Testing Inhibitor Combinations

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EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

Susceptible (S)

A micro-organism is defined as susceptible by a level of antmicrobial activity associated with a high likelihood of therapeutic success. A micro-organism is categorized as susceptible by applying the appropriate breakpoint in a defined phenotypic test system.

Note: This breakpoint may be altered with legitimate changes in circumstances

Intermediate (I)
A micro-organism is defined as intermediate by a level of antimicrobial activity associated with indetermiate therapeutic effect. A micro-organism is categorized as intermediate by applying the appropriate breakpoints in a defined phenotypic test system.
Note: This breakpoints may be altered with legitimate changes in circumstances.
Resistant (R)
bacteria are defined as resistant by a level of antimicrobial activity associated with a high likelihood of therapeutic failure. A micro-organism is categorized as resistant by applying the appropriate breakpoint in a defined phenotypic test system.
Note: This breakpoint may be altered with legitimate changes in circumstances

Cephalosporins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Cefaclor	-	-		-	-
Cefadroxil (uncomplicated UTI only)	16	16	30	12	12
Cefalexin (uncomplicated UTI only)	16	16	30	14	14
Cefazolin	-	-		-	-
Cefepime	1	4	30	24	21
Cefixime (uncomplicated UTI only)	1	1	5	17	17
Cefotaxime	1	2	5	20	17
Cefoxitin (screen)[2]	NA	NA	30	19	19
Cefpodoxime (uncomplicated UTI only)	1	1	10	21	21
Ceftaroline	0.5	0.5	5	23	23
Ceftazidime	1	4	10	22	19
Ceftibuten (UTI only)	1	1	30	23	23
Ceftobiprole	0.25	0.25	IP	IP	IP
Ceftriaxone	1	2	30	23	20
Cefuroxime iv	8[3]	8	30	18[A]	18
Cefuroxime oral (uncomplicated UTI only)	8	8	30	18	18

Carbapenems[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Doripenem	1	2	10	24	21
Ertapenem	0.5	1	10	25	22
Imipenem[2]	2	8	10	22	16
Meropenem	2	8	10	22	16

Cephalosporins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
	S ≤	R >		S ≥	R <	
Cefaclor	-	-		-	-	**1.** The cephalosporin breakpoints for Enterobacteriaceae will detect all clinically important resistance mechanisms (including ESBL and plasmid mediated AmpC). Some isolates that produce beta-lactamases are susceptible or intermediate to 3rd or 4th generation cephalosporins with these breakpoints and should be reported as tested, *i.e.* the presence or absence of an ESBL does not in itself influence the categorisation of susceptibility. In many areas, ESBL detection and characterisation is recommended or mandatory for infection control purposes. **2.** The cefoxitin ECOFF (8 mg/L) has a high sensitivity but poor specificity for identification of AmpC-producing Enterobacteriaceae as this agent is also affected by permeability alterations and some carbapenemases. Classical non-AmpC producers are wild type, whereas plasmid AmpC producers or chromosomal AmpC hyperproducers are non-wild type. **3A.** The breakpoint relates to a dosage of 1.5 g x 3 and to *E. coli*, *Klebsiella* spp. and *P. mirabilis* only.
Cefadroxil (uncomplicated UTI only)	16	16	30	12	12	
Cefalexin (uncomplicated UTI only)	16	16	30	14	14	
Cefazolin	-	-		-	-	
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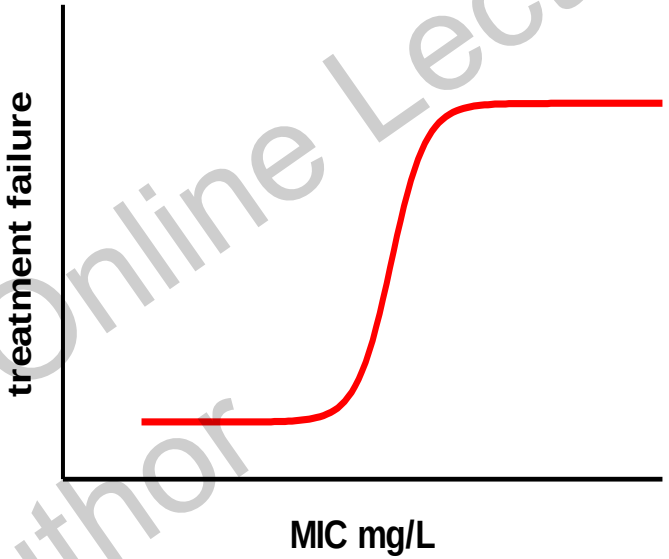
	S ≤	R >		S ≥	R <	
Doripenem	1	2	10	24	21	**1.** The carbapenem breakpoints for Enterobacteriaceae will detect all clinically important resistance mechanisms (including the majority of carbapenemases). Some isolates that produce carbapenemase are categorised as susceptible with these breakpoints and should be reported as tested, *i.e.* the presence or absence of a carbapenemase does not in itself influence the categorisation of susceptibility. In many areas, carbapenemase detection and characterisation is recommended or mandatory for infection control purposes. **2.** Low-level resistance is common in *Morganella* spp., *Proteus* spp. and *Providencia* spp.
Ertapenem	0.5	1	10	25	22	
Imipenem[2]	2	8	10	22	16	
Meropenem	2	8	10	22	16	

Is susceptibility (MICs) related to (clinical) outcome?



If yes, which values (breakpoints) make the difference?

ACTVITY
***in vitro* (MIC)**

CONCENTRATIONS
***in vivo* (PK)**

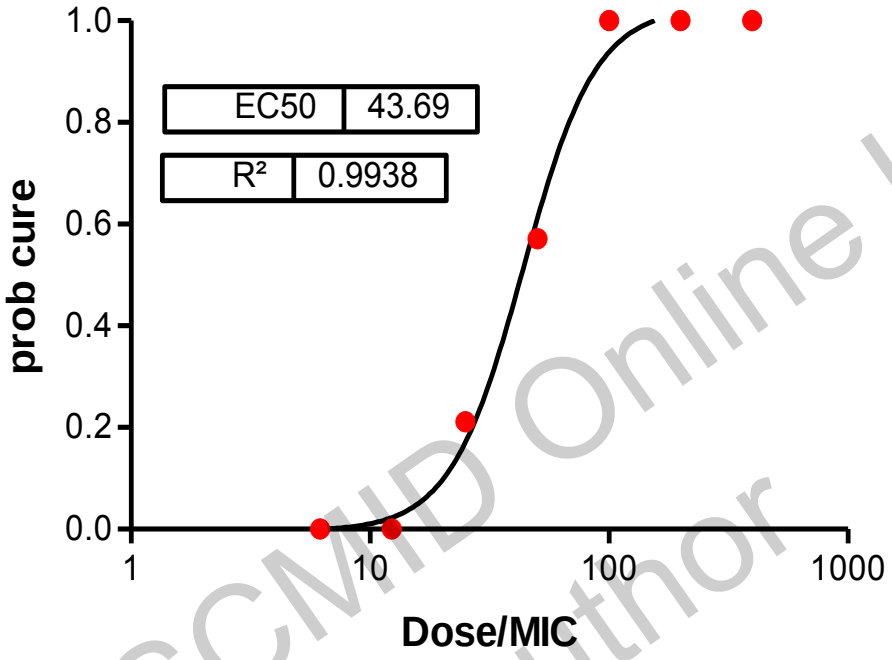
DOSING
regimen

ANTMICROBIAL EFFICACY
(*Microbiological Cure*)

Other factors

CLINICAL EFFICACY
(*Clinical Cure*)

Probability of cure after treatment with fluconazole Oropharygeal Candidiasis n=132

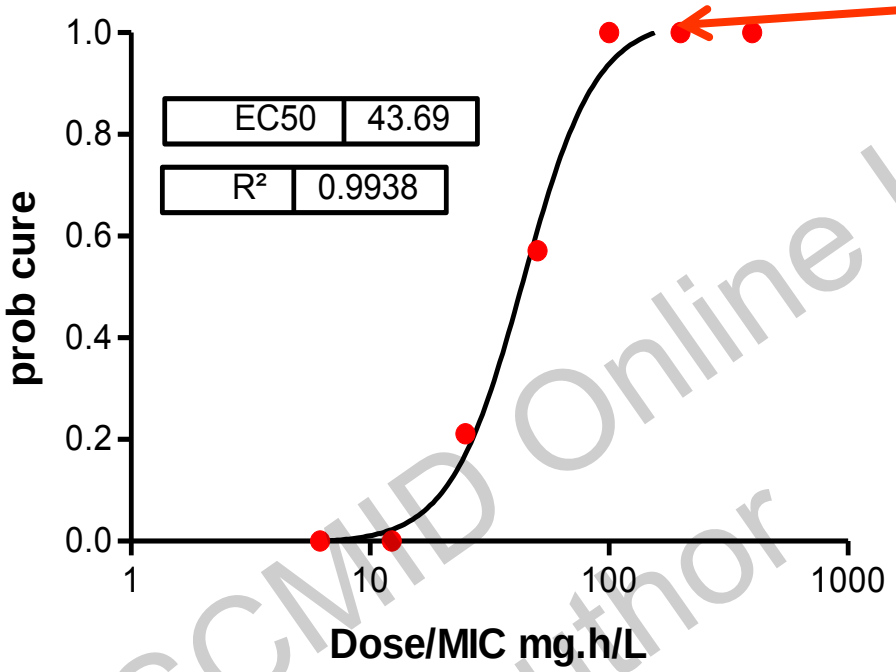


- Prob cure correlates with Dose/MIC
- POSITIVE correlation with dose
- INVERSE correlation with MIC

Each data point represents the proportion of patients cured within a group representing a certain AUC/MIC value

Rodriguez- Tudela et al, AAC 2007

Probability of cure after treatment with fluconazole Oropharygeal Candidiasis n=132



Pharmacodynamic Target

NOTE : MICs by EUCAST method

Rodriguez- Tudela et al, AAC 2007

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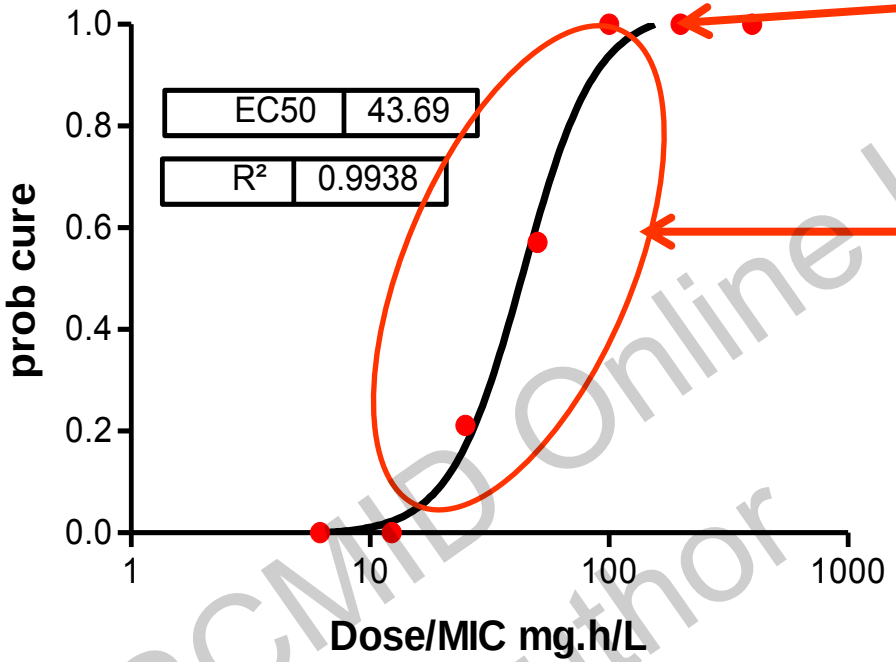

Pharmacodynamic Target

Uncertainty

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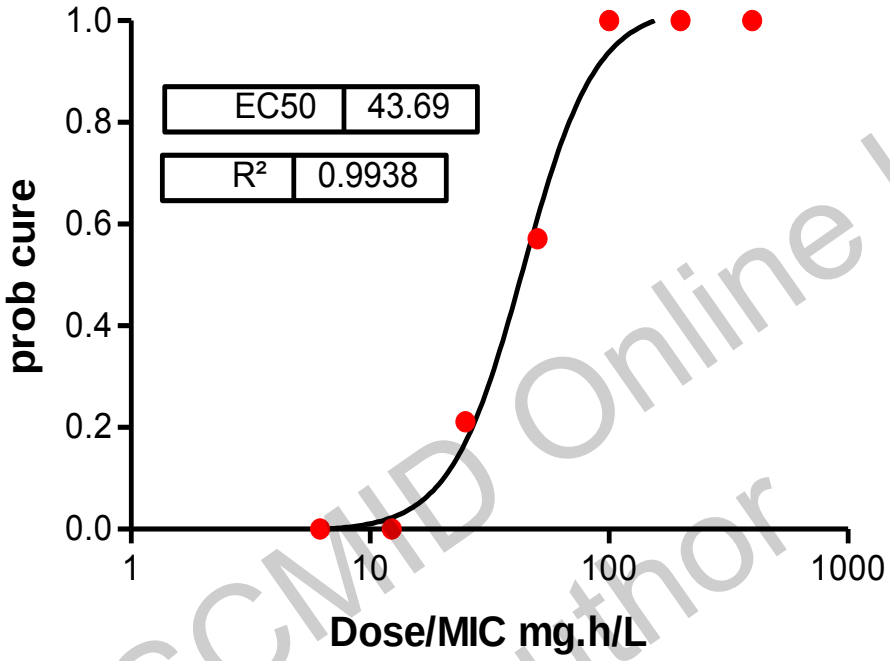

- If Dose is known
- And an Dose/MIC of 100 is required
- It follows that the breakpoint is 400/100 = 4 mg/L

Rodriguez- Tudela et al, AAC 2007

SETTING A BREAKPOINT –PK/PD

DETERMINE THE PK/PD TARGET e.g. *value of the PK/PD Index*
(animal studies, clinical studies)

ESTIMATE EXPOSURE from the dosing regimen and PK, including population variability

CALCULATE PK/PD BREAKPOINT from *PK/PD target = PK/PD Index*

The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach

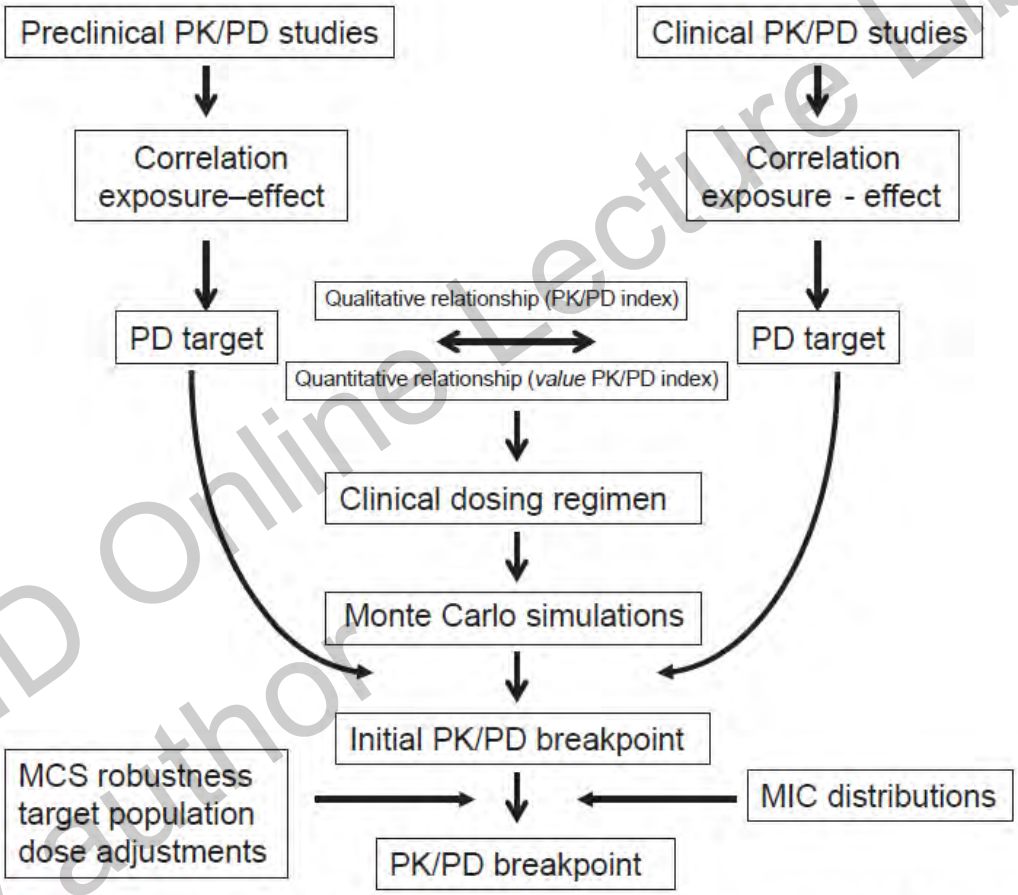

FIG. 7. Summary of the process of setting pharmacokinetic/pharmacodynamic (PK/PD) breakpoints by EUCAST.

Mouton et al, CMI 2012

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Clinical phase 3 study

PK-data

PK population model

Individual PK parameters

Culture-results with MIC-values

MIC-values per individual

Individual exposure to CAZ
%*f*T>MIC

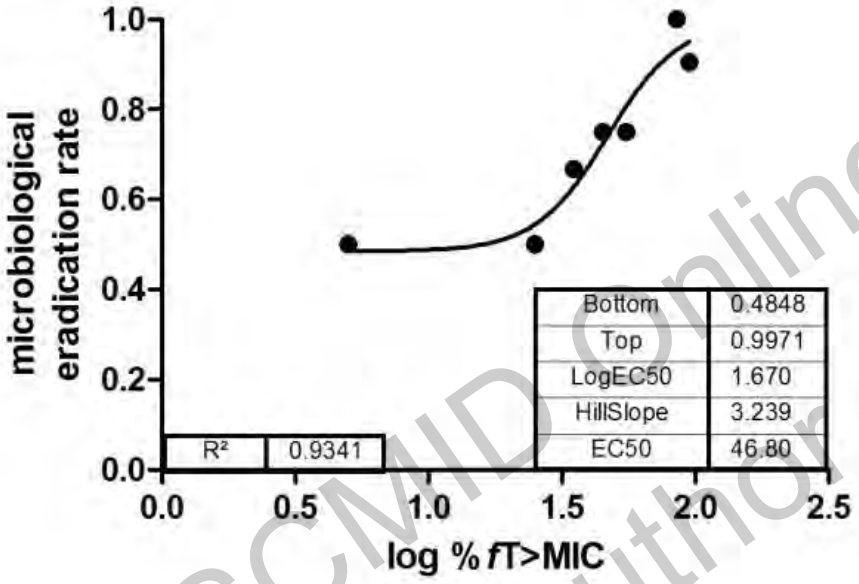
Microbiological outcome
Clinical outcome

PK/PD of ceftazidime in Clinical Study

- 154 patients with nosocomial pneumonia (including VAP)
- PK parameters determined in every patient
  - Sparse sampling; covariates; population PK
- MICs of infecting micro-organisms
- Individual exposures to CAZ (%*f*T>MIC)
  Categorised (%*f*T>MIC per 10%)
- Eradication rate per exposure group

Muller et al, JAC 2013 68:900-906

Exposure-response Emax model
microbiological eradication

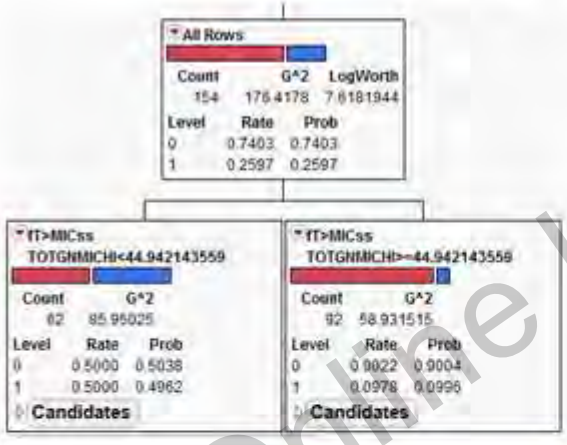


- Individual exposures to CAZ
- Categorised (%*f*T>MIC per 10%)
- Eradication rate per category
- 154 patients

Muller et al, JAC 2013 68:900-906

Ceftazidime in patients with nosocomial pneumonia

CART analysis



- to differentiate between lower and higher response rate

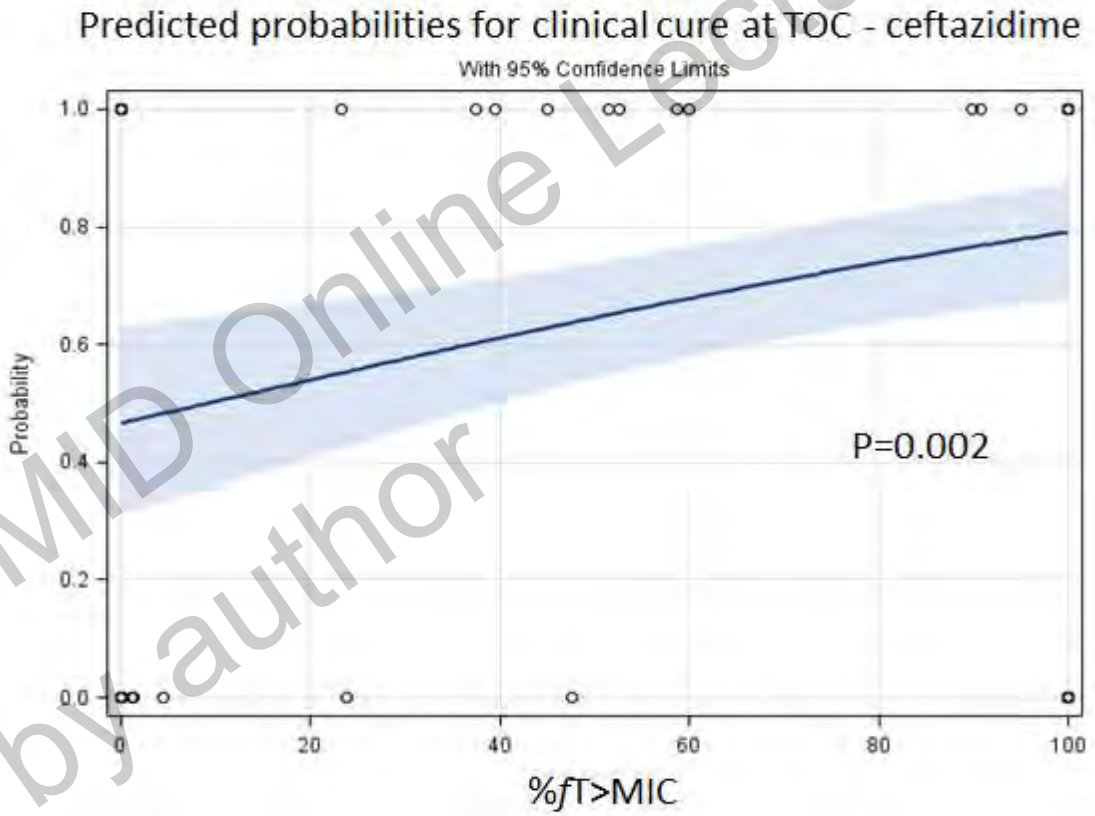
%*f*T>MIC breakpoint = 44.9 %

P< 0.0001

%*f*T>MIC	Success	Failure
≥44.9	83 (90.2%)	9 (9.8%)
<44.9	31 (50%)	31 (50%)

Muller et al, JAC 2013 68:900-906

Probability plot of the logistic regression analysis for ceftazidime showing the relationship between %*f*T>MIC (Gram-negatives at baseline/EOT) and probability of cure at TOC



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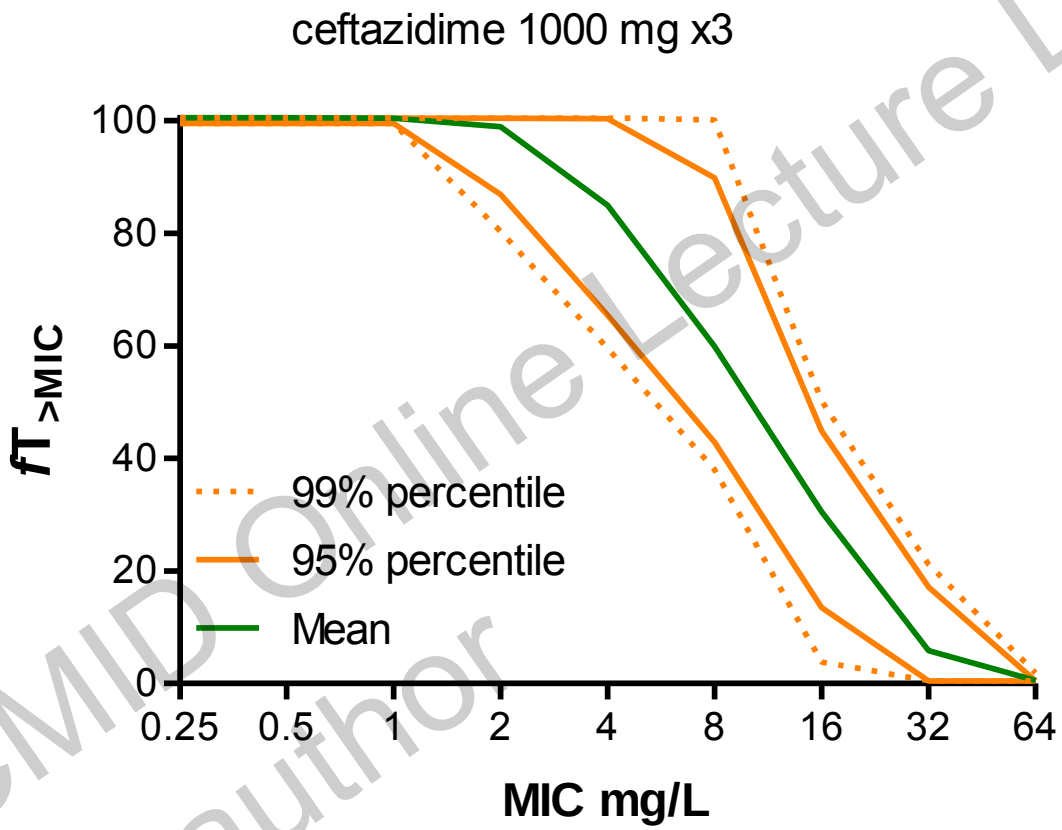
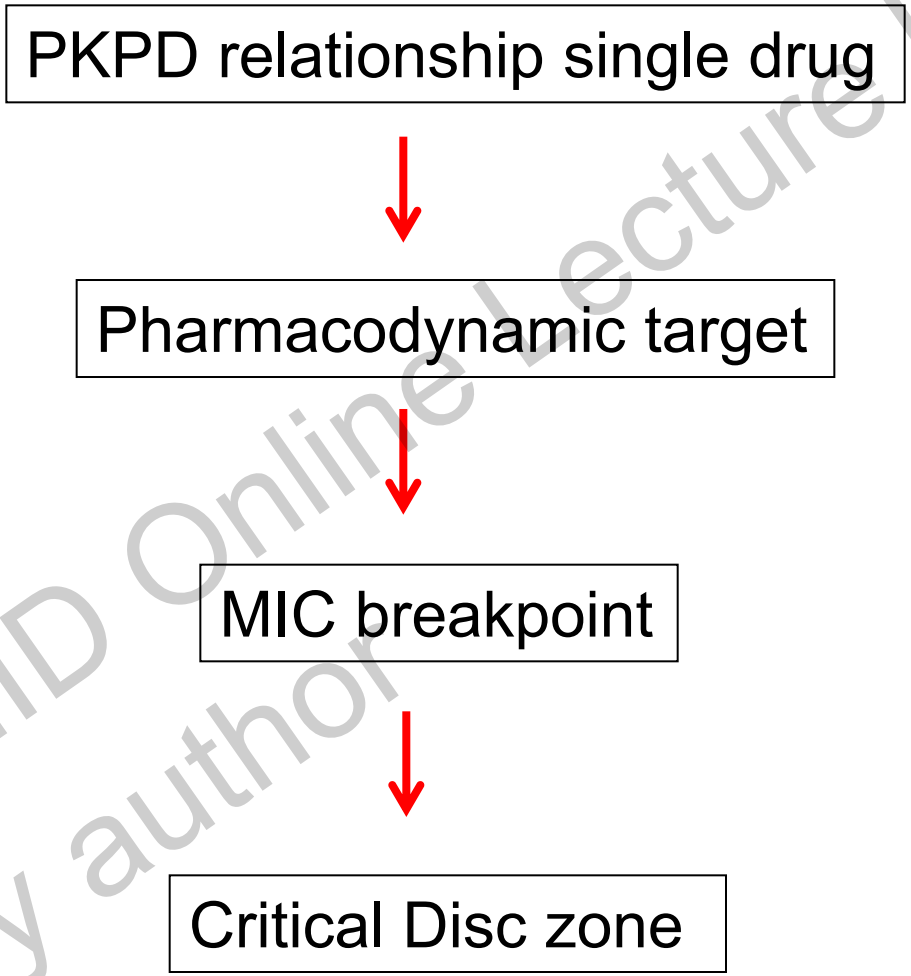
Monte Carlo Simulations : Exposure –MIC relationship



EUCAST rationale document

Deriving breakpoints and diskzones



Deriving breakpoints and diskzones

	ceftazidime
PKPD relationship single drug	$\%fT_{>MIC}$
↓	↓
Pharmacodynamic target	45-50%
↓	↓
MIC breakpoint (dose-dependent)	4 mg/L 1g q8h
↓	↓
Critical Diskzone	19 mm 10 ug disk

Purpose of BLI inhibitor

Restore the activity of the parent drug

e.g. ceftazidime, imipenem

Deriving breakpoints and disc zones Combinations

	ceftazidime
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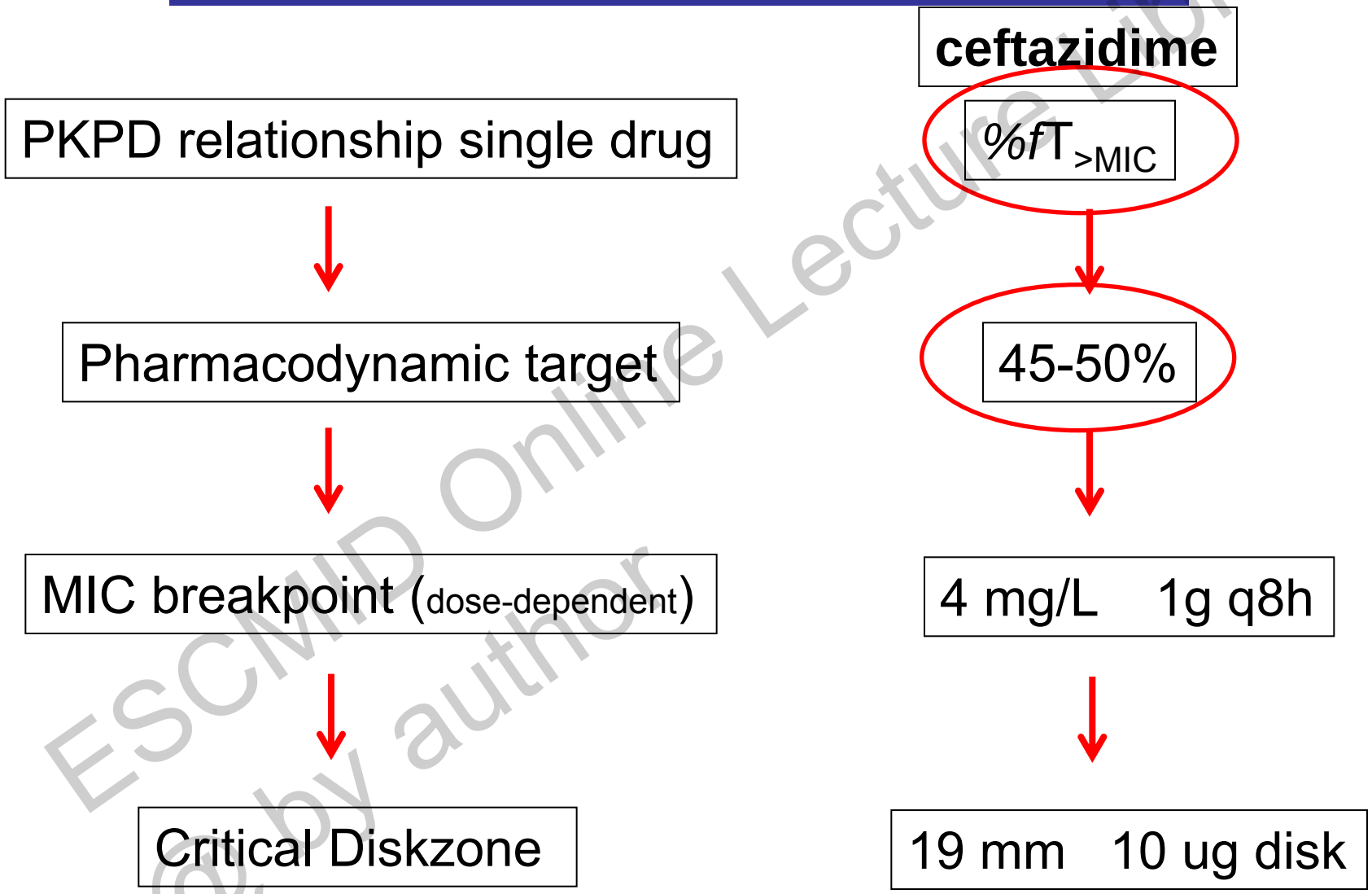
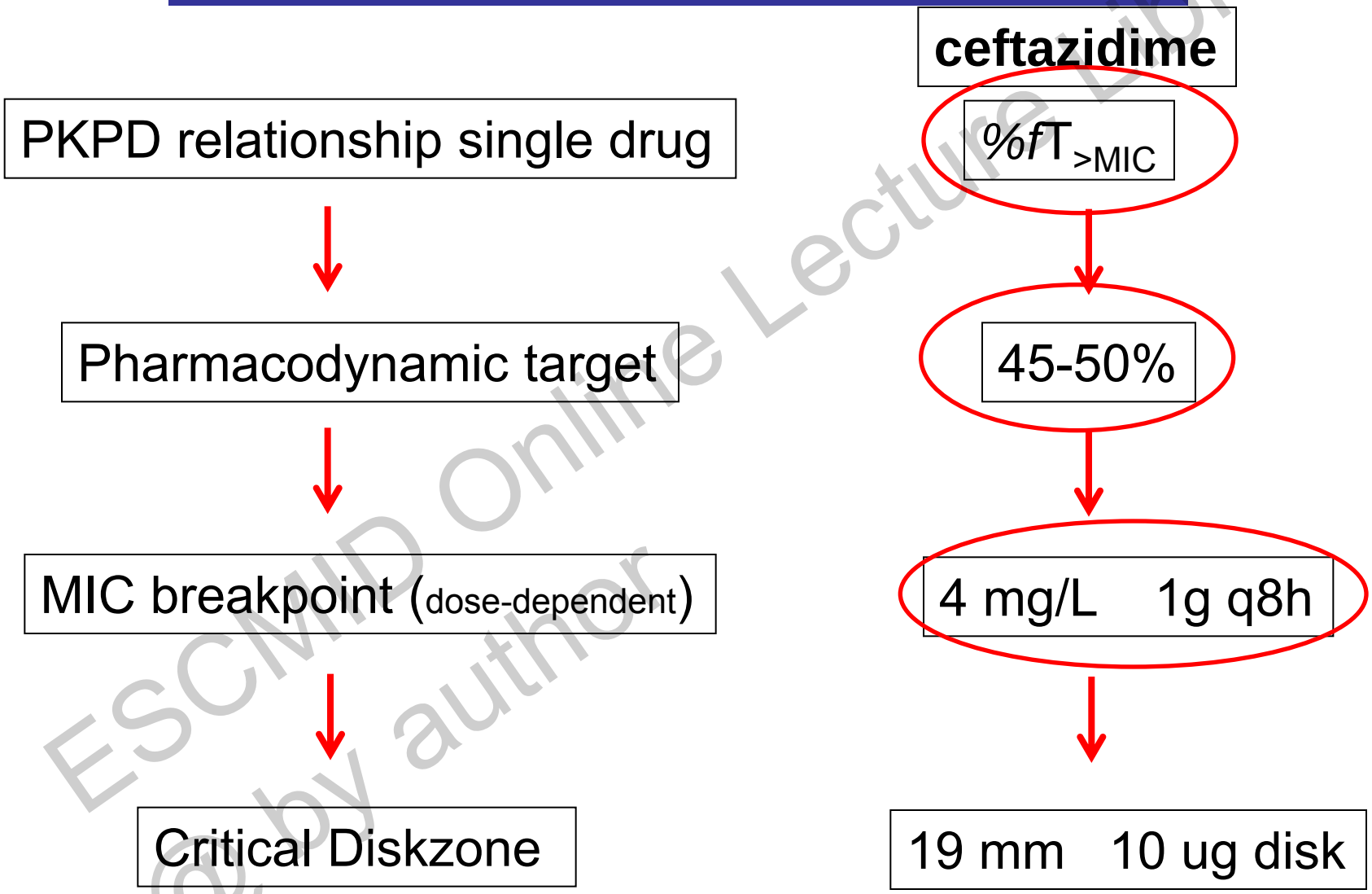
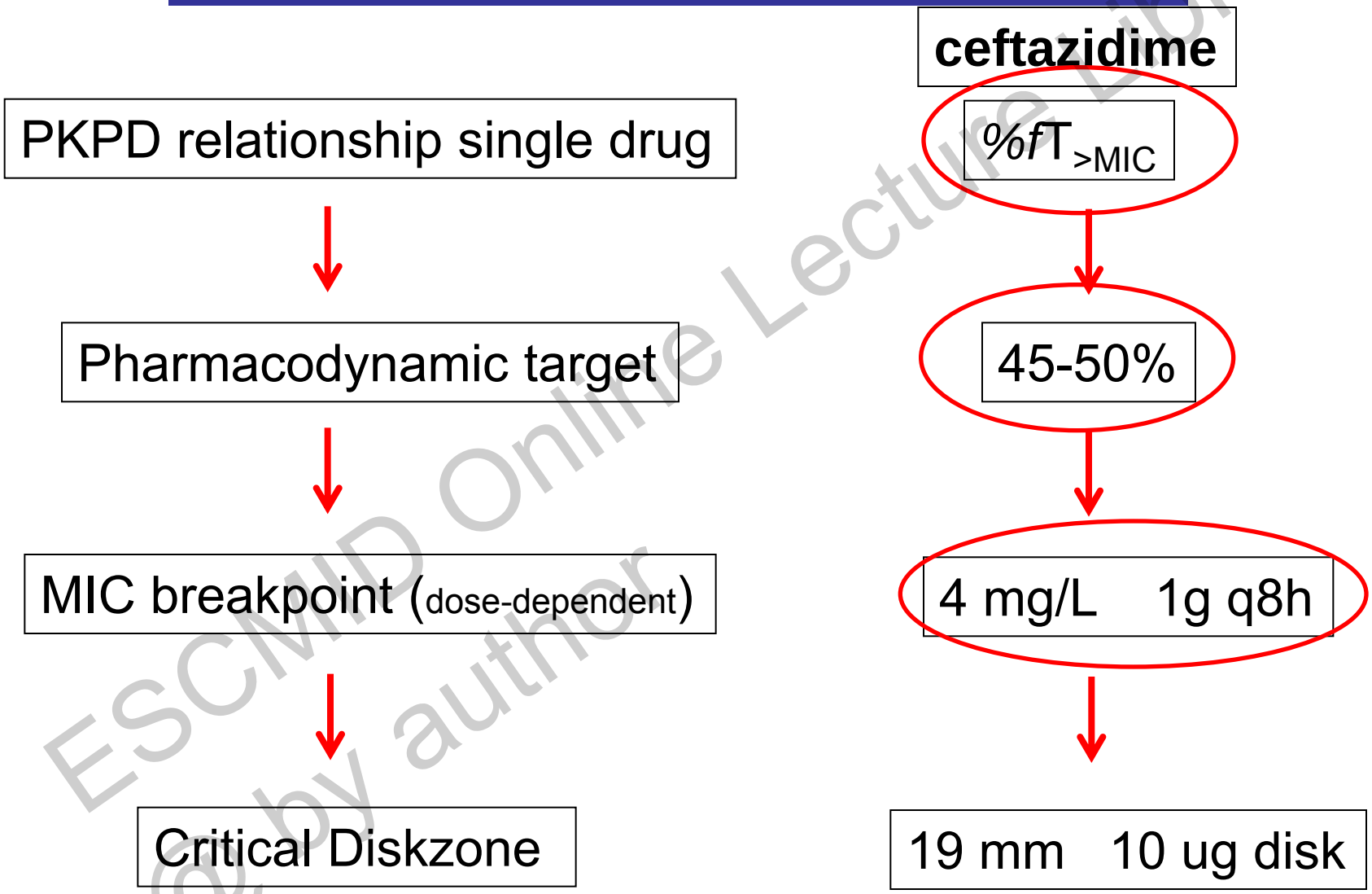


Deriving breakpoints and disc zones Combinations



Deriving breakpoints and disc zones Combinations



Which Questions to answer?

- Which concentration of BLI inhibitor is required in the MIC test system?
  - Reproducible results
  - Maximum effects?

- Which dose of BLI inhibitor is required for treatment?
  - Pharmacodynamic properties
  - Exposure response relationship
  - Pharmacodynamic targets

- Exposure res ponse relationship ‘parent’ drug
  - Other dosing regimens?

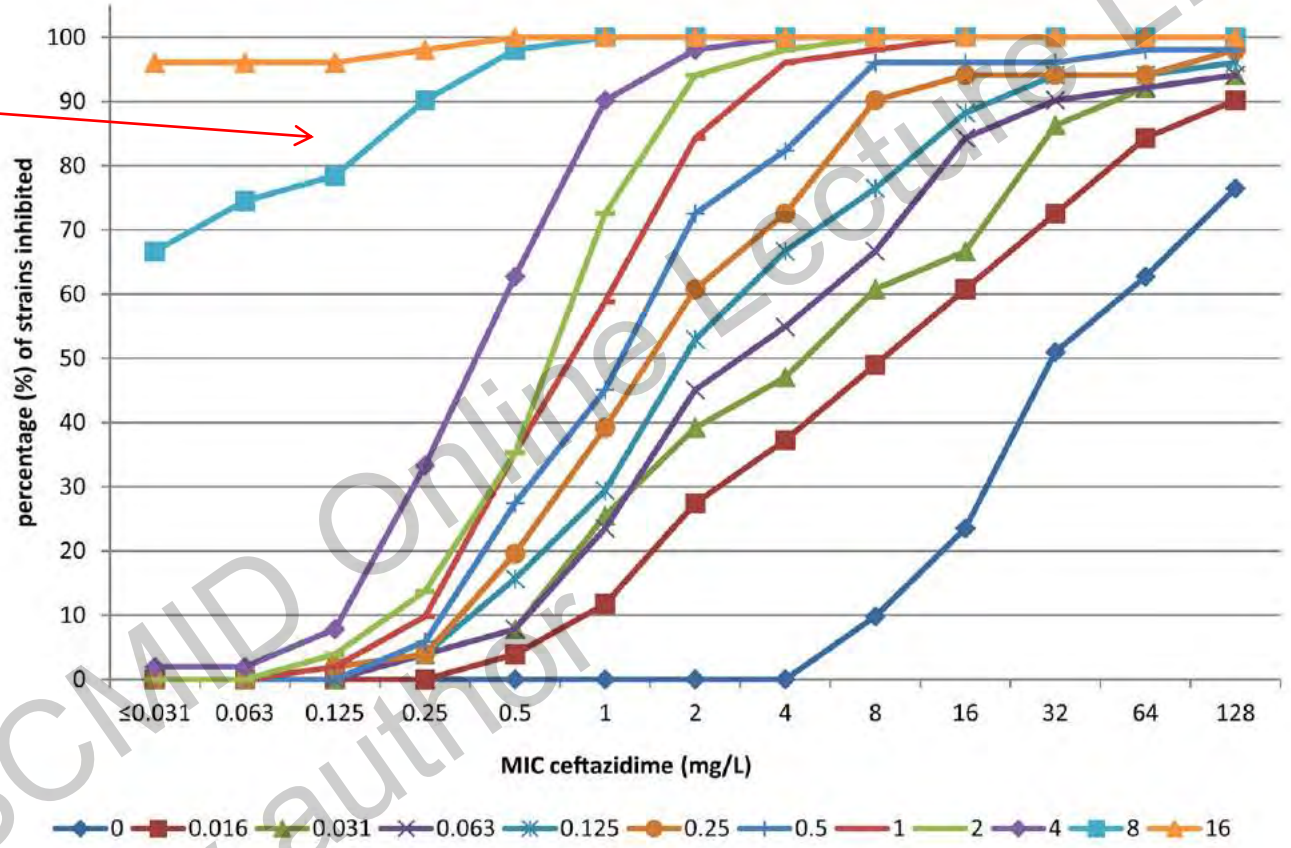
- Diskzone correlates
  - What is the optimal diskload combination?

Which Questions to answer?

- Which concentration of BLI inhibitor is required in the MIC test system?
  - Maximum effects?
  - Reproducible results

Cumulative Cumulative % inhibition of Avibactam 51 *Enterobacteriaceae*

Avibactam activity alone



Berkhout et al, AAC 2015

Inhibition of BL by tazobactam differs by strain
Cefepime + Tazobactam

Max effect?

strain	no. Anits	0	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16
kpn	44	16	8	8	16	4	16	8	4	2	4	0.25
ecl	68	16	16	8	8	8	1	4	4	4	2	0.5
ecl	18	>32	32	16	32	8	4	4	2	2	1	0.5
eco	73	>32	2	2	1	0.125	1	0.125	0.125	0.125	0.125	0.125
eco	81	>32	2	2	2	0.25	0.064	0.125	0.064	0.064	0.064	0.064
ecl	32	>32	4	4	4	8	1	1	0.25	0.125	0.125	0.125
eco	46	>32	4	8	4	2	0.125	2	2	0.064	0.064	0.064
eco	14	>32	8	4	8	4	0.25	0.25	0.125	0.064	0.064	0.25
eco	66	>32	8	4	4	4	2	0.25	0.125	0.125	0.125	0.064
ecl	33	>32	8	8	4	4	4	2	1	1	1	0.25
eco	16	>32	8	8	8	8	4	0.25	0.064	0.032	0.032	0.032
eco	56	>32	8	8	8	2	0.25	2	0.125	0.125	0.064	0.125
eco	78	>32	8	8	4	2	0.125	0.5	0.125	0.125	0.125	1
kpn	11	>32	8	8	8	8	1	0.125	0.125	0.064	0.032	0.016
ecl	72	>32	8	16	8	16	8	8	8	1	1	1
eco	34	>32	8	16	8	16	2	4	0.125	2	0.125	0.125
kpn	52	>32	16	8	16	16	8	16	32	4	1	0.5
kpn	53	>32	16	8	8	4	4	4	2	1	0.5	1
ecl	27	>32	16	16	16	16	4	1	2	0.5	0.5	0.5

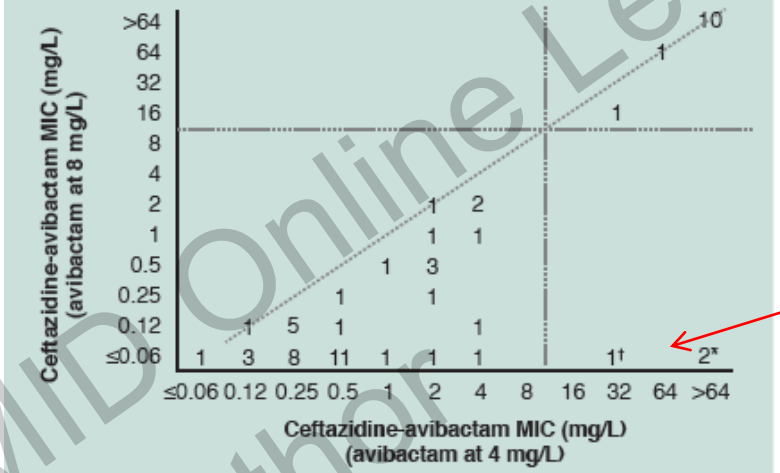
Mouton et al, abstr 2251 ICAAC 2010

P1290

Ceftazidime-avibactam: use of a predictor panel to evaluate and optimize avibactam concentrations for *in vitro* susceptibility testing

Michael D. Huband[1], Wright W. Nichols[2], Gregory G. Stone[2], Linda G. Otterson[2], Patricia A. Bradford[2]
[1]Formerly of AstraZeneca Pharmaceuticals LP, Waltham, MA, USA; [2]Development Microbiology, AstraZeneca Pharmaceuticals LP, Waltham, MA, USA

Figure 2. *Enterobacteriaceae: ceftazidime-avibactam MICs compared: avibactam 4 versus 8 mg/L*



misclassified

E. coli ARC3531 (TEM-1, VEB-5)
E. coli ARC3805 (NDM-1, CTX-M-15, CMY-4, OXA-1, OXA-2)
†*K. pneumoniae* ARC3804 (NDM-1, TEM-1, CTX-M-15, SHV-5, OXA-2)

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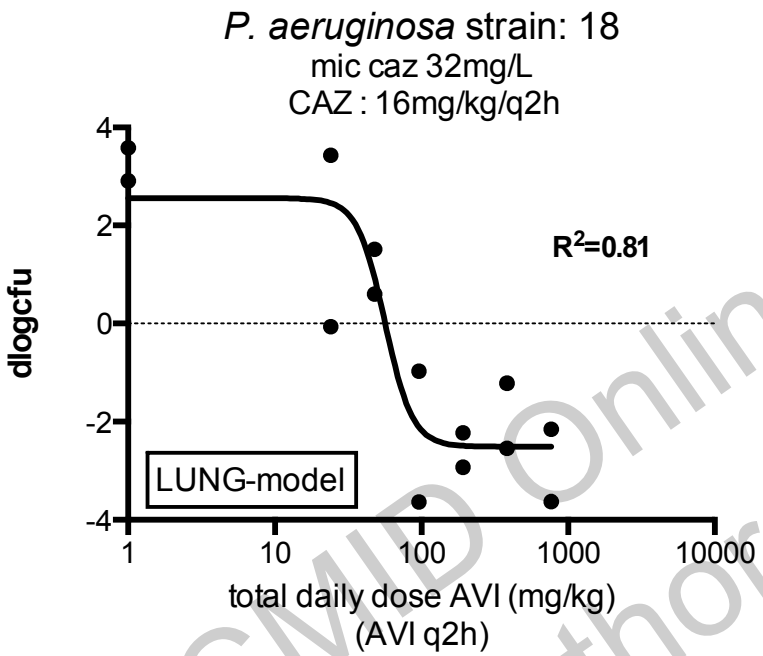
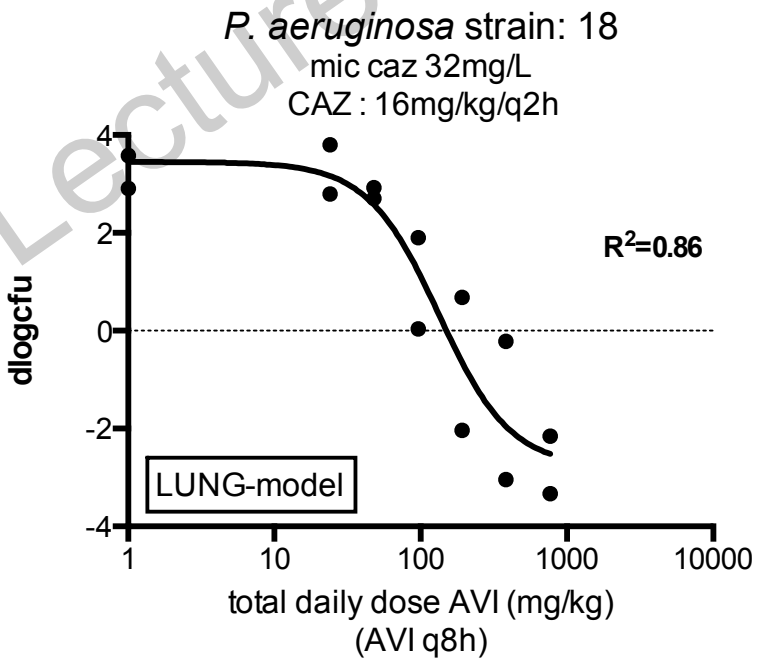
Time above Ct of Avibactam



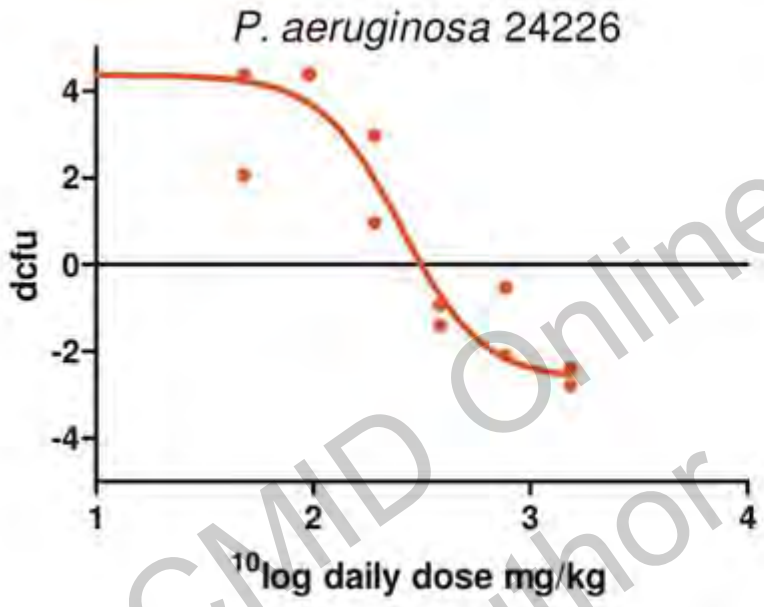


Less frequent dosing : more inhibitor required

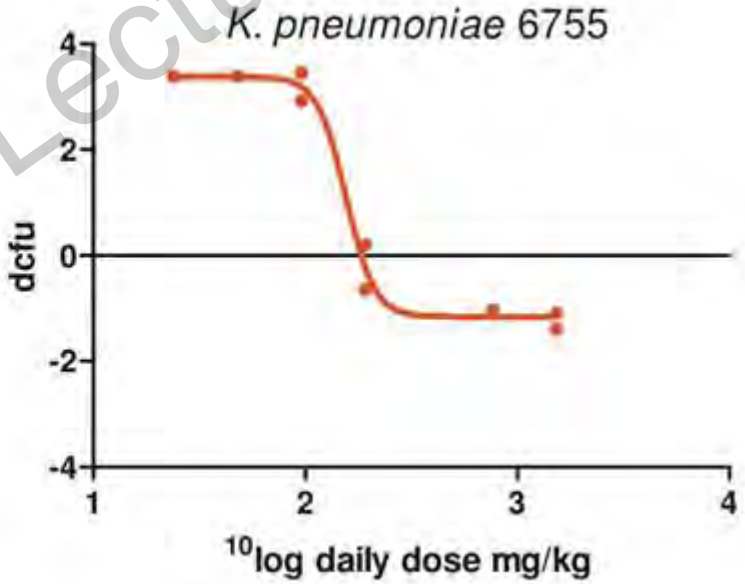
Berkhout ICAAC 2013

ER of Imipenem alone

MIC 8 mg/L



MIC 4 mg/L



Static

*f*T>MIC = 36.4%

*f*T>MIC = 33.0%

Mavridou et al. AAC 2015

Pharmacodynamics of MK7655 - imipenem

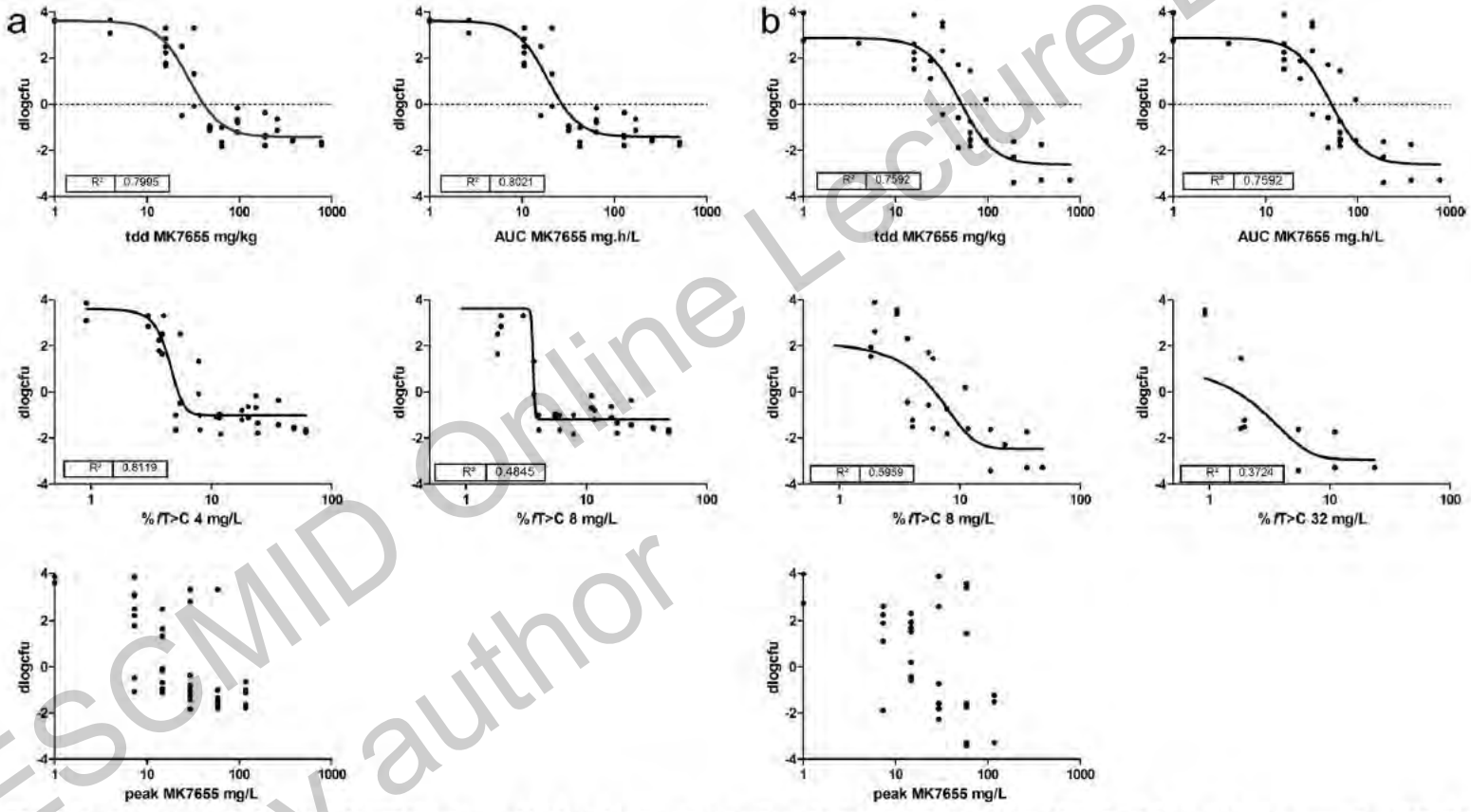


FIG 2 Dose-response relationship of MK7655 observed in dose fractionation studies with IMP/C administered q2h at fixed doses. (a) *K. pneumoniae* 6755; (b) *P. aeruginosa* 24228. tdd, total daily dose.

Mavridou et al. AAC 2015

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Optimal diskload

- Should differentiate between R , I and S
- In principle, the diskload of active drug is similar
- The inhibitor load optimized.

Conclusions

- The efficacy of the combination should reflect the efficacy of the active drug (ceftazidime, imipenem)
- The BLI inhibitor should restore the activity of the active drug
- Exposure of the BLI inhibitor should be maximized (dose, PDI)
- Breakpoints of the combinations are similar to the active compound **IF THE SAME DOSE IS USED**
- Diskzones reflect MIC breakpoint cut-offs of combinations

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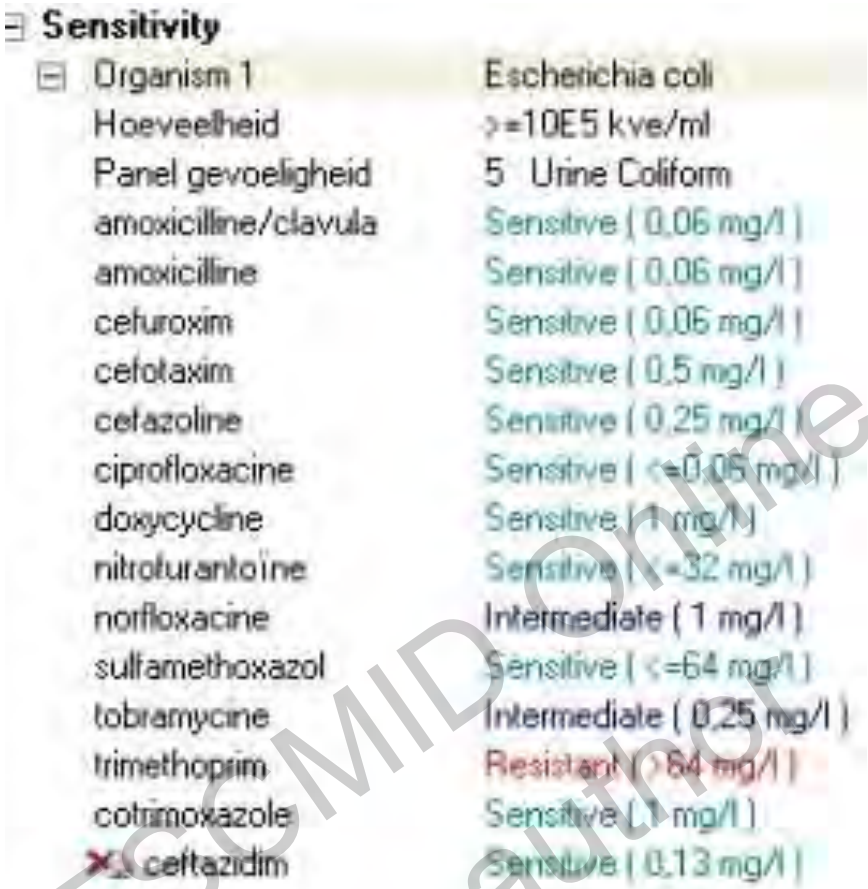
Note: This breakpoint may be altered with legitimate changes in circumstances

LAB REPORT



Sensitivity	
Organism 1	Escherichia coli
Hoeveelheid	>=10E5 kve/ml
Panel gevoeligheid	5 Urine Coliform
amoxicilline/clavula	Sensitive (0,06 mg/l)
amoxicilline	Sensitive (0,06 mg/l)
cefuroxim	Sensitive (0,06 mg/l)
cefotaxim	Sensitive (0,5 mg/l)
cefazoline	Sensitive (0,25 mg/l)
ciprofloxacine	Sensitive (<=0,06 mg/l)
doxycycline	Sensitive (1 mg/l)
nitrofurantoïne	Sensitive (<=32 mg/l)
norfloxacine	Intermediate (1 mg/l)
sulfamethoxazol	Sensitive (<=64 mg/l)
tobramycine	Intermediate (0,25 mg/l)
trimethoprim	Resistant (>64 mg/l)
cotrimoxazole	Sensitive (1 mg/l)
ceftazidim	Sensitive (0,13 mg/l)

- Provides Clinician/Consultant guidelines how to optimally treat a patient (Freely translated from EUCAST guideline)

Conclusions

- PK/PD explains and predicts the effects of antimicrobials
- Dose optimization includes MICs AND patient variability
- For beta-lactams, %*f*T>MIC is the most important predictor
- Increasing target attainment by adjusting the dosing regimens and/or choosing the right antimicrobial is helpful, especially at borderline MICs (Pseudomonas!)
- Therapeutic drugmonitoring in ICU patients should be discussed

SETTING A BREAKPOINT –PK/PD (example 1)

DETERMINE THE PK/PD TARGET e.g. *value of the PK/PD Index*

(animal studies, clinical studies)

Levofloxacin target ~34

ESTIMATE EXPOSURE from the dosing regimen and PK, including population variability

CALCULATE PK/PD BREAKPOINT from *PK/PD target = PK/PD Index*