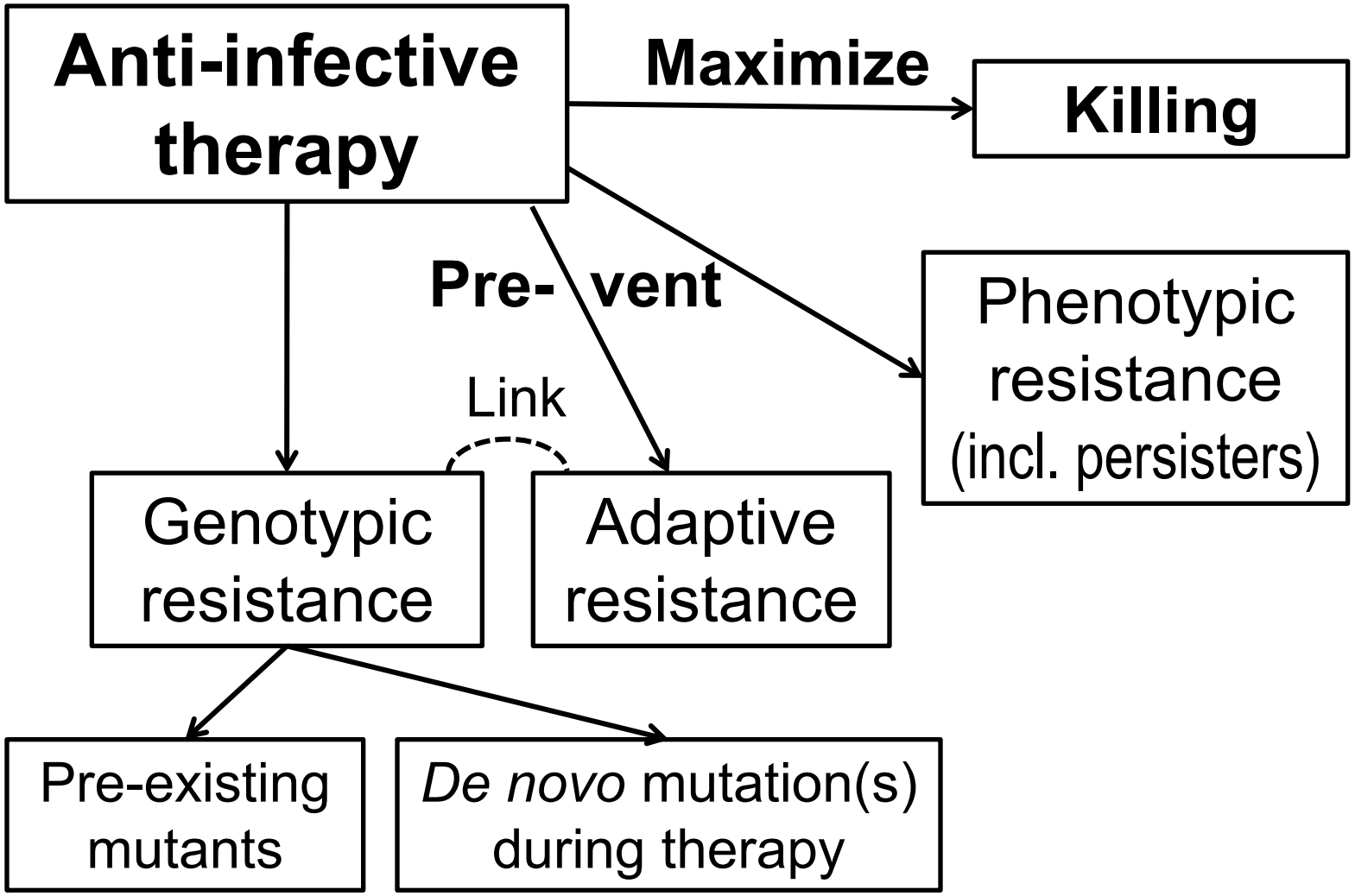
Combination therapy of *P. aeruginosa* with special reference to modeling of polymyxins *in vitro* and to preliminary animal models

April 20th, 2010

Jürgen B. Bulitta, PhD

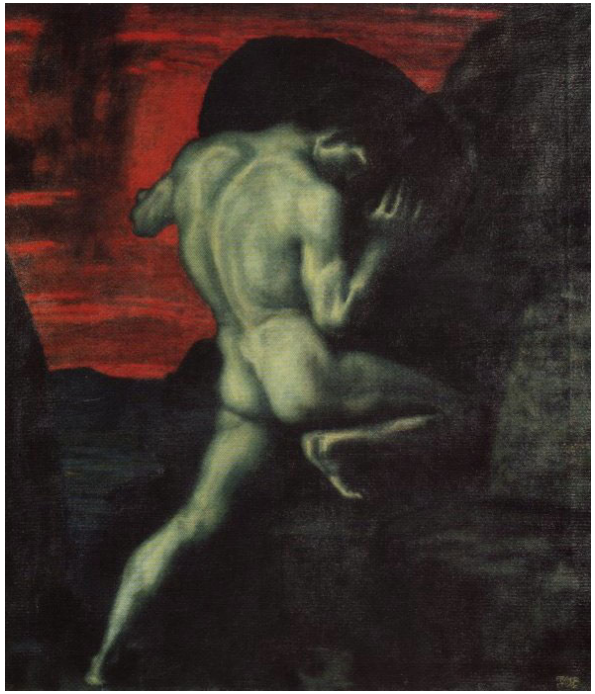
Target Goals



Sometimes a Single Drug (Man) just cannot Achieve the Target Goals

Most problematic infections:

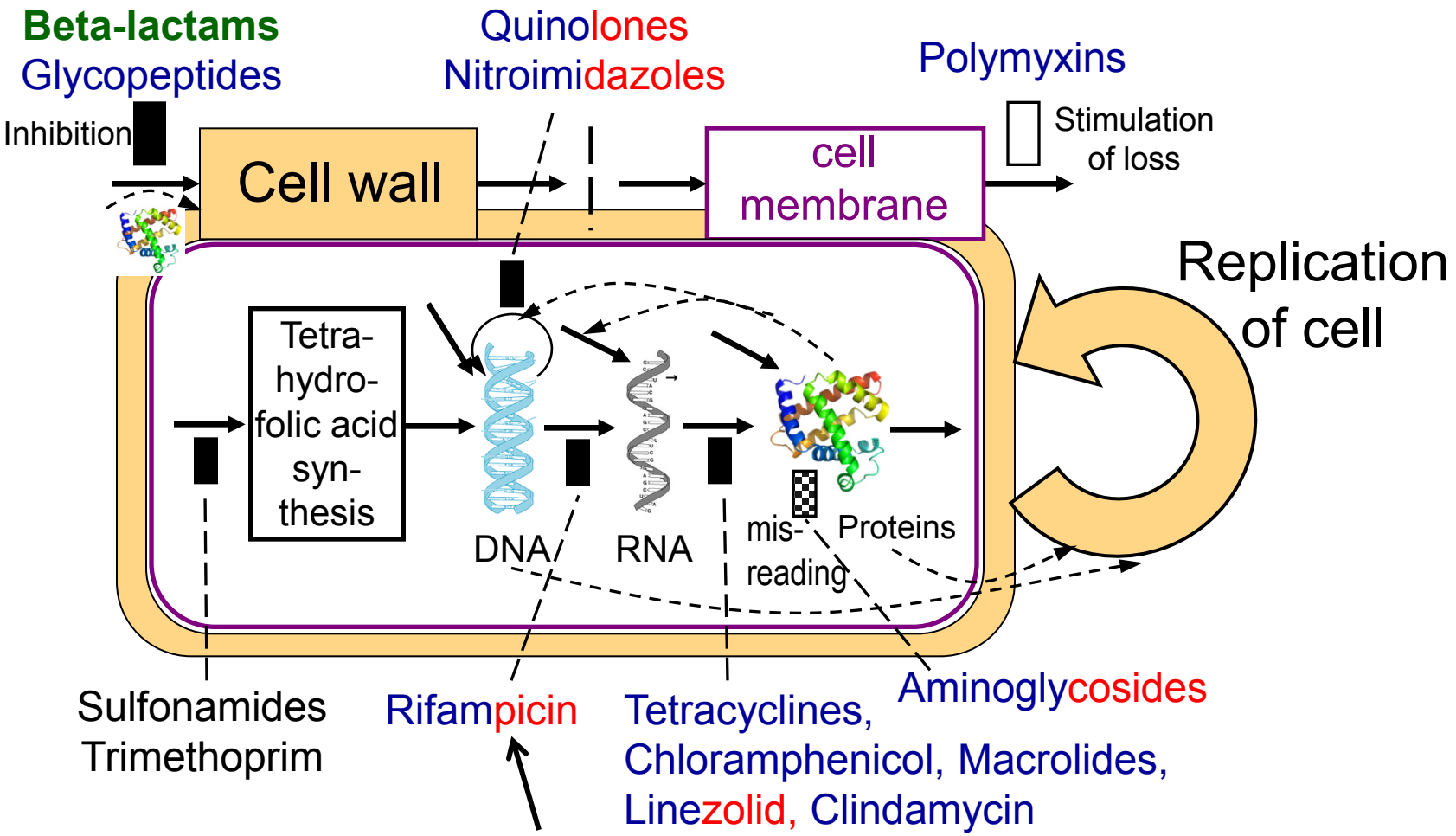
1. Pre-existing resistant bacteria present in a high initial inoculum.
2. *De novo* formation of resistant mutants during long therapy or due to error prone replication.
3. Phenotypic tolerance of bacteria at the infection site (CSF, CF / mucus).
4. Sequestered infection sites.
5. Immuno-compromised patients.



Sisyphos

by Franz von Stuck, 1920

Best PK/PD index: T>MIC, AUC/MIC, C_{max}/MIC

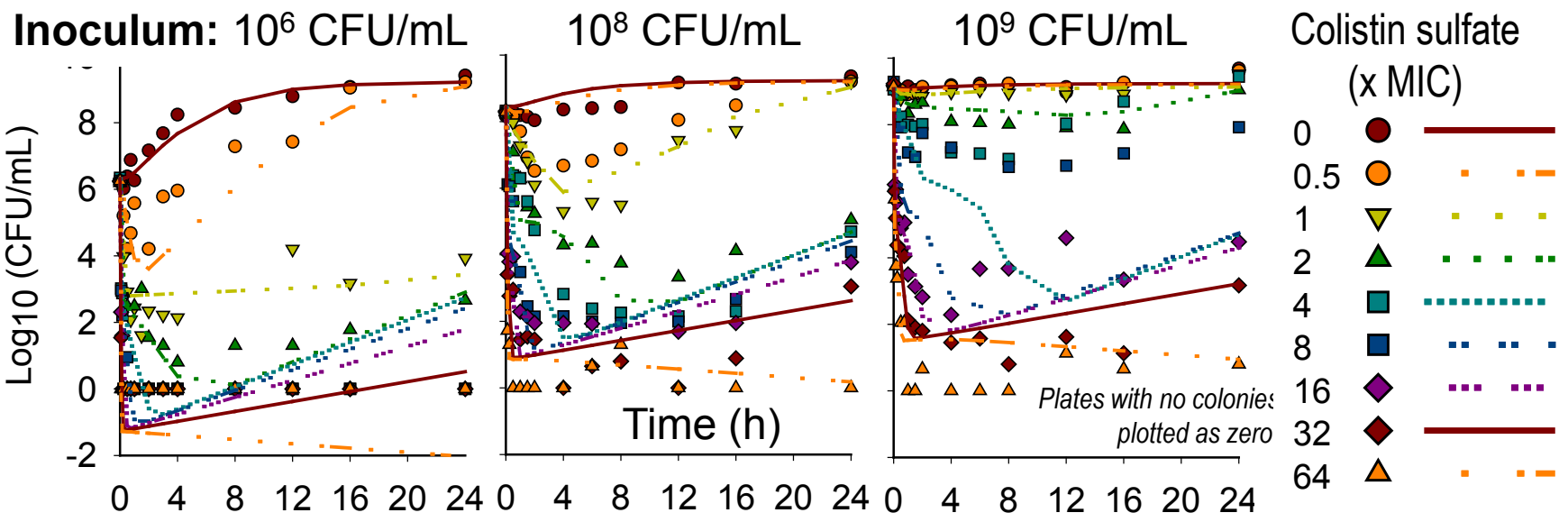


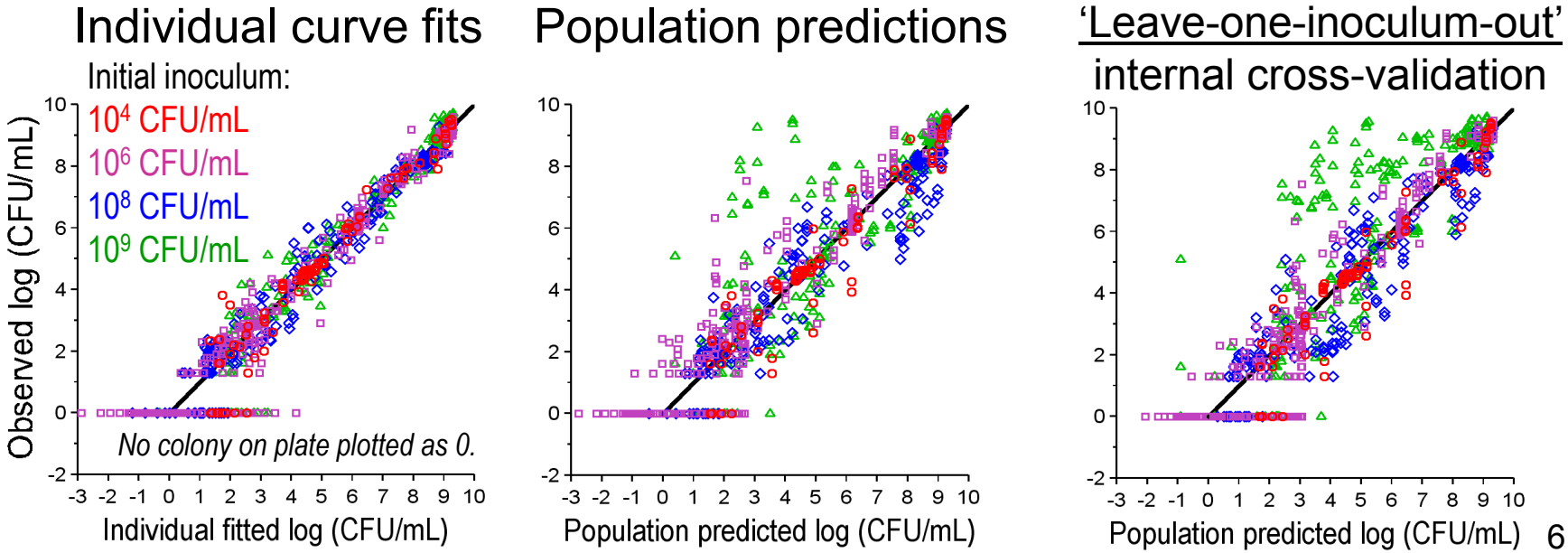
PK/PD indices for cell kill and for prevention of resistance differ within the same drug!

Ambrose PG et al. CID 2007, 44:79-86.
Gumbo T et al. AAC 2007, 51:3781-8.
Louie A et al. AAC 2008, 52: 2486-96.

Rapid killing and inoculum effect of colistin *in vitro*

Inoculum effect of colistin vs. *P. aeruginosa* PAO1





Bulitta JB et al. Antimicrob Agents Chemother, 2010 March.

Funding : R01AI079330, NIAID.

Structural model for colistin vs. *P. aeruginosa*

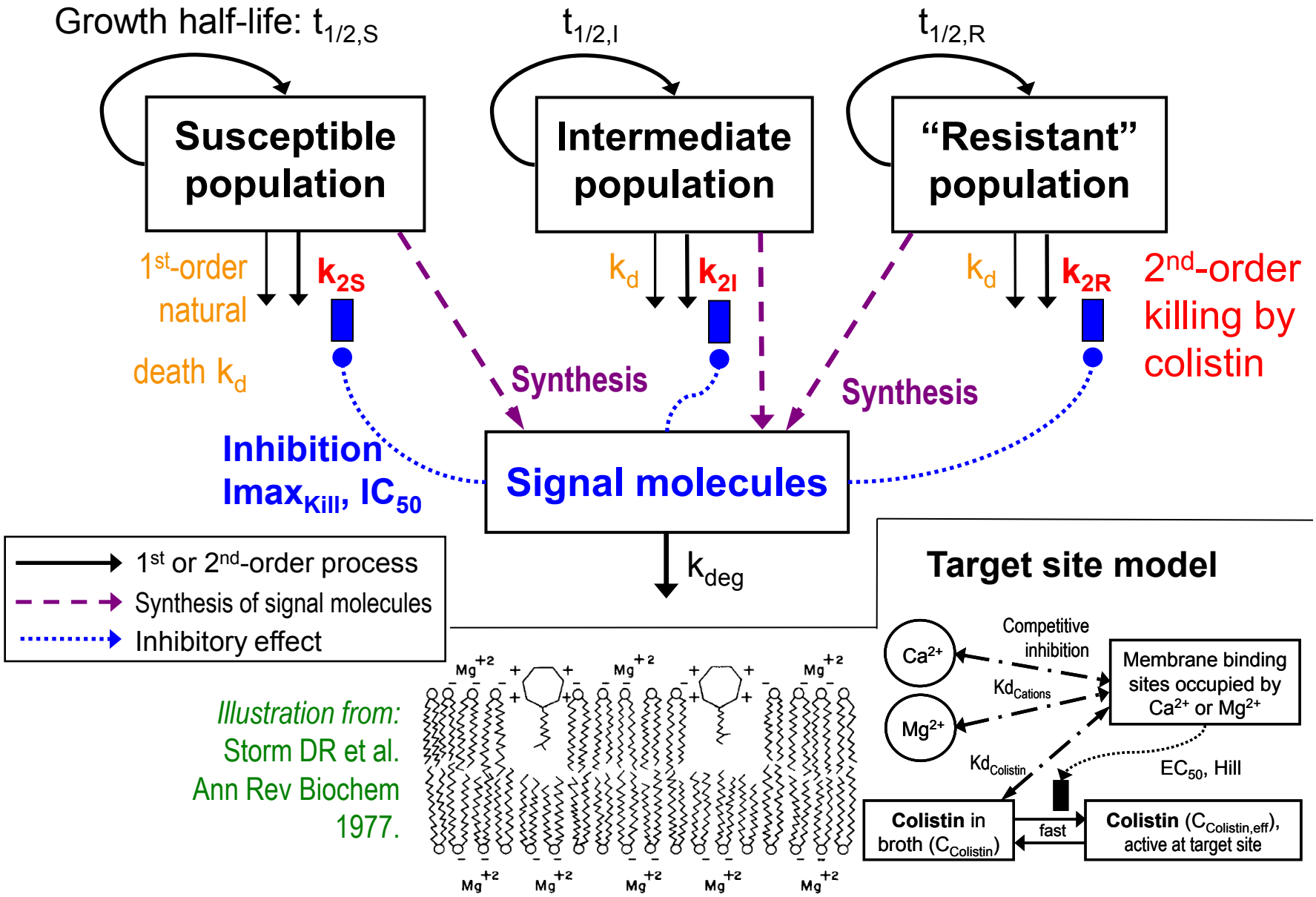


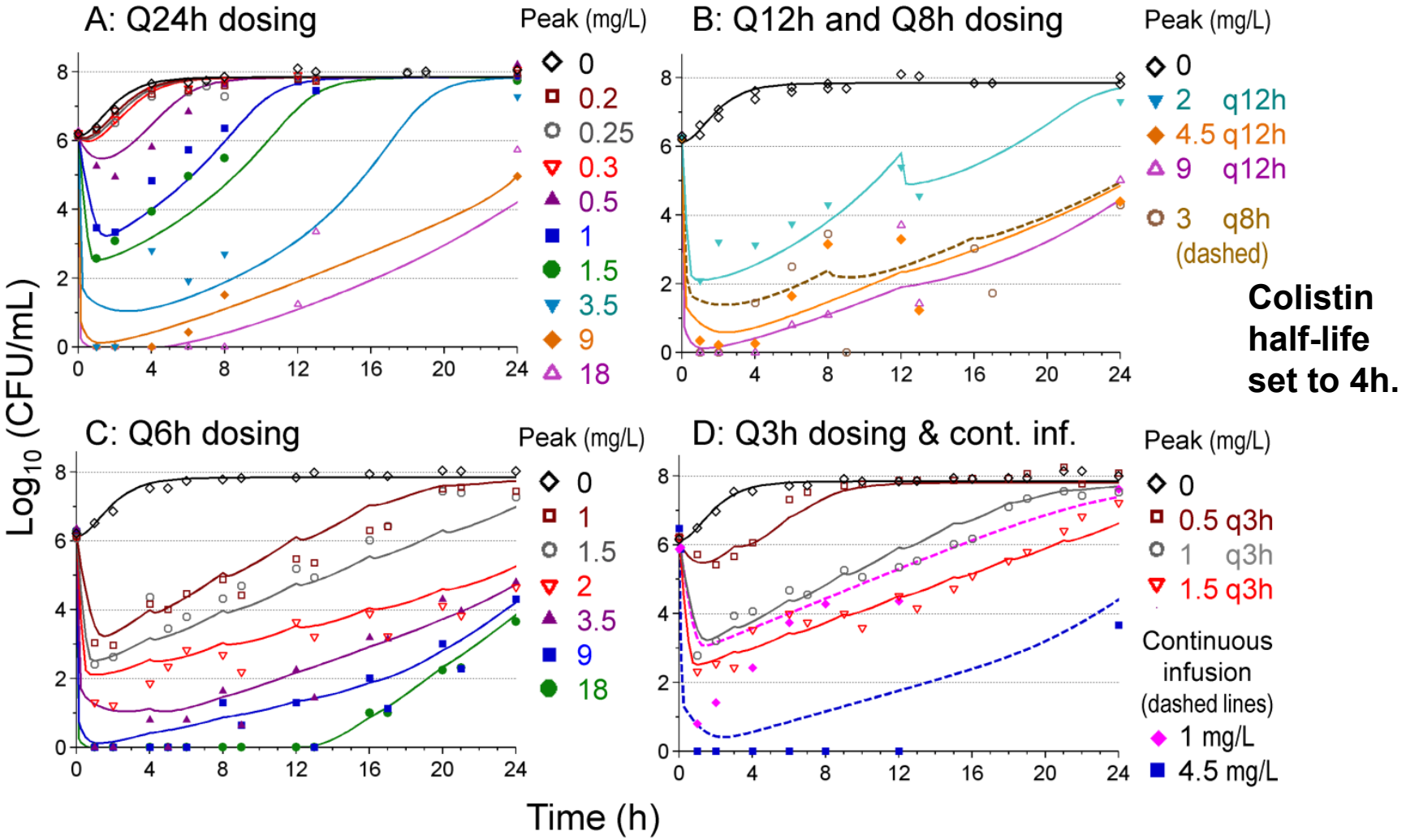
Illustration from:
Storm DR et al.
Ann Rev Biochem
1977.

Bulitta JB et al. Antimicrob Agents Chemother, 2010 March.

Funding : R01AI079330, NIAID.

Adaptive resistance to colistin and inter-conversion of sub-populations

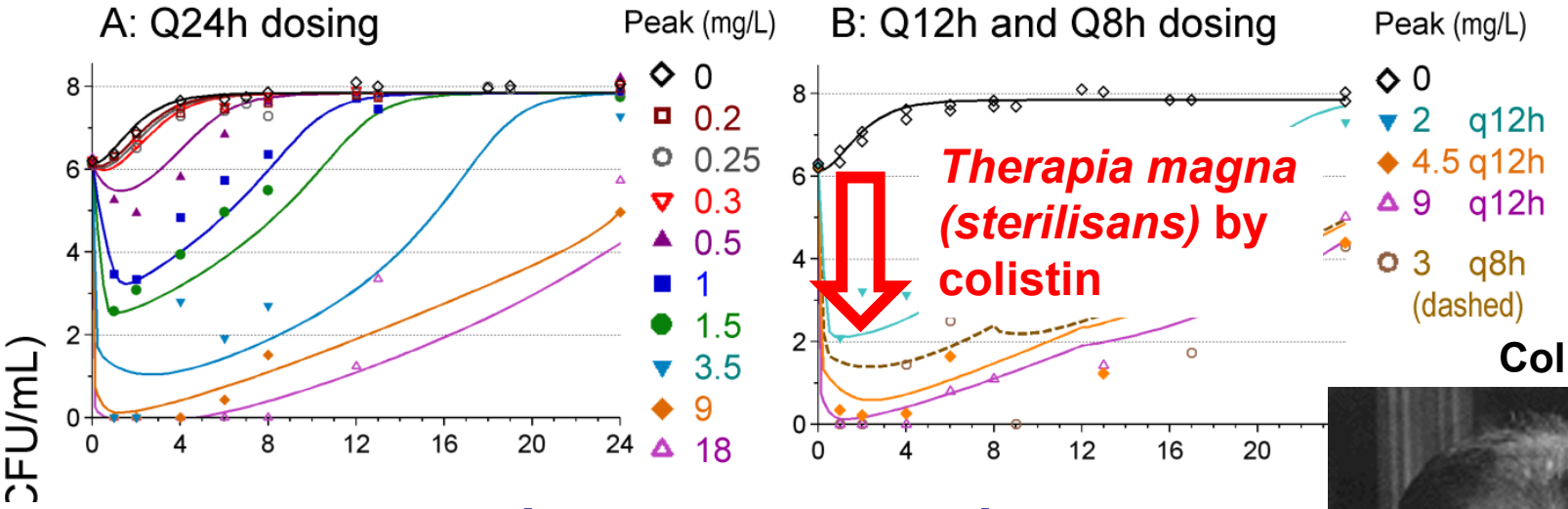
Translation to 1-compartment infection model: Colistin vs. *P. aeruginosa* ATCC 27853



Data: Bergen PJ et al., ICAAC 2008, A-1671. Modeling: Bulitta JB et al., American Conf. on Pharmacometrics, 2009.

Funding : R01AI079330, NIAID.

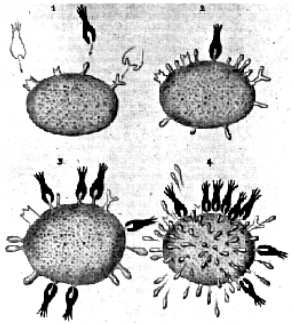
Translation to 1-compartment infection model: Colistin vs. *P. aeruginosa* ATCC 27853



Paul Ehrlich (1854 - 1915)

Therapia magna sterilisans:
Eradication therapy with ONE large dose.

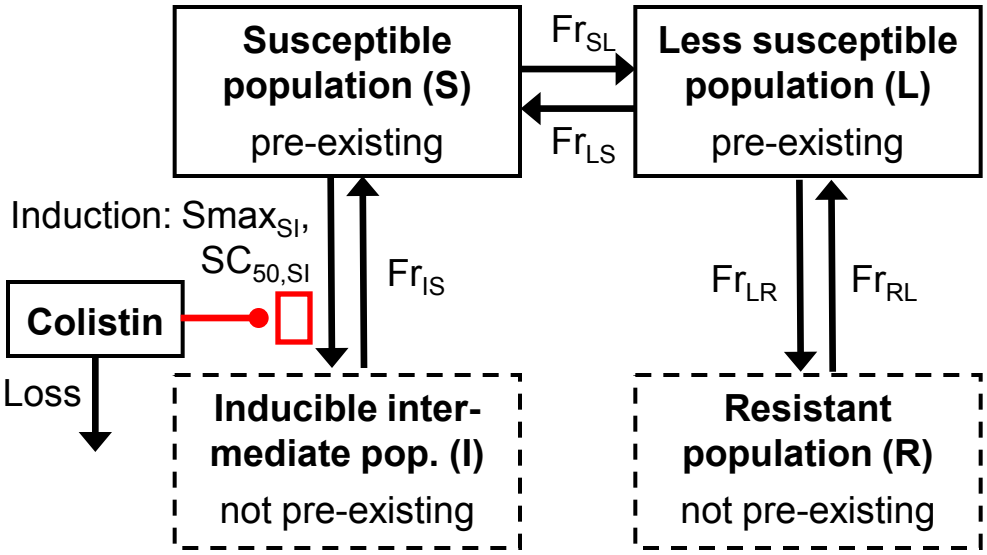
Therapia fractionata sterilisans:
Eradication therapy with fractionated doses.



Colistin

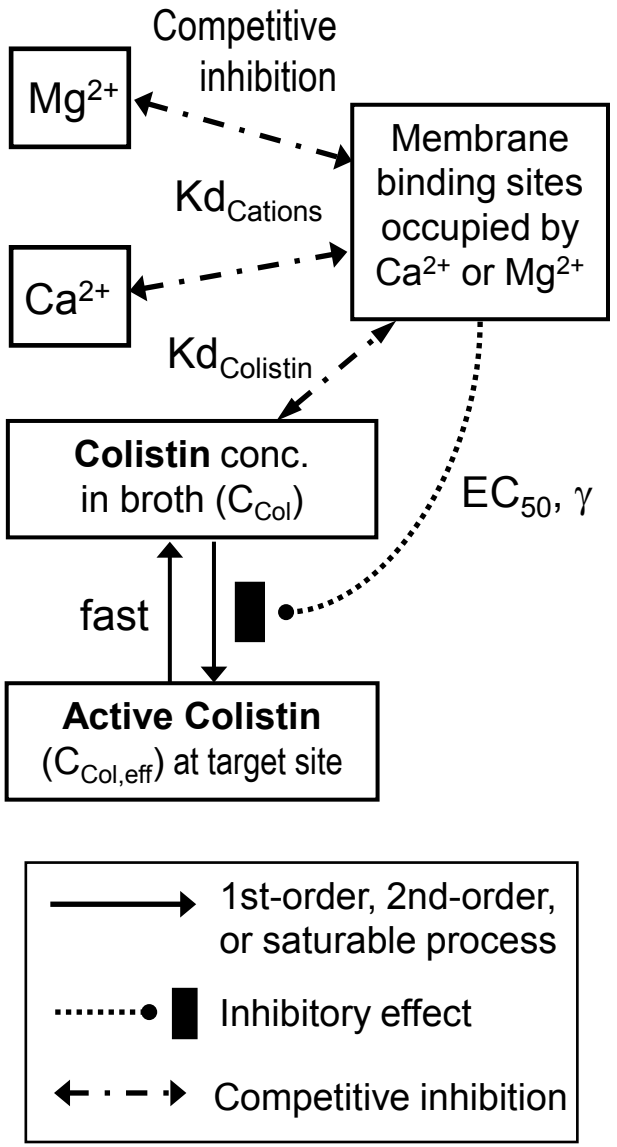
Mechanism-based model for colistin vs. *P. aeruginosa* ATCC 27853

Sub-population dynamics model
with four sub-populations; formation of one intermediate sub-population is induced by colistin



Bulitta JB et al., American Conf. on Pharmacometrics, 2009.
Bergen PJ et al., ICAAC 2008, A-1671.

Target site model



Funding : R01AI079330, NIAID.

Mathematical modeling methods

- Nonlinear mixed-effects modeling using the state-of-the-art Monte Carlo Parametric Expectation Maximization (MC-PEM) algorithm in S-ADAPT (version 1.56) parallelized on a computer cluster or pooled analysis in NONMEM VI.
- LSODA differential equation solver that can handle both stiff and non-stiff systems.
- Life-cycle model [1] to describe bacterial replication.
- All viable counts (including plates with no colonies) for each antibiotic alone and for the combination fitted simultaneously.
- Additive error on log-scale for CFU counts ≥100 CFU/mL. Low CFU counts were fit on linear scale as number of colonies per plate. Poisson error was included for these low colony counts.

1: Bulitta et al. Antimicrob Agents Chemother 2009, 53:46-56.
2: Bulitta & Yang et al. Antimicrob Agents Chemother 2010 Mar 8.

Parameter estimates from nonlinear mixed-effects modeling (S-ADAPT) and a pooled fit (NONMEM)

Parameter	Symbol	Unit	Estimate (%SE)		5-95% percentile from leave 20% out cross-validation
			NONMEM	S-ADAPT	
Log_{10} (Initial inoculum)	Log_{10} CFUo		6.14 (3.9%)	6.16 (2.8%)	6.14 [6.12 - 6.16]
Half-life of growth lag-time	Ln(2) / k_{lag}	min	31.5 (60%)	26.8 (13%)	31.7 [22.9 - 41.3]
Mean generation time at low signal molecule conc.	MTT_{12} = k_{12}^{-1}	min	20.5 (12%)	23.5 (22%)	20.5 [17.0 - 25.5]
Doubling rate constant	k_{21}	h^{-1}	50 (fixed)	50 (fixed)	50 (fixed)
Maximum population size	CFU_{max}	CFU/mL	7.93 (0.9%)	7.99 (0.8%)	7.94 [7.90 - 7.99]
Ratio of transfer rate constant (k_{12}) from state 1 to state 2 relative to the susceptible pop.					
for less susceptible population	$frc_{12,L}$		0.237 (13%)	0.306 (32%)	0.242 [0.205 - 0.992]
for resistant population	$frc_{12,R}$		1 (fixed)	1 (fixed)	1 (fixed)
for inducible intermediate pop.	$frc_{12,I}$		1 (fixed)	1 (fixed)	1 (fixed)
Second order killing rate constants relating colistin (base) concentrations at the target site to the rate of killing					
for susceptible population	k_{2S}	L/(mg·h)	30.1 (12%)	27.8 (34%)	29.3 [26.4 - 43.8]
for less susceptible population	k_{2L}	L/(mg·h)	0.0689 (16%)	0.0591 (49%)	0.063 [0.033- 0.095]
for resistant population	k_{2R}	L/(mg·h)	0 (fixed)	0 (fixed)	0 (fixed)
for inducible intermediate pop.	k_{2I}	L/(mg·h)	1.03 (16%)	0.969 (63%)	1.04 [0.653 - 1.36]
Log_{10} fraction of cells converting from one population to another during one growth cycle					
from population L to S	Log_{10} Fr_{LS}		-2.78 (26%)	-2.83 (29%)	-2.83 [-8.73 to -0.46]
from population R to L	Log_{10} Fr_{RL}		-0.512 (10%)	-0.551 (13%)	-0.52 [-0.88 to -0.47]
Log_{10} (Fr_{SL} / Fr_{LS})			-6.58 (2.6%)	-7.28 (9.0%)	-6.60 [-7.27 to -6.06]
Log_{10} (Fr_{LR} / Fr_{RL})			-5.02 (23%)	-5.00 (7.9%)	-4.98 [-11.9 to -4.28]
from population I to S	Log_{10} Fr_{IS}		-0.493 (5.1%)	-0.550 (26%)	-0.49 [-0.57 to -0.44]
Maximum fraction of cells converting from pop. S to I	Log_{10} $Smax_{SI}$		-0.364 (66%)	-0.291 (63%)	-0.364 [-0.504 to -0.00364]
Colistin (base) conc. causing with 50% of $Smax_{SI}$	$SC_{50,SI}$	mg/L	50 (fixed)	50 (fixed)	50 (fixed)

→ **Both estimation methods (programs) yielded consistent results.**

Bulitta JB et al., American Conf. on Pharmacometrics, 2009.

Bergen PJ et al., ICAAC 2008, A-1671.

Sometimes, single agent therapy just can't get the "job done"

WHAT ABOUT COMBINATION THERAPY AND PREVENTION OF RESISTANCE?

T>MIC, AUC/MIC, C_{max}/MIC

How can these indices be applied to optimize drug combinations?

Case I:
Drug A: AUC/MIC
Drug B: AUC/MIC

Combination:
Sum of AUC/MIC?

Quo vadis?

Case II:
Drug A: T>MIC
Drug B: AUC/MIC

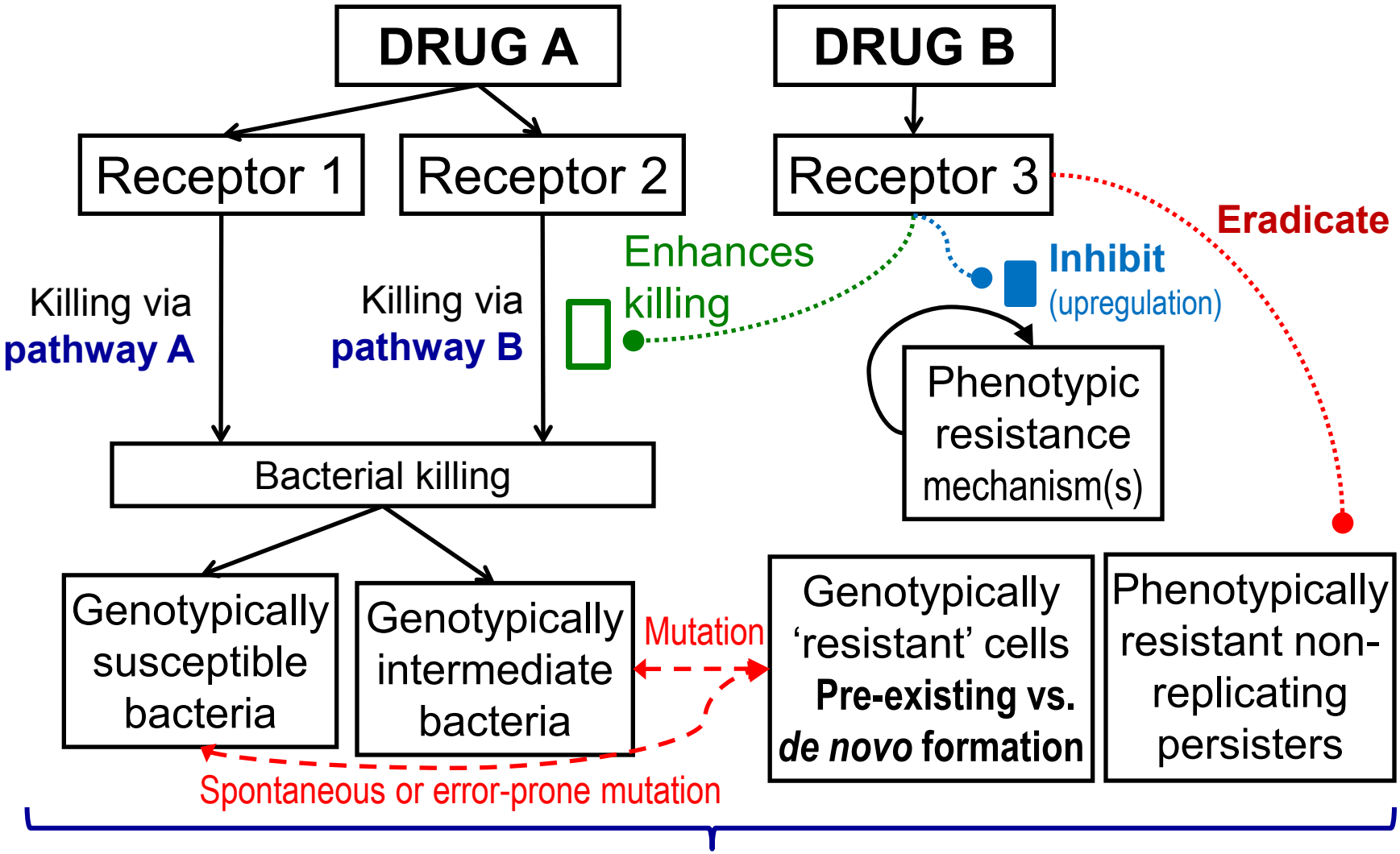
Combination:
???

→ **Applying PK/PD indices to combination therapy is difficult.**

→ **Many antibiotics bind to more than one receptor.**

→ **Mechanistic knowledge about the relationship between receptor occupancy and bacterial responses (incl. resistance) is critical.**

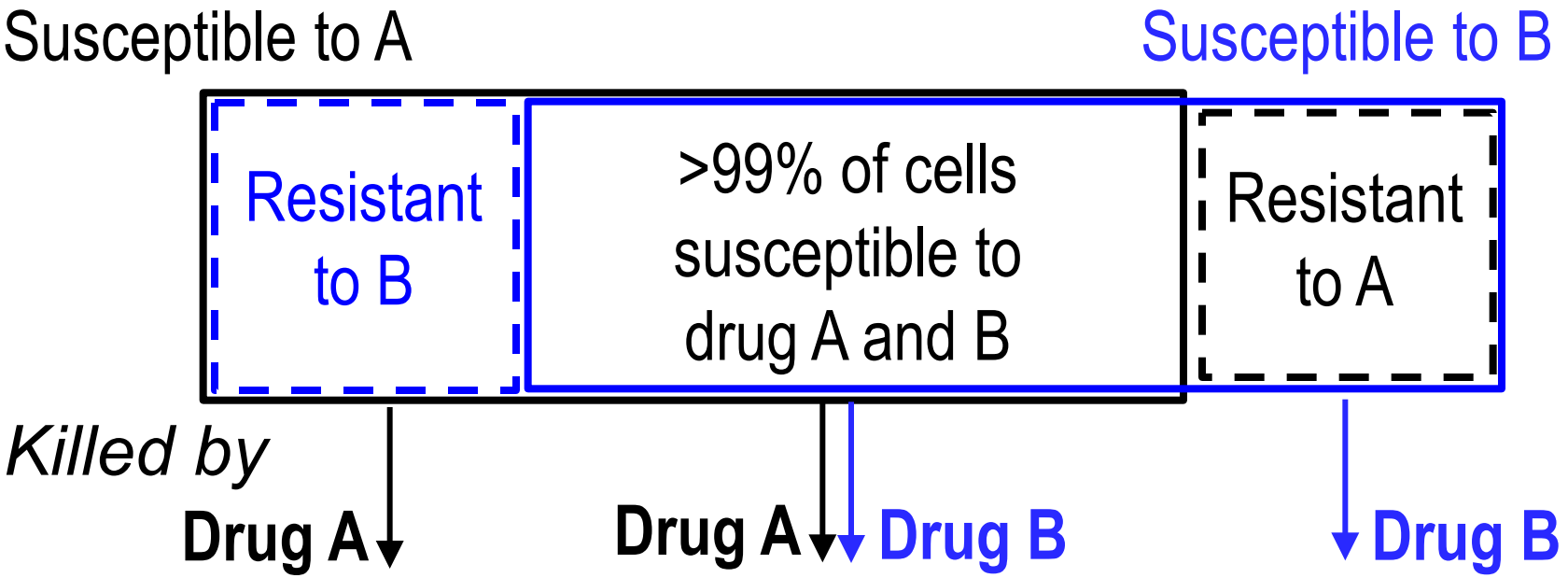
Unique Receptor Occupancy Patterns can be used to Rationally Optimize Combination Chemotherapy



Mechanism-based modeling integrates time course & probabilities

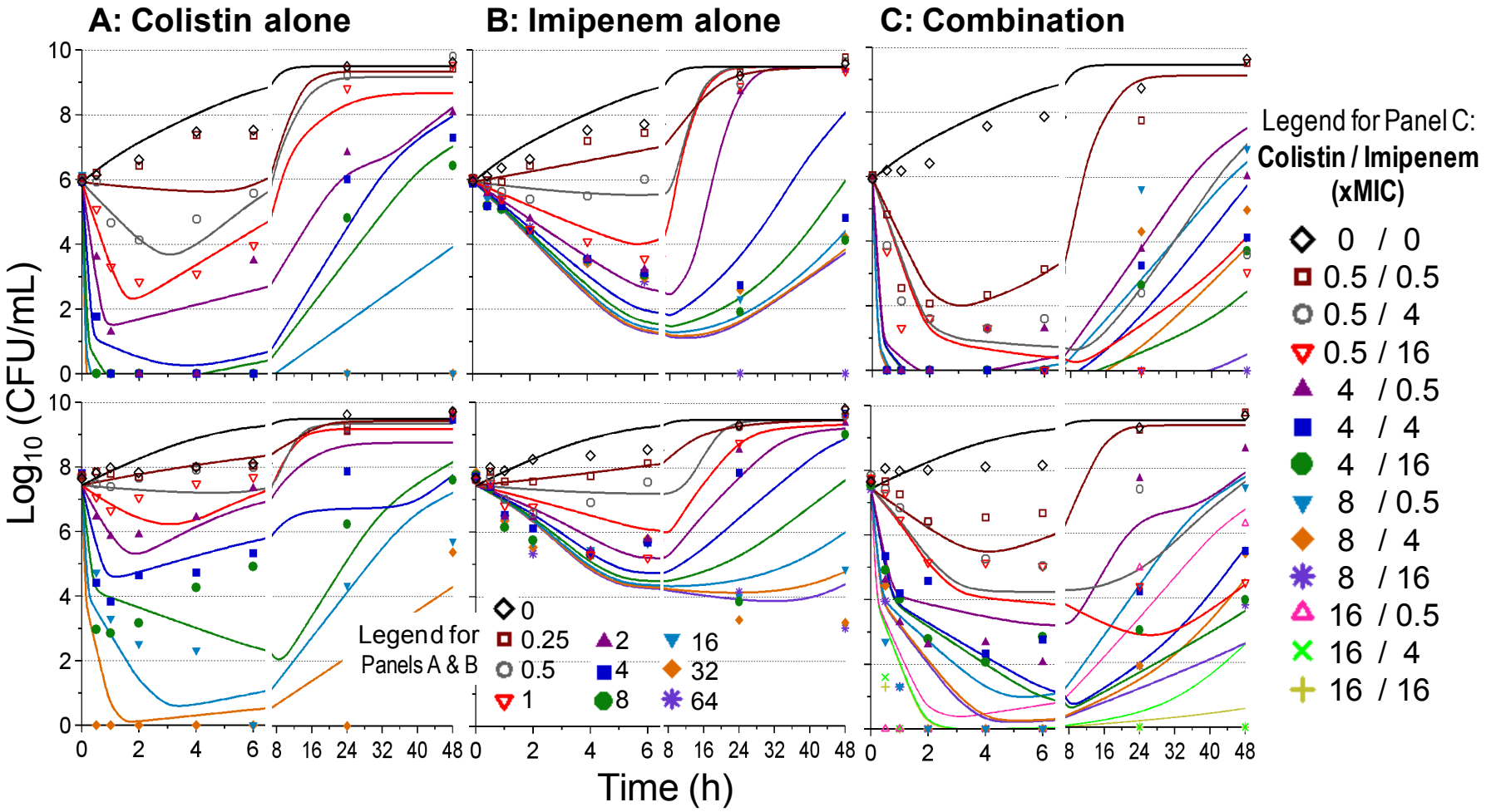
Sub-population synergy

Drug A kills the resistant sub-population of drug B & vice versa.



Example of sub-population synergy: Imipenem & colistin vs. *P. aeruginosa*
Bergen PJ et al., ICAAC 2009, poster A1-575.

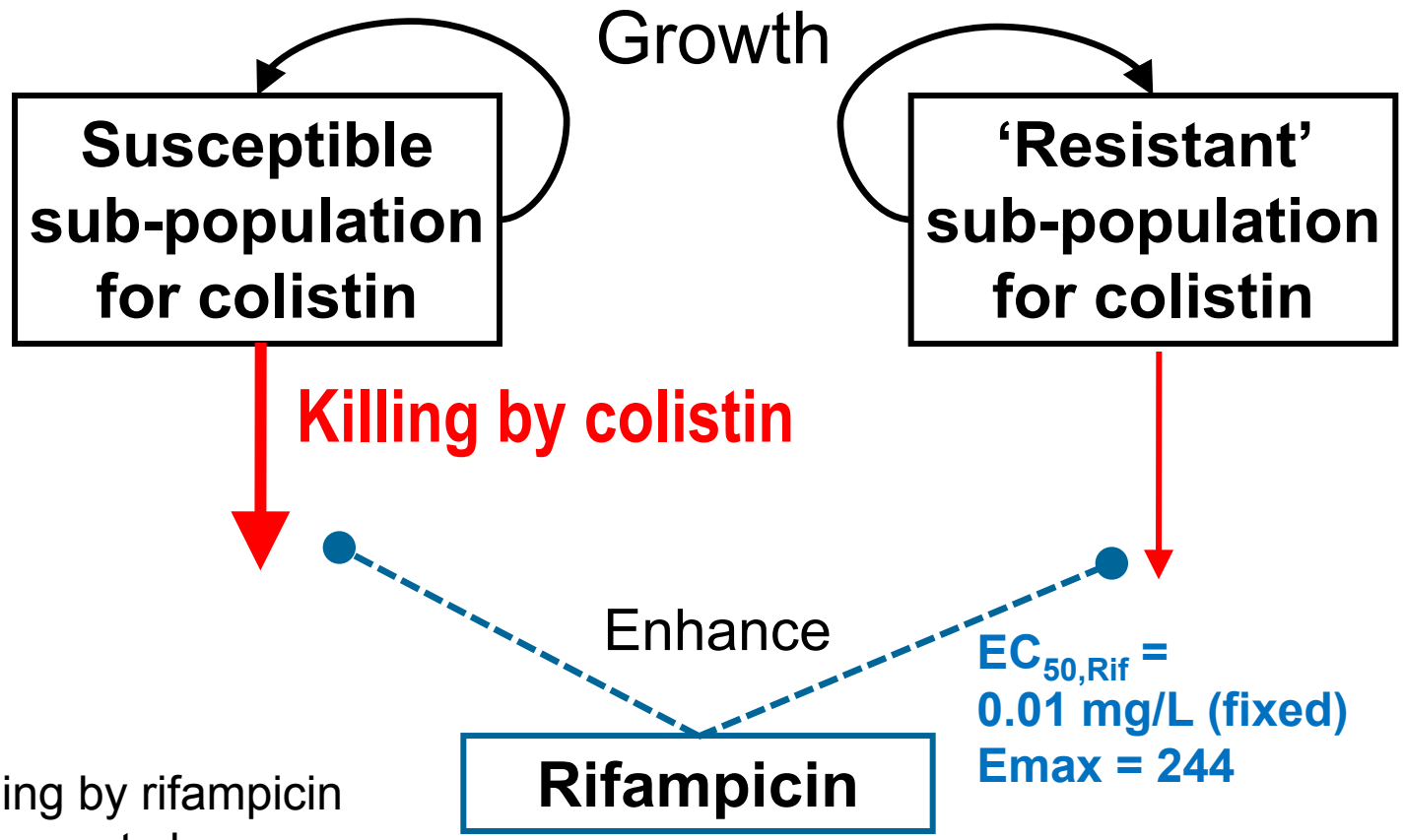
Colistin and imipenem alone & in combination against *Pseudomonas aeruginosa* at two initial inocula



Bergen PJ et al., ICAAC 2009, poster A1-575.

Funding : R01AI079330, NIAID.

Mechanism-based Synergy for Antibacterial Combinations



Killing by rifampicin alone not shown in the diagram.

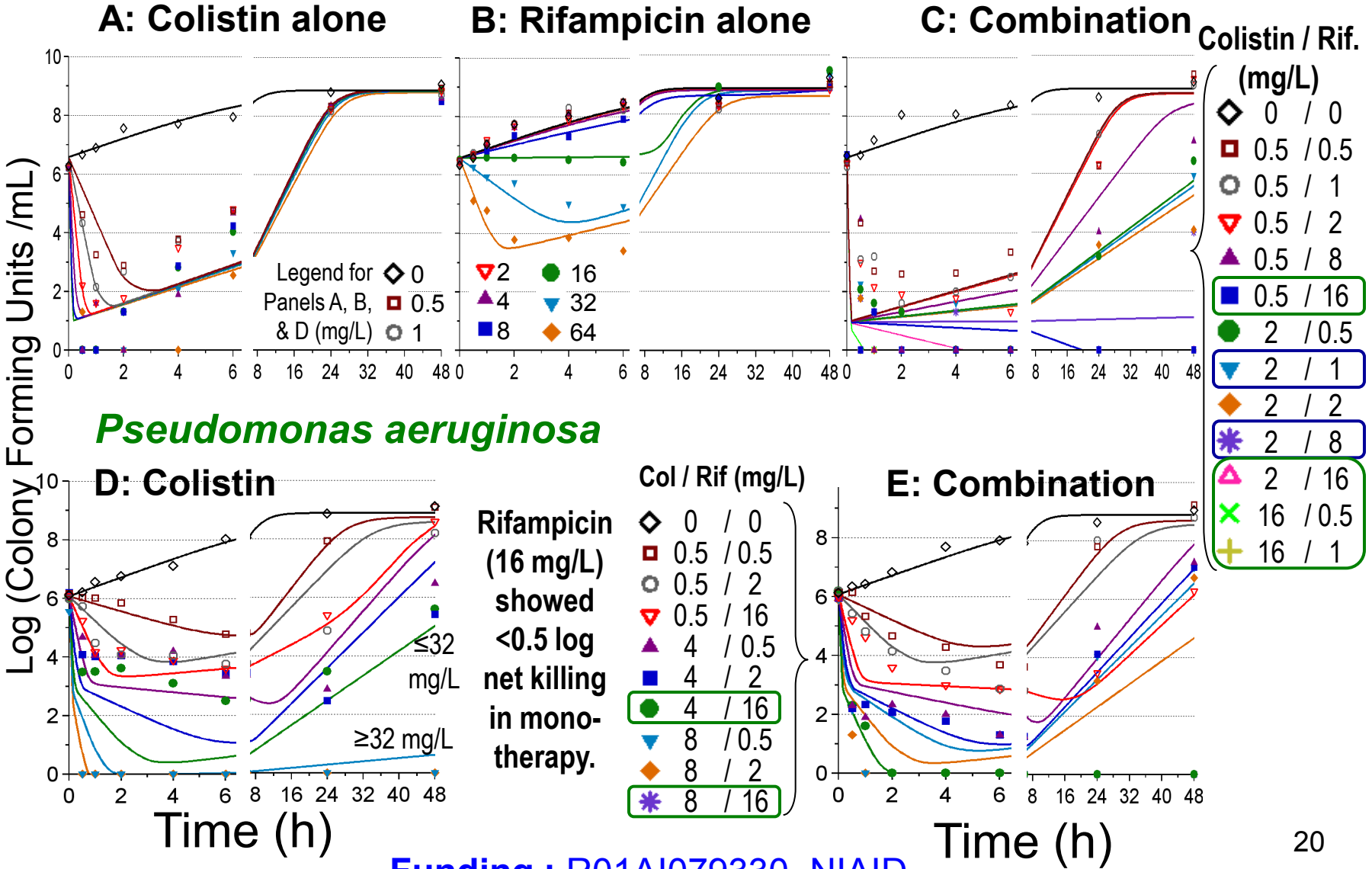
Funding :
R01AI079330, NIAID.

Bulitta JB et al., ICAAC 2009, poster A1-573.

Li J et al., ICAAC 2009, poster A1-574.

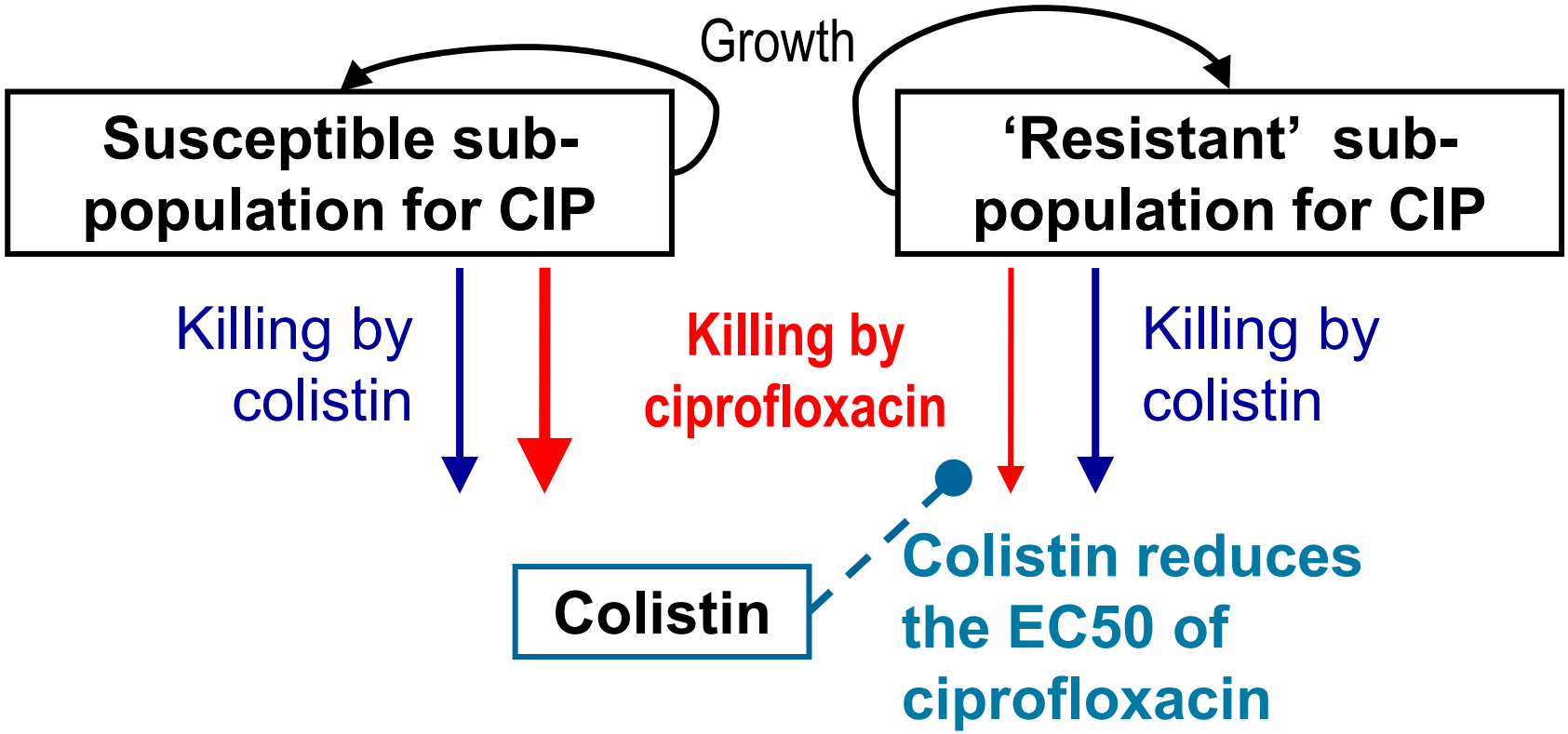
Rifampicin Enhances Rate of Killing by Colistin – time-kill studies

Acinetobacter baumannii



Funding : R01AI079330, NIAID.

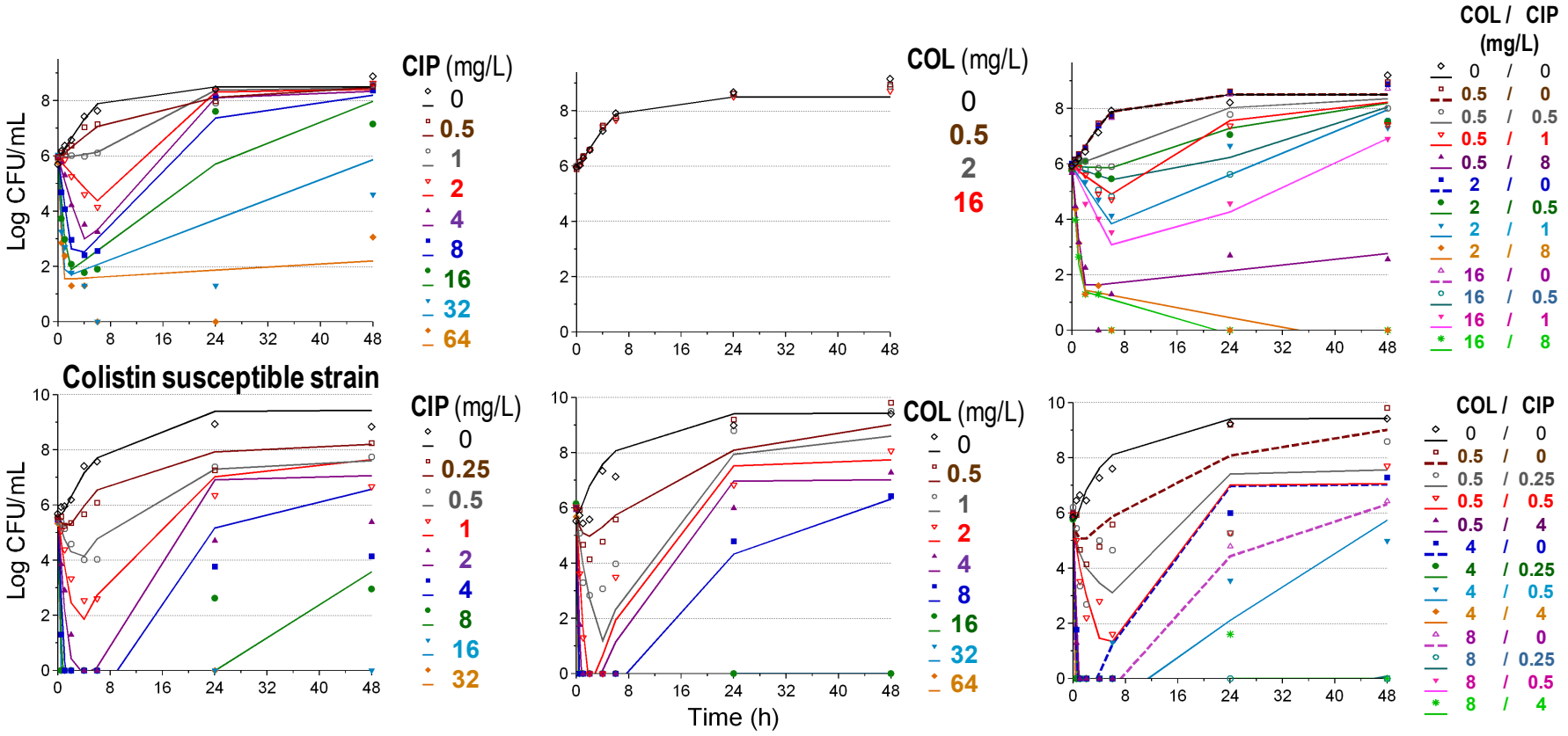
Mechanistic synergy: Colistin increases the effective intracellular concentration of ciproflox. potentially via interference with efflux transporters



Bulitta JB et al., ECCMID 2009.

Funding :
R01AI079330, NIAID.

Curve Fits: Colistin + ciprofloxacin vs. *P. aeruginosa*



Bulitta JB et al., ECCMID 2009.

Funding :
R01AI079330, NIAID.

Transition to man

In vivo protein binding – a truly exciting story for colistin

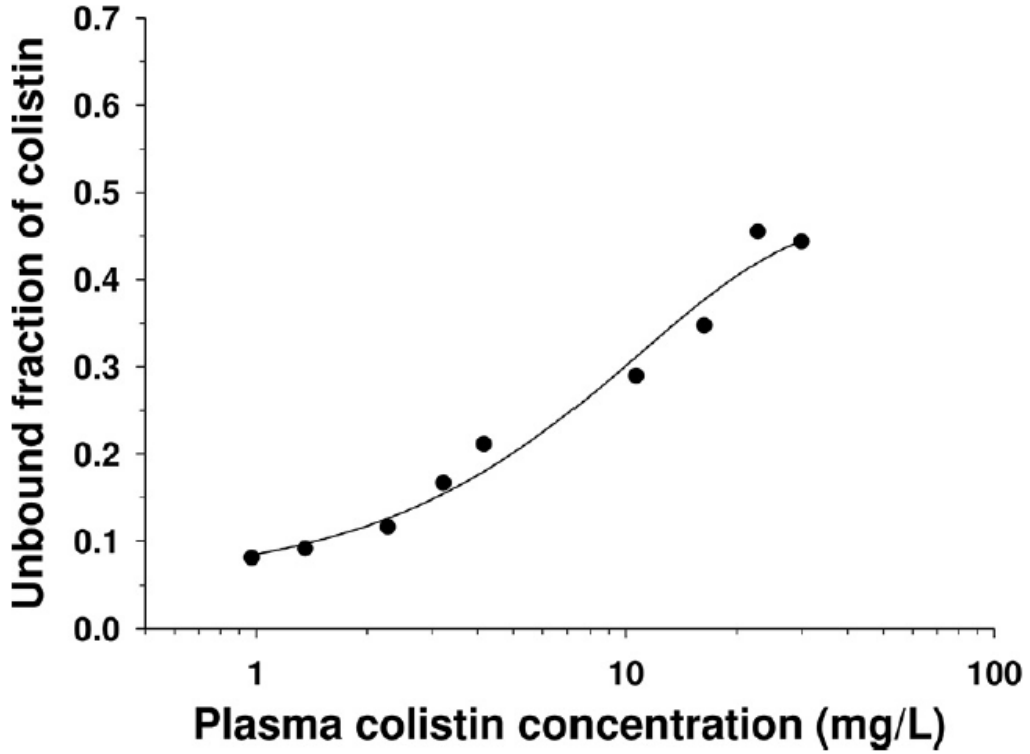


FIG. 1. f_u of colistin against the end-dialysis plasma concentration of colistin in the equilibrium dialysis study. The solid line is a four-parameter model fit obtained by nonlinear least-squares regression ($R^2 = 98\%$) of the experimental data: $f_u = -3.45 + 3.91/(1 + \exp\{-[x - (-21.41)]\}/10.09)$, where x is the plasma colistin concentration.

Dudhani RV. et al. Antimicrob Agents Chemother 2010; 54: 1117-1124

PK of colistin (base) in mice

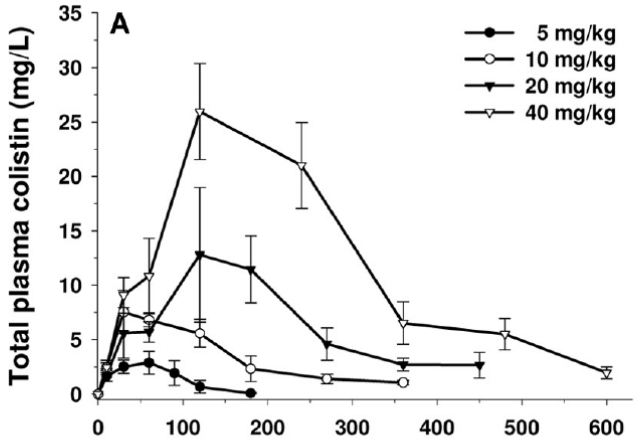


TABLE 1. Values of pharmacokinetic parameters for unbound colistin following subcutaneous administration of single doses (5 to 40 mg/kg) in neutropenic infected mice

Parameter	Result for a single subcutaneous dose of:			
	5 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg
C_{max} (mg/liter)	0.31	1.44	3.29	8.89
T_{max} (min)	60	30	120	120
V/F (L/kg)	10.1	4.63	4.88	3.27
CL/F (ml/min/kg)	169	46.8	29.2	16.4
$t_{1/2}$ (min)	17.9	68.7	80.2	83.6

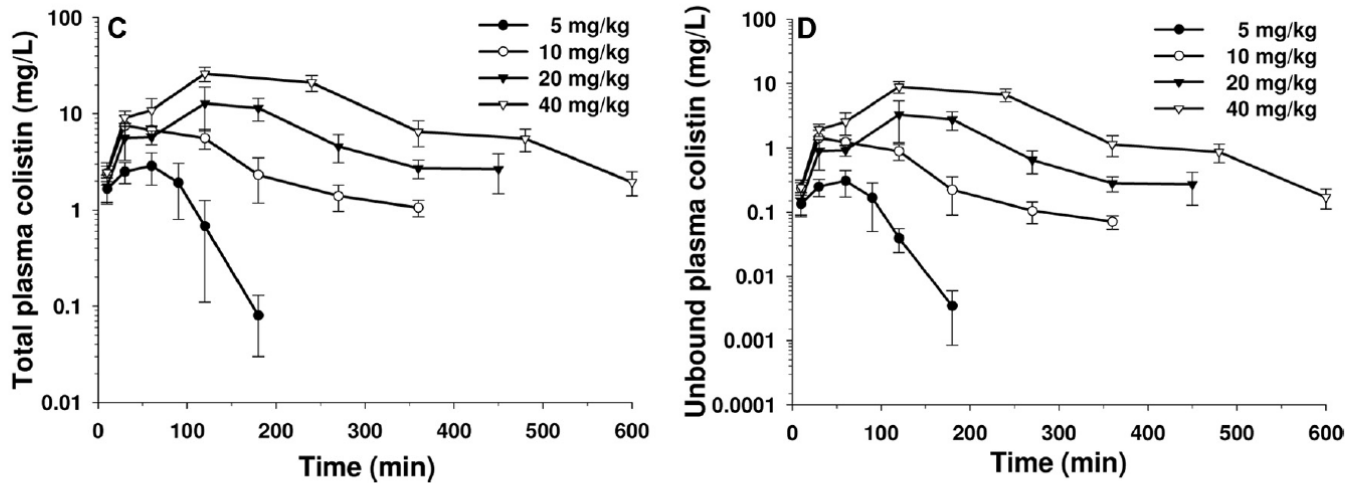
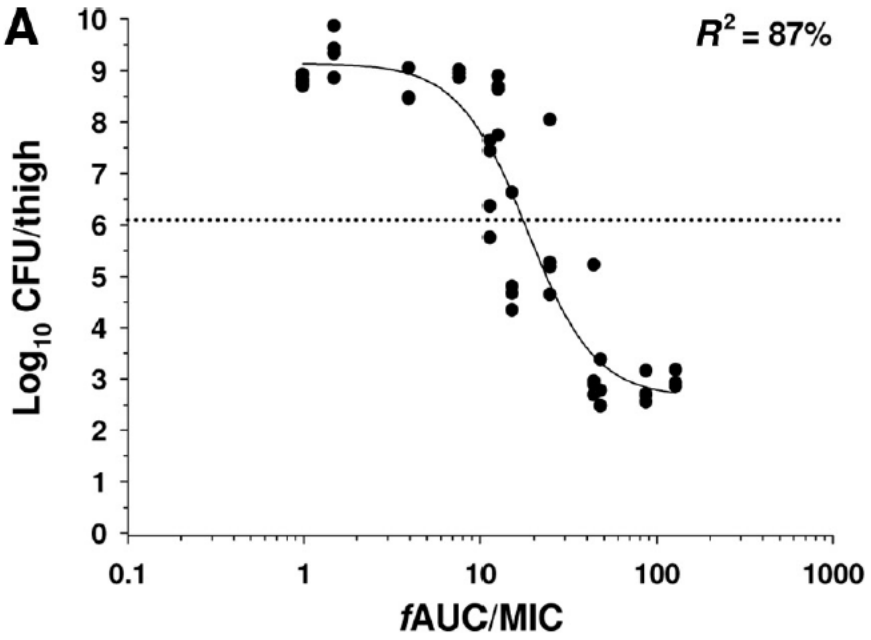
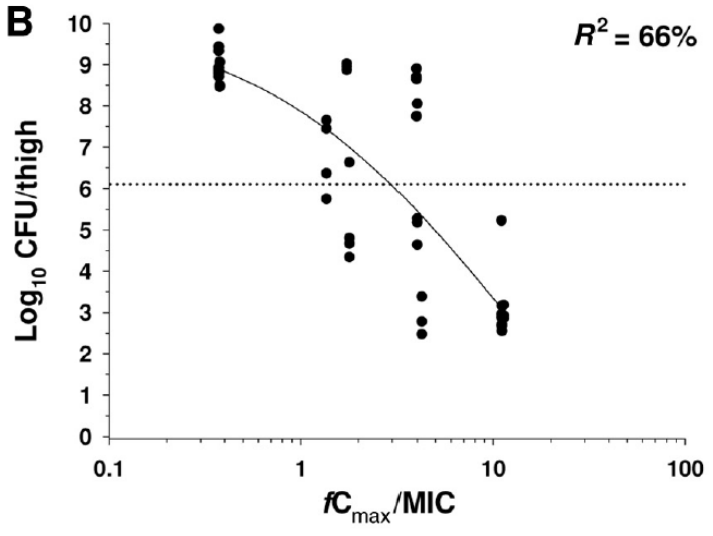


FIG. 2. Total (A) and unbound (B) plasma colistin concentrations versus time after administration of single subcutaneous doses of 5, 10, 20, or 40 mg/kg colistin (sulfate) in neutropenic infected mice. (C and D) Corresponding data on semilogarithmic coordinates. Each symbol represents the mean ± standard deviation for four mice.

Dudhani RV. et al. Antimicrob Agents Chemother 2010; 54: 1117-1124

PK/PD indices in neutropenic animals





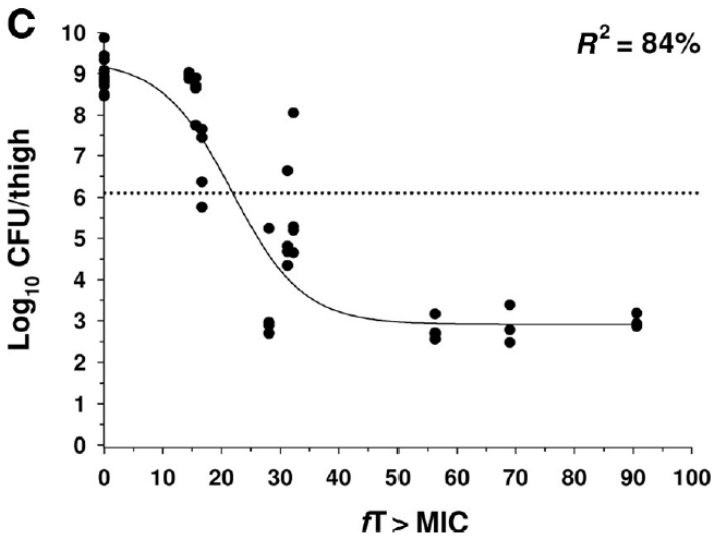


FIG. 3. Relationships for *P. aeruginosa* ATCC 27853 between the log_{10} CFU per thigh at 24 h and the PK/PD indices *f*AUC/MIC (A), fC_{max}/MIC (B), and $fT > MIC$ (C). Each symbol represents the datum from a single thigh. The dotted lines represent the mean bacterial burden in the thighs at the start of treatment.

Dudhani RV. et al. Antimicrob Agents Chemother 2010; 54: 1117-1124

PK/PD parameter estimates in mice

TABLE 2. PK/PD model parameter estimates predicting viable counts at 24 h for the *f*AUC/MIC index for colistin against all three strains of *P. aeruginosa* in the thigh and lung infection models

Model and strain	E_{max} (log_{10} CFU/organ)	E_0 (log_{10} CFU/organ)	EC_{50}	γ
Thigh infection				
ATCC 27853	6.29 (8.2)[a]	8.97 (2.9)	18.8 (11.8)	2.36 (23.1)
PAO1	5.97 (6.1)	8.34 (1.9)	22.7 (12.6)	1.51 (16.2)
19056[b]	6.23 (10.1)	7.98 (3.0)	19.5 (20.4)	1.13 (24.0)
Lung infection				
ATCC 27853	7.58 (16.1)	9.34 (3.4)	16.8 (48.8)	0.61 (20.2)
PAO1	7.36 (26.1)	8.97 (3.4)	31.7 (87.9)	0.54 (30.0)
19056[b]	6.86 (12.7)	8.85 (2.9)	12.4 (40.0)	0.54 (18.4)

[a] Data in parentheses are the percent relative standard error.
[b] Multidrug-resistant mucoid strain.

Dudhani RV. et al. Antimicrob Agents Chemother 2010; 54: 1117-1124

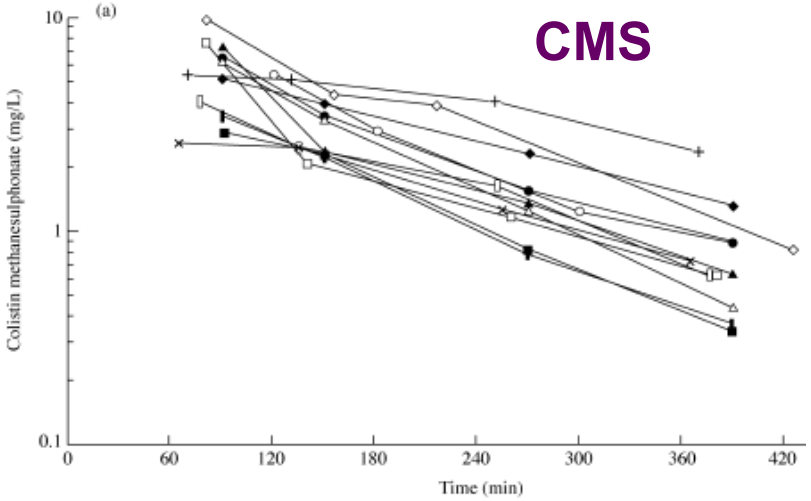
PK/PD index value for certain killing endpoints

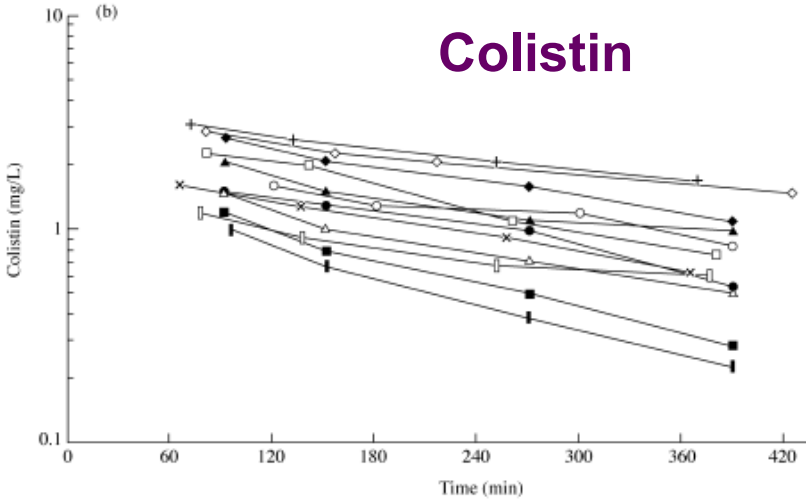
TABLE 3. Target values of colistin *f*AUC/MIC for stasis and 1-, 2-, and 3-$\log_{10}$ kill against all three *P. aeruginosa* strains in the thigh and lung infection models

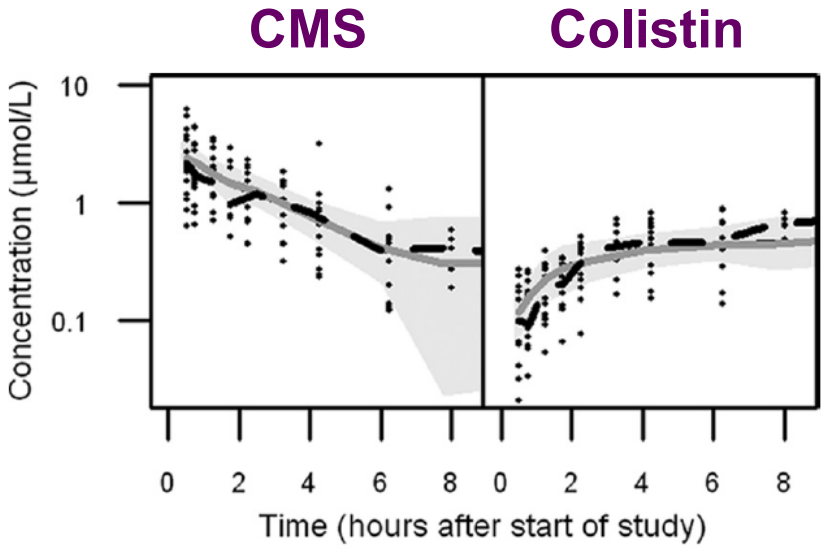
Model and kill effect	Target value of colistin *f*AUC/MIC for strain:		
	ATCC 27853	PAO1	19056
Thigh infection			
Static effect	17.3	14.4	8.34
1-$\log_{10}$ kill	22.7	22.8	15.6
2-$\log_{10}$ kill	31.2	36.1	27.6
3-$\log_{10}$ kill	55.1	66.7	53.3
Lung infection			
Static effect	6.43	5.42	4.07
1-$\log_{10}$ kill	15.6	16.7	12.2
2-$\log_{10}$ kill	37.9	45.9	36.9
3-$\log_{10}$ kill	105	135	141

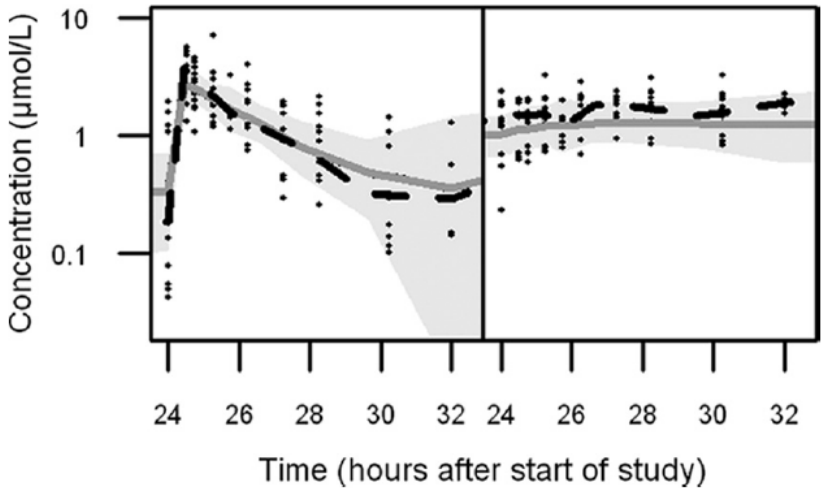
Dudhani RV. et al. Antimicrob Agents Chemother 2010; 54: 1117-1124

Population PK of colistin in CF-patients and in Cystic Fibrosis and Critically ILL patients









Li J et al. JAC 2003; 52: 987-92.

Plachouras D. et al. AAC 2009; 53: 3430-6.

Modeling the impact of the immune system

$$\frac{dX(4)}{dt} = K_{gmax} \times \left(1 - \frac{X(4)}{POPMAX}\right) \times X(4)$$

$$\times 1 - \left(\frac{[AmB]^{H_g}}{C_{50}g^{H_g} + [AmB]^{H_g}}\right)$$

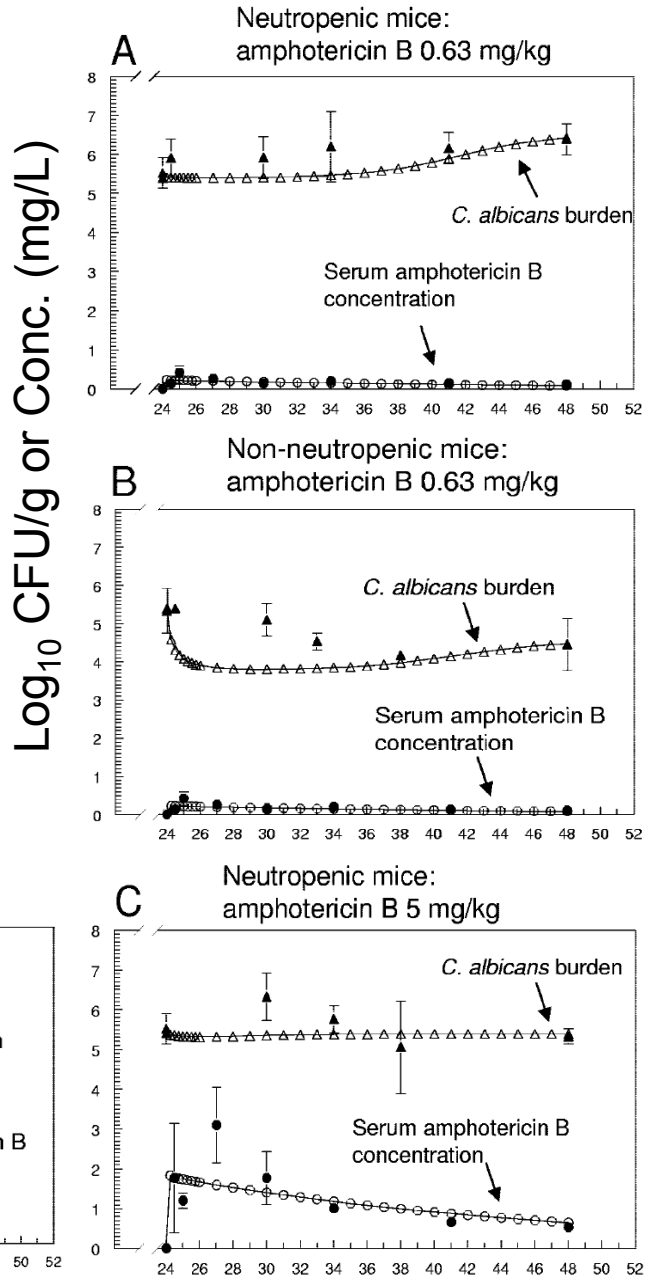
$$- K_{kmax} \times \frac{[AmB]^{H_k}}{C_{50}k^{H_k} + [AmB]^{H_k}} \times X(4)$$

$$- WBCKILL_{max} \times \frac{X(4)}{WBCKILL_{50} + X(4)}$$

$$\times R(1) \times X(4)$$

10^4 to 10^5 CFU/g

Hope WW, Drusano GL, et al. AAC 2007, 51:285-95.



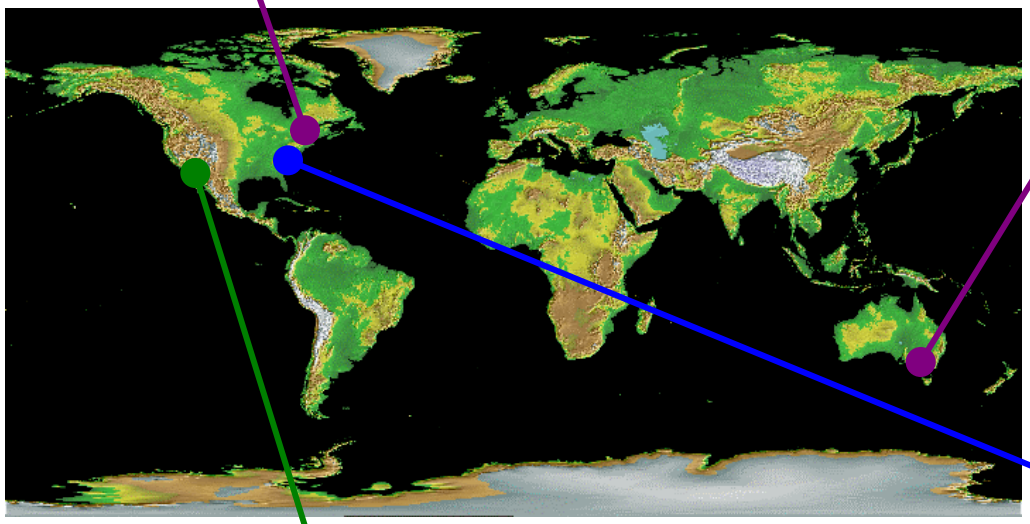
Conclusions

1. Colistin is a very promising component of our armamentarium against MDR gram-negatives.
2. The rapid killing and rapid emergence of resistance to colistin *in vitro* suggests administering a large initial dose of colistin and a short duration of therapy.
3. PK in special patient groups needs to be considered.
4. Synergy in cell kill and prevention of resistance of colistin with a variety of compounds *in vitro* warrants studies *in vivo* and in the hollow fiber system.
5. Rational development of combination regimens with colistin supported by mathematical modeling holds great promise.

A Global Team Approach

Team of Alan Forrest, Brian T. Tsuji (Buffalo, NY, USA) and Jurgen Bulitta (Albany, NY)

Roger Nation's and Jian Li's Team in Melbourne, Australia



Funding: Colistin work supported by R01AI079330 from NIAID, NIH.

And a series of other collaborators, including our colleagues (David Z D'Argenio & Robert J Bauer, et al.) writing the mathematical software tools.