10%	20%
Time to reach peak concentration	1-6 h	1-3 h	1-2.5 h	1-2 h	0.5-6.0 h
Volume of distribution	4-6 L/kg	4-20 L/kg	kb. 4 L/kg	4-20 L/kg	0.3-2.36 L/kg
Protein binding	90-99% (lipoproteins)	75%	>98%	90%	99%
Elimination half-life	6-27 h. Depends on age in pediatric patients	18-35 h	8-18 h	46-78 h	3.9-34.8 h
Metabolism	>30 metabolites CYP3A4	Kb. 20 metabolites CYP3A4, p-glycoprotein	>4 metabolites UGT	>7 metabolites CYP3A, p-glycoprotein	>9 metabolites
Elimination	Bile	Bile	Urine	Bile	Bile

TEST SAMPLES FOR MONITORING IMMUNOSUPPRESSANTS

- WHOLE BLOOD
  - CYCLOSPORINE A
  - EVEROLIMUS
  - SIROLIMUS
  - TACROLIMUS
- SERUM OR PLASMA
  - MYCOPHENOLATE

METHODS FOR MONITORING IMMUNOSUPPRESSANTS

- IMMUNOASSAYS
  - MICROPARTICULAR ENZYME IMMUNOASSAY (MEIA)
  - CLONED ENZYME DONOR IMMUNOASSAY (CEDIA)
  - ANTIBODY CONJUGATE MAGNETIC IMMUNOASSAY (ACMIA)
  - CHEMILUMINESCENT MICROPARTICULATE IMMUNOASSAY (CMIA)
  - ELECTROCHEMILUMINESCENT IMMUNOASSAY (ECLIA)
  - PARTICLE-ENHANCED TURBIDIMETRIC INHIBITION IMMUNOASSAY (PETINIA)

- HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV/VIS DETECTION
  - MYCOPHENOLATE

- LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY
  - CYCLOSPORINE A, EVEROLIMUS, SIROLIMUS, TACROLIMUS, MYCOPHENOLATE

INTERFERENCES WITH IMMUNOASSAYS EMPLOYED FOR MONITORING IMMUNOSUPPRESANTS

- CROSS REACTIONS
  - CYCLOSPORINE: METABOLITES, ENDOGENOUS HETEROPHILIC ANTIBODIES
  - EVEROLIMUS: METABOLITES
  - MYCOPHENOLATE: ACID GLUCURONIDE METABOLITE
  - SIROLIMUS: METABOLITES
  - TACROLIMUS: METABOLITES, ENDOGENOUS AND ENDOGENOUS HETEROPHILIC ANTIBODIES
- LOW HEMATOCRIT
  - TACROLIMUS
- POOR REPRODUCIBILITY AT LOW CONCENTRATIONS
  - TACROLIMUS

TARGETS IN IMMUNOSUPPRESSANT MONITORING

- CYCLOSPORIN A
  - TROUGH CONCENTRATIONS (MAINTENANCE THERAPY: <150 NG/ML), C2 CONCENTRATIONS
  - DEPENDS ON TYPE OF TRANSPLANT, TIME FROM TRANSPLANT, AND TESTING METHOD
- EVEROLIMUS
  - 3-8 NG/ML (TROUGH)
- MYCOPHENOLATE
  - 1.0-3.5 MG/L (TROUGH)
- SIROLIMUS
  - 5-20 NG/ML (MONOTHERAPY)
  - 5-15 NG/ML (COMBINED TREATMENTS)
- TACROLIMUS
  - 5-15 NG/ML (TROUGH)
  - 0-12 AREAS UNDER THE CONCENTRATION-TIME CURVE HAVE A BETTER PREDICTIVE VALUE

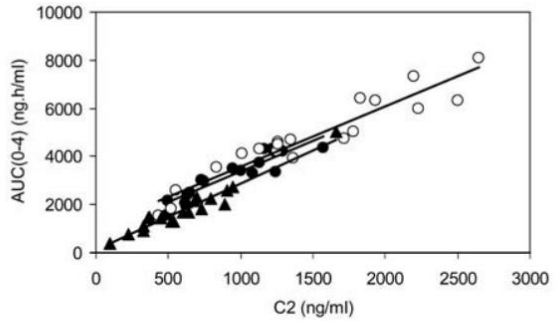


Fig. 2 Regression of the cyclosporine concentration 2 h post dose (C2) and the area under the concentration-time curve in the first 4 h post dose [AUC(0–4)] in pediatric de novo kidney (*open circles*), maintenance kidney (*filled circles*), and maintenance liver (*filled triangles*) allograft recipients. Shown are the associated regression lines. The corresponding regression parameters are listed in Table 2

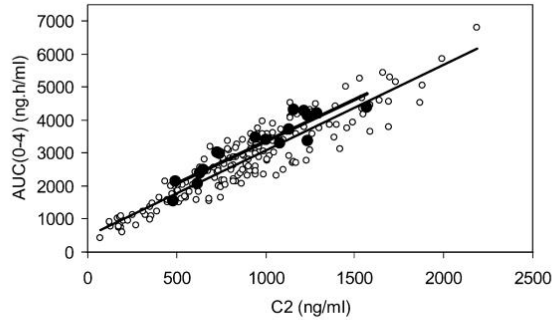


Fig. 3 Regression of the cyclosporine concentration 2 h post dose (C2) and the area under the concentration-time curve in the first 4 h post dose [AUC(0–4)] in pediatric (*filled circles*) and adult (*open circles*) maintenance kidney allograft recipients. Shown are the corresponding regression lines. The pediatric data are the same as in Fig. 2 for maintenance kidney transplant patients and serve as a link between the figures

Kovarik JM et al. Pediatr Nephrol 2003;18:1275.

MONITORING TACROLIMUS USING MICROSAMPLING

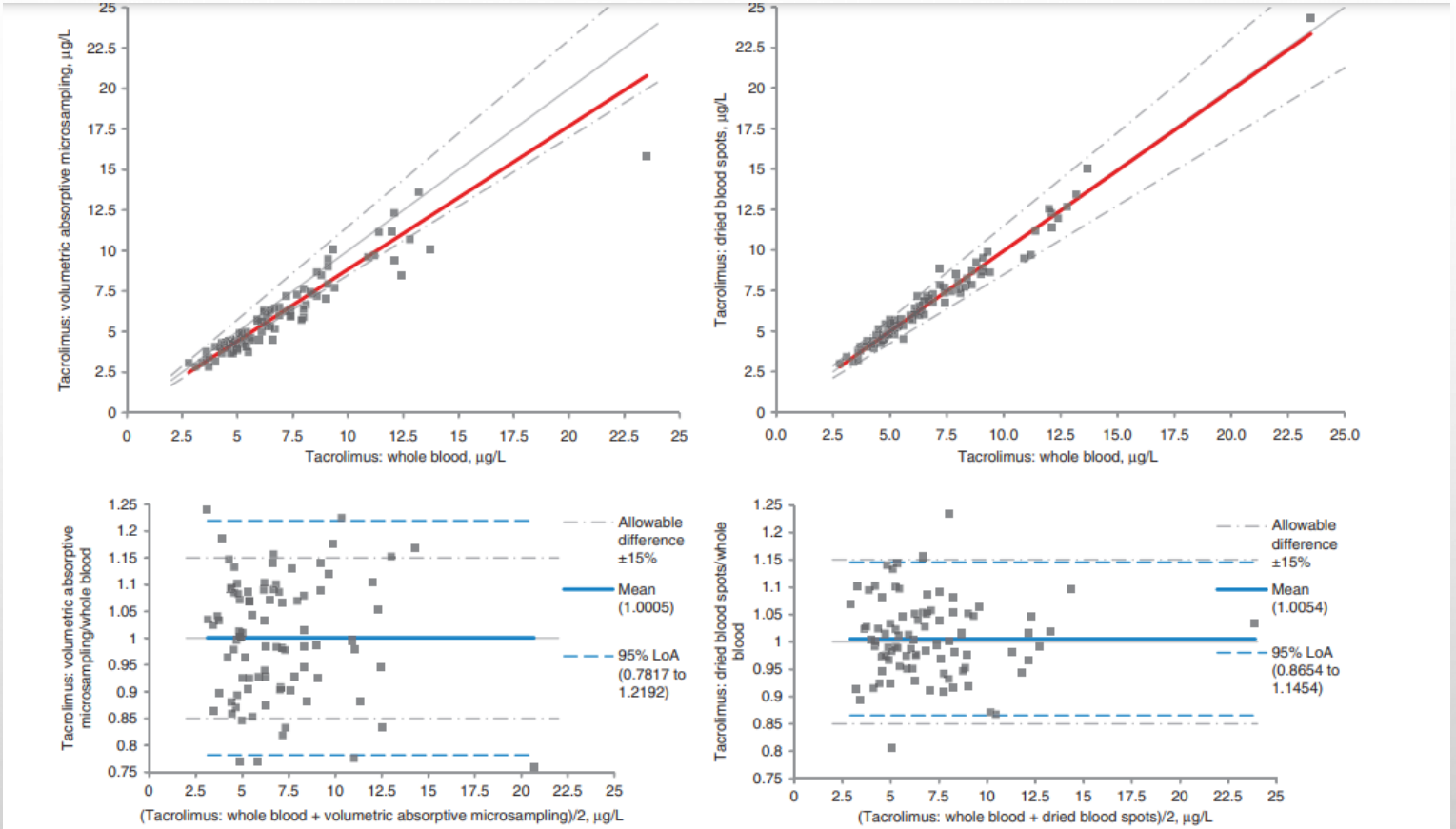


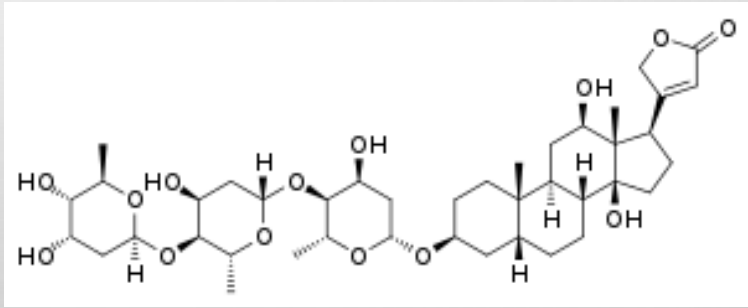
Figure 2: Method comparison between whole blood (WB) tacrolimus levels, volumetric absorptive microsampling (VAMS) tacrolimus levels and dried blood spot (DBS) tacrolimus levels for 88 matched samples.
In the upper left panel, the bold red continuous line is the Passing-Bablok regression line $y = 0.88x + 0.01$ (95% CI slope, 0.81–0.97; 95% CI intercept, −0.47–0.39) for WB vs. VAMS. The dotted/dashed line is the 15% limit of clinical acceptance. In the upper right panel, the bold red continuous line is the Passing-Bablok regression line $y = 0.99x + 0.02$ (95% CI slope, 0.95–1.04; 95% CI intercept, −0.26–0.28) for WB vs. DBS. The dotted/dashed line is the 15% limit of clinical acceptance. The lower left panel shows the Bland-Altman analysis bias estimation based on recalculated values for VAMS using the formula [tacrolimus WB concentration] = [tacrolimus VAMS concentration]/0.88. Calculated bias is 1.00 (95% CI 0.98–1.02). The dotted/dashed line is the 15% limit of clinical acceptance. The dashed line is the 95% limits of agreement (LoA). The lower right panel shows the Bland-Altman analysis bias estimation for WB vs. DBS of 1.01 (95% CI 0.99–1.02). The

Veenhof H. et al. Clin Chem Lab Med 2020;58:1687.

MONITORING CARDIOVASCULAR DRUGS: DIGOXIN

- INDICATIONS OF THERAPY:
  - HEART FAILURE DUE TO IMPAIRED PUMP FUNCTION
  - ATRIAL FIBRILLATION
  - ATRIAL FLUTTER
  - PAROXYSMAL SUPRAVENTRICULAR TACHYCARDIA

- MECHANISM OF EFFECT:
  - NA/K ATP-ASE INHIBITOR
  - POSITIVE INOTROPIC AGENT



MONITORING CARDIOVASCULAR DRUGS: DIGOXIN

- TOXIC AGENT WITH NARROW THERAPEUTIC RANGE
  - ADVERSE EFFECTS: ARRYTHMIA, HEART BLOCKADE, NEUROLOGICAL SYMPTOMS, GI SYMPTOMS
  - ANTIDOTE: DIGIBIND. DIGIFAB
- PER OS AND IV. FORMULATIONS
- INDIVIDUALIZED DOSE ADJUSTMENT INDICATED:
  - TREATING ATRIAL ARRYTHMIAS USUALLY REQUIRES DOSES HIGHER THAN THOSE ADMINISTERED TO TREAT HEART FAILURE
  - DOSE MUST BE CALCULATED FOR LEAN BODY MASS
  - RENAL FUNCTION
  - HORMONAL STATUS (EG. THYROIDS)
  - COMORBIDITIES
  - CO-ADMINISTERED MEDICATIONS (EG. ANTIBIOTICS)

MONITORING CARDIOVASCULAR DRUGS: DIGOXIN

- PER OS BIOAVAILABILITY: 50-100%
- VOLUME OF DISTRIBUTION: 5-7.5 L-KG
  - ATTAINS 10-30X PLASMA CONCENTRATIONS IN CARDIAC MUSCLE
  - DISTRIBUTES IN TOTAL BODY WATER
- RATE OF PROTEIN BINDING: 25%
- ELIMINATION HALF-LIFE: 26-48 H
- METABOLISM: DEGLYCOSYLATION, CONJUGATION, REDUCTION (INTESTINAL MICROBES)
- ELIMINATION: FIRST-ORDER KINETICS, PRIMARILY THROUGH KIDNEYS
- NON-DIALYSABLE

MONITORING CARDIOVASCULAR DRUGS: DIGOXIN

- TARGET TROUGH RANGE: 0.5-1.0 NG/ML
- TOXICITY: FROM 2 NG/ML
- TEST SAMPLE: SERUM, PLASMA
- TEST METHOD: IMMUNOASSAY

MONITORING BIOLOGICAL THERAPY AGENTS

- PROTEINS EMPLOYED FOR TREATING INFLAMMATORY BOWEL DISEASE AND OTHER IMMUNE-MEDIATED INFLAMMATORY DISORDERS
- TNF-ALPHA INHIBITORS AND IMMUNOGLOBULIN G1 ANTIBODIES
- FORMATION OF ANTIBODIES AGAINST THE DRUG MAY LEAD TO SUBOPTIMAL EFFICACY, THESE ANTIBODIES ARE ALSO MONITORED
- ADVERSE EFFECTS CAN BE SEVERE
- ADALIMUMAB, INFLIXIMAB, GOLIMUMAB, USTEKINUMAB, VEDOLIZUMAB ETC.
- TESTING METHOD: ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

IMPACT OF DRUGS ON LABORATORY TESTS

- BIOLOGICAL IMPACT (ADVERSE AFFECTS) AND TECHNICAL INTERFERENCES ARE DIFFERENT!

- <u>EXAMPLES OF LAB TEST-RELATED ADVERSE EFFECTS OF DRUGS:</u>
  - PROTON PUMP INHIBITORS DECREASE ION CONCENTRATIONS
  - STATINS – CREATINE KINASE (CK), GLUTAMATE OXALOACETATE TRANSAMINASE, GLUTAMATE PYRUVATE TRANSAMINASE
  - SELECTIVE SEROTONINE REUPTAKE INHIBITORS MAY CAUSE HYPONATRAEMIA
  - NON-SELECTIVE BETA-BLOCKERS MAY CAUSE HYPERKALAEMIA
- <u>EXAMPLES OF TECHNICAL INTERFERENCES:</u>
  - BIOTIN (VITAMIN B7) INFLUENCES THE RESULTS OF BIOTIN-BASED IMMUNOASSAYS
  - CEFALOSPORINES AND OTHER BETA LACTAMS MAY PRESENT FALSE POSITIVE URINE GLUCOSE AND KETONE TESTS, AND ALTERED COOMBS TEST RESULTS

EXAMPLE: CROSS-REACTIVITY OF AMPHETAMINE IMMUNOASSAYS WITH MDMA (ECSTASY) AND MDA

Immunoassay	MDMA cross reactivity (%)	MDA cross reactivity (%)
DRI-amphetamine	11-44	51-99
CEDIA-amphetamine	57-68	0
EMIT d.a.u. monoclonal IA	7-46	175-214
SYNCHRON-CX-amphetamine	49-100	58-73
COBAS Integra amphetamine	38-48	24-42

EXAMPLE: CROSS-REACTIVITY OF AMPHETAMINE IMMUNOASSAYS WITH DRUGS

- BUPROPION. MEXILETINE. PHENYLEPHRINE. PROMETHAZIN. RANITIDINE

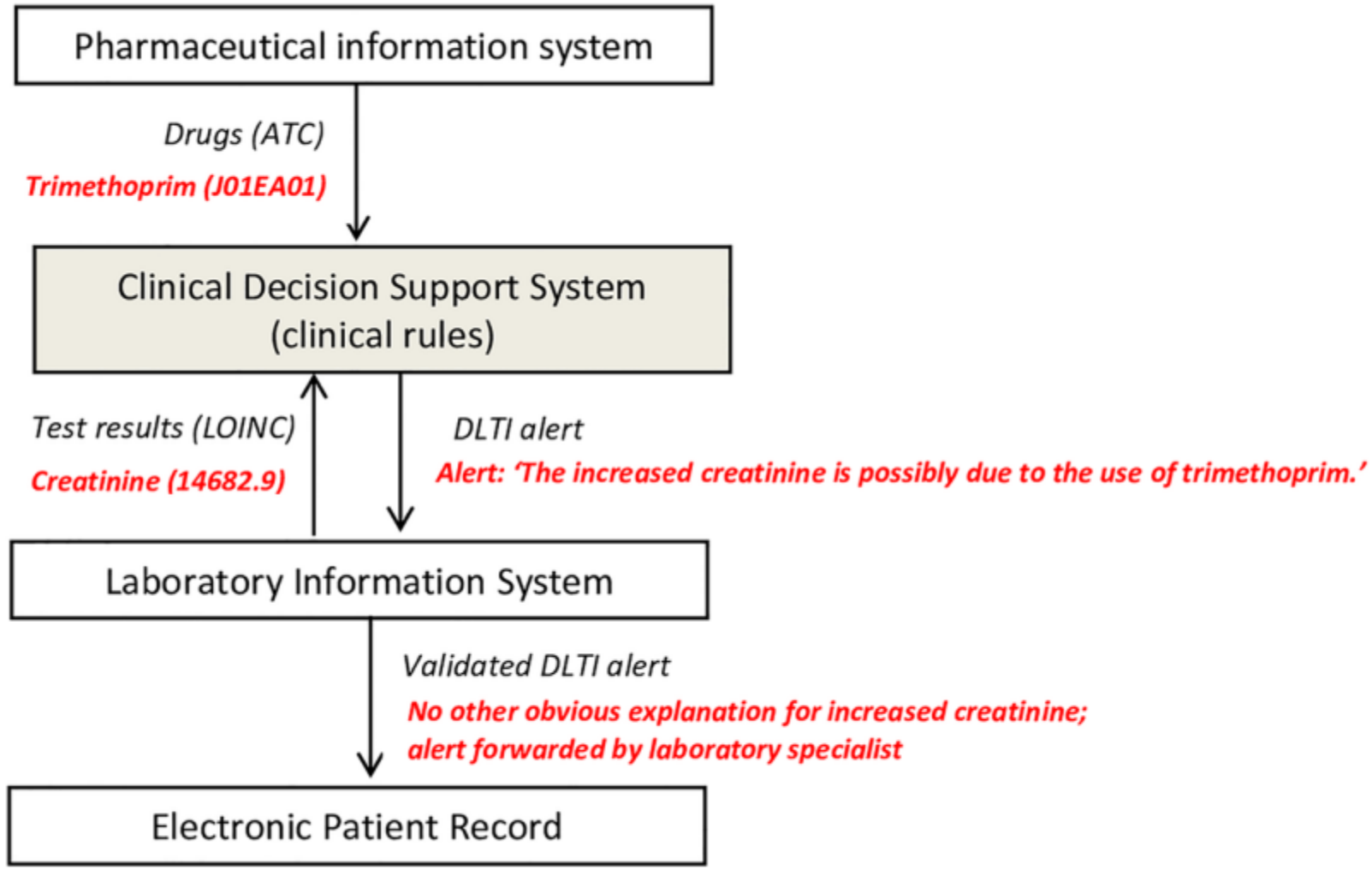


Figure 2: Connections of electronic decision support system (containing clinical rules) to receive real-time patient data and consequently, send DLTI messages. ATC, anatomical therapeutic chemical (drug classification system of the World Health Organization); LOINC, logical observation identifiers names and codes (universal standard for identifying medical laboratory observations, such as laboratory tests); DLTI, drug laboratory test interaction.

Van Balveren JA et al. Clin Chem Lab Med 2021;59:235.

THANK YOU FOR YOUR ATTENTION

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