Development and PK Challenges of a Murine Model for *Acinetobacter baumannii* infection

Brian Luna, PhD, and Jürgen Bulitta, PhD

Team Effort

University of Southern California
- Brian Luna, PhD
- Brad Spellberg, MD

University of Florida
- Jürgen Bulitta, PhD
- George Drusano, MD
- Arnold Louie, MD

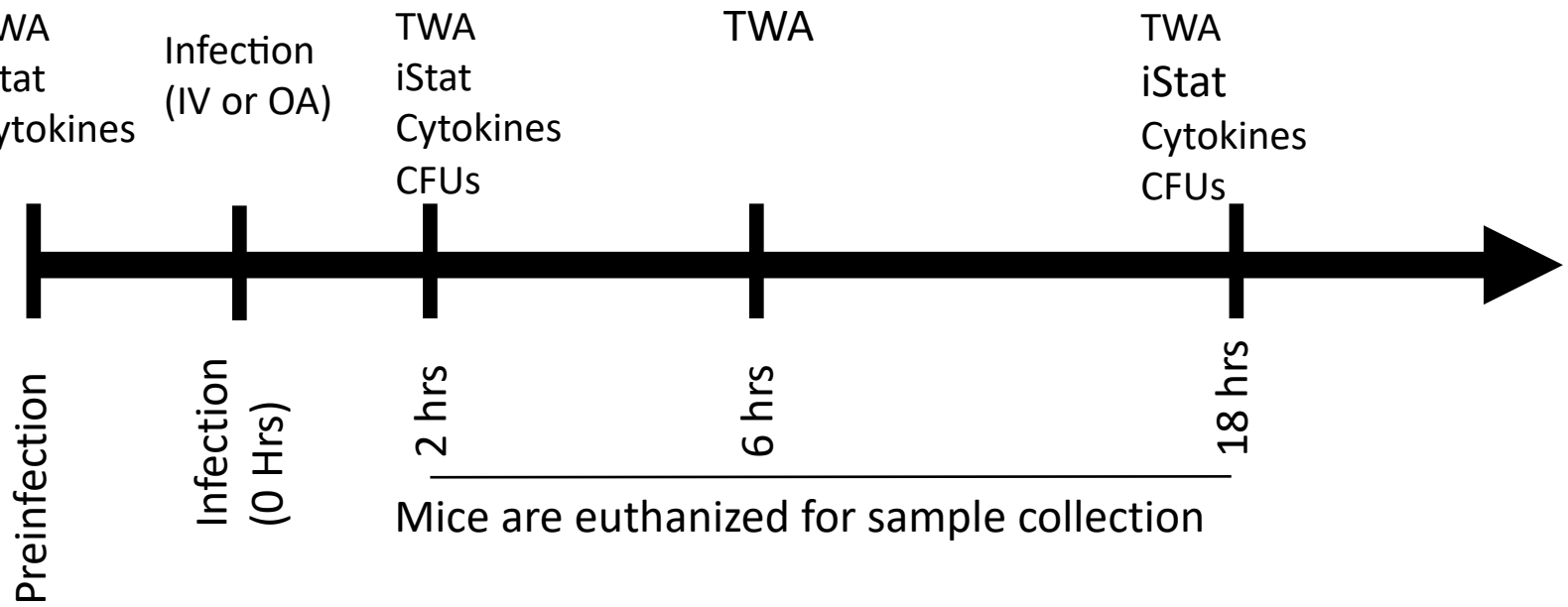
Case Western
- Robert Bonomo, MD

Rationale behind animal chosen

- Mouse model
- Murine models have proven useful historically for the study of host/pathogen interactions and preclinical drug efficacy studies.

NIH workshop review paper: Bulitta JB, Hope WW, Eakin AE, Guina T, Tam VH, Louie A, Drusano GL, Hoover JL. Antimicrob Agents Chemother. 2019; 63. pii: e02307-18. PMCID: PMC6496039.

Study Design



TWA = Temperature, Weight, and Activity Scores
CFUs = Blood (and lung homogenate for OA model)
iStat= blood pH, serum bicarbonate, lactate, and base deficit
Cytokines = Measure blood, lung and ELF compartments.
proinflammatory IL-1B, IL-6, and TNF and the anti-inflammatory IL-10.

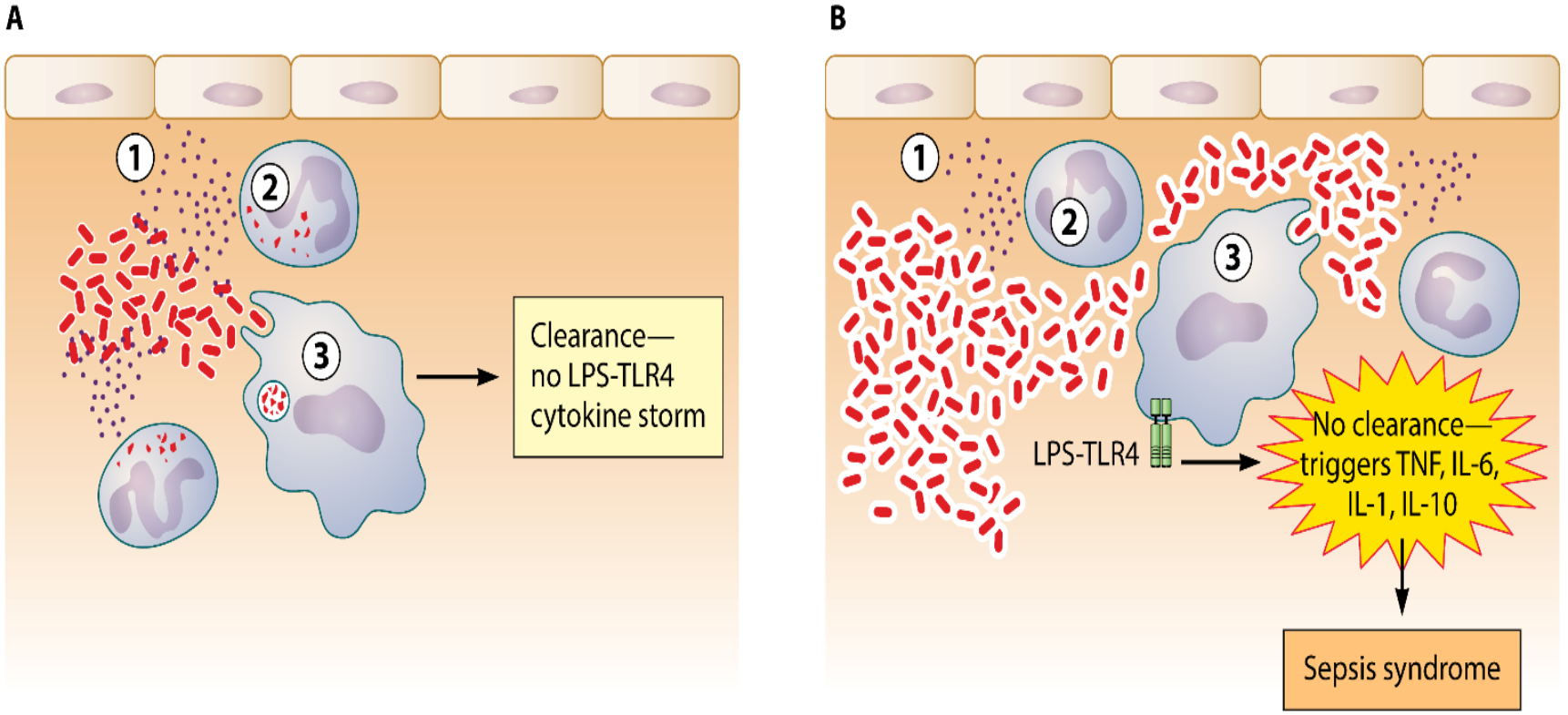
Hypotheses

- Treatment of mice with FDA-approved antibiotics that are active *in vitro* against the infecting isolate will result in clinical success
  - Survival, clinical endpoints, decrease in CFU burden, inflammatory and physiological endpoints

- In contrast, treatment of mice infected with strains that are resistant to the FDA approved antibiotics will result in clinical failure.

A. baumannii virulence

Integrated Overview of *A. baumannii virulence*. A) Early clearance of the microbe by the three primary innate effectors—(1) complement, (2) neutrophils, and (3) macrophages —prevents sustained LPS-TLR4 activation and subsequent cytokine storm. B) If the organism can resist initial innate effector clearance and replicate, it triggers sustained LPS activation of TLR4, resulting in cytokine storm and sepsis syndrome.



Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B. Clinical and Pathophysiological Overview of Acinetobacter Infections: a Century of Challenges. Clin Microbiol Rev. 2017; 30:409–447. PMCID: PMC5217799

A. baumannii clinical isolates

Table 1. Antibiotic MICs for Clinical *A. baumannii* isolates.

A. baumannii Strain	MIC (μg/ml) AMK	MER	PMB
***HUMC1**	**128**	**128**	**0.25**
***VA-AB41**	**8**	**64**	**0.50**
LAC-4	128	4	0.25
***LAC-4 Col^{R}**	**64**	**1**	**64**
ATCC 17978	8	0.25	0.5
C-14	2	1	8
C-8	8	8	16

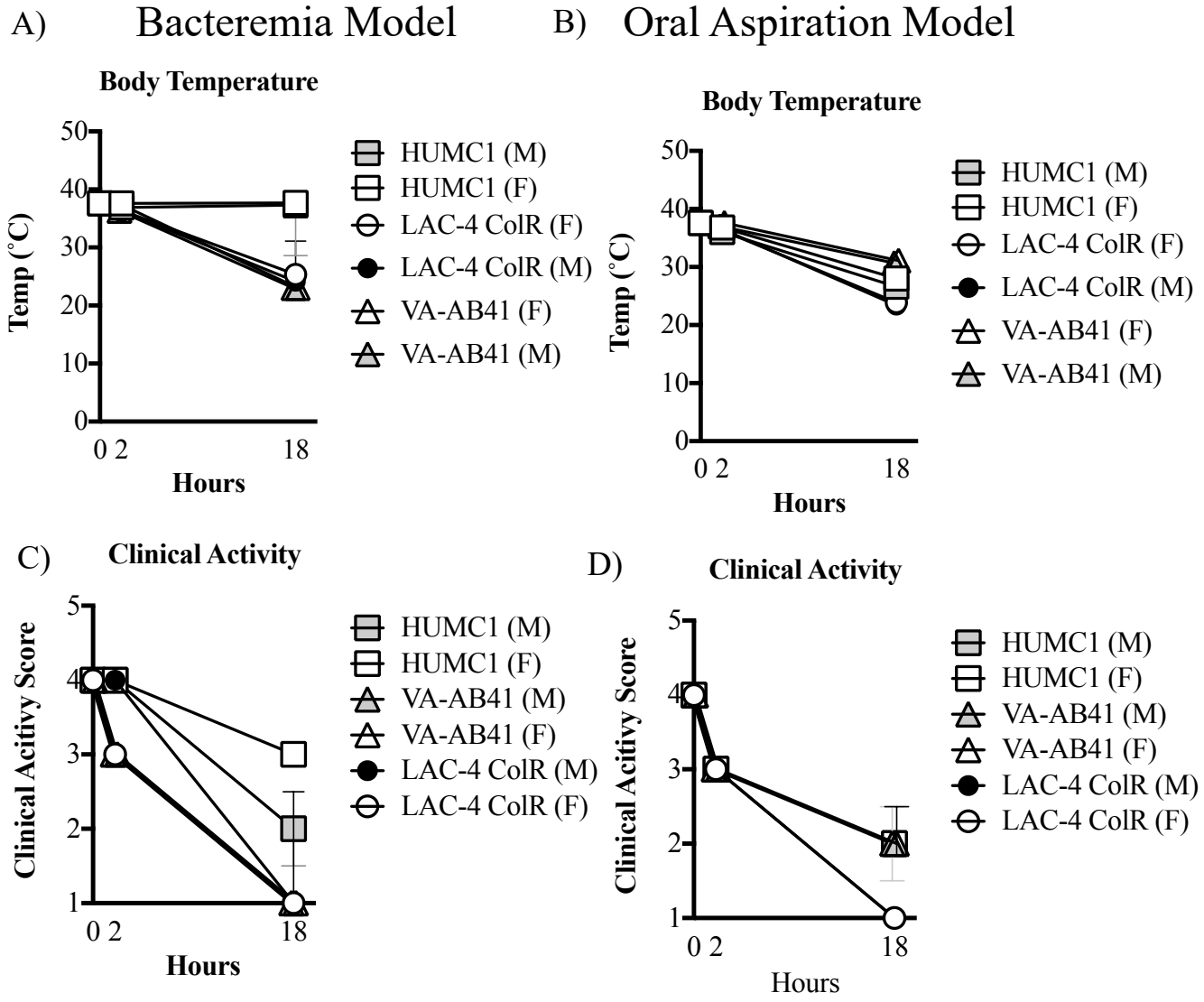
Table 2. LD100s of *A. baumannii* Isolates.[1]

	IV bacteremia model		OA pneumonia model	
	Sublethal CFU	LD_{100} CFU	Sublethal CFU	LD_{100} CFU
***HUMC1**	**$5.4x10^{6}$**	**$2.0x10^{7}$**	**$2.5x10^{8}$**	**$4.7x10^{8}$**
LAC-4	$1.0x10^{7}$	$2.5x10^{7}$	$1.9x10^{7}$	$2.8x10^{7}$
***LAC-4 Col^{R}**	**$5.7x10^{7}$**	**$9.7x10^{7}$**	**$2.4x10^{7}$**	**$7.6x10^{7}$**
***VA-AB41**	**$1.0x10^{7}$**	**$4.3x10^{7}$**	**$3.2x10^{8}$**	**$6.2x10^{8}$**
ATCC 17978	$5.0x10^{8}$	$9.0x10^{8}$	N/A	N/A
C-14	$5.0x10^{8}$	N/A	N/A	N/A
C-8	$8.6x10^{8}$	$9.6x10^{8}$	N/A	N/A

[1]The number shown reflect the highest sublethal inocula or lowest lethal inocula identified

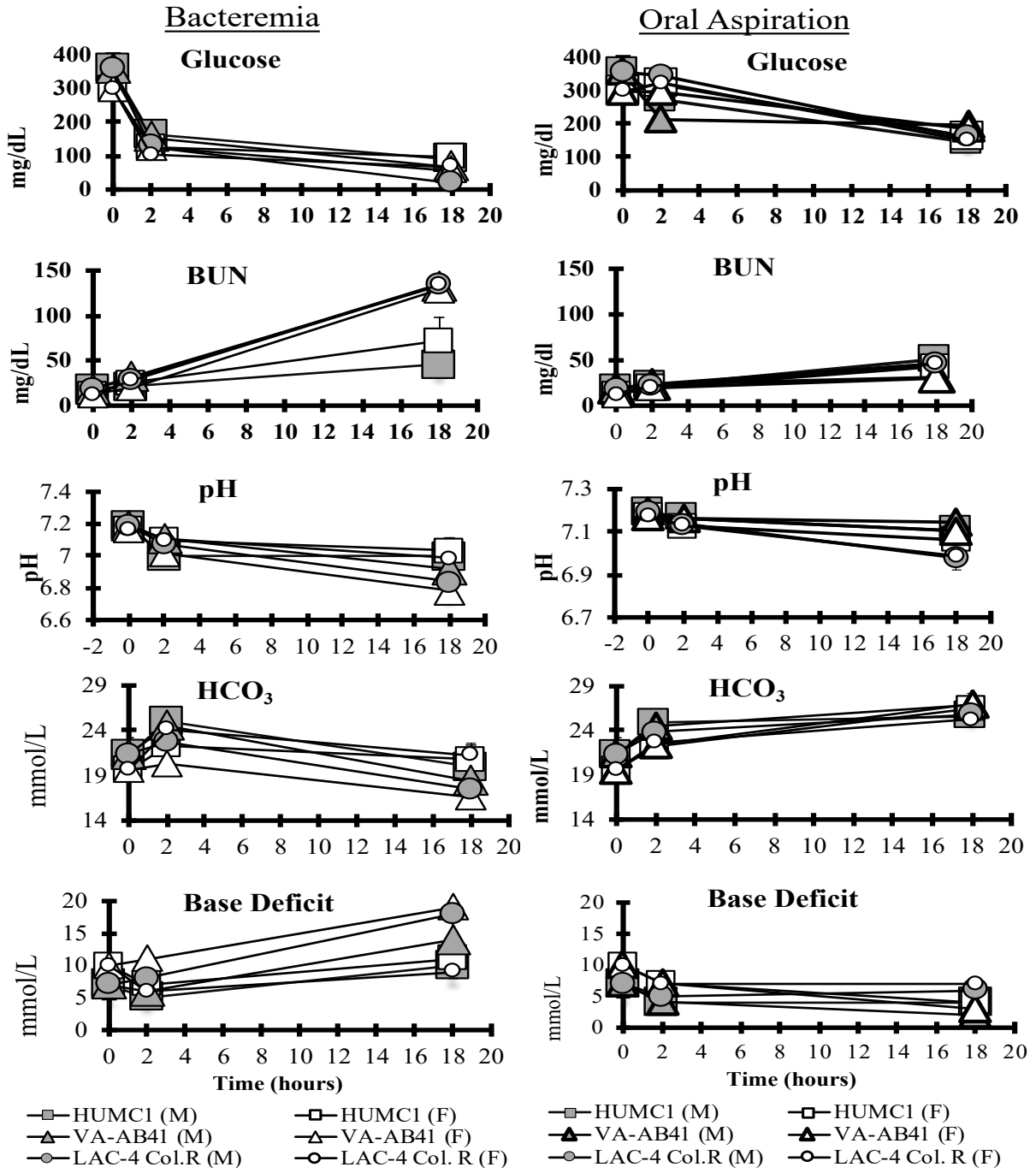
Temperature and Activity Scores

- Blood infection model: The physiology and host response indicates that the mice die due to sepsis.
- Oral aspiration pneumonia: The physiology and host response indicates that the mice die due to respiratory failure.



Sepsis Biomarkers

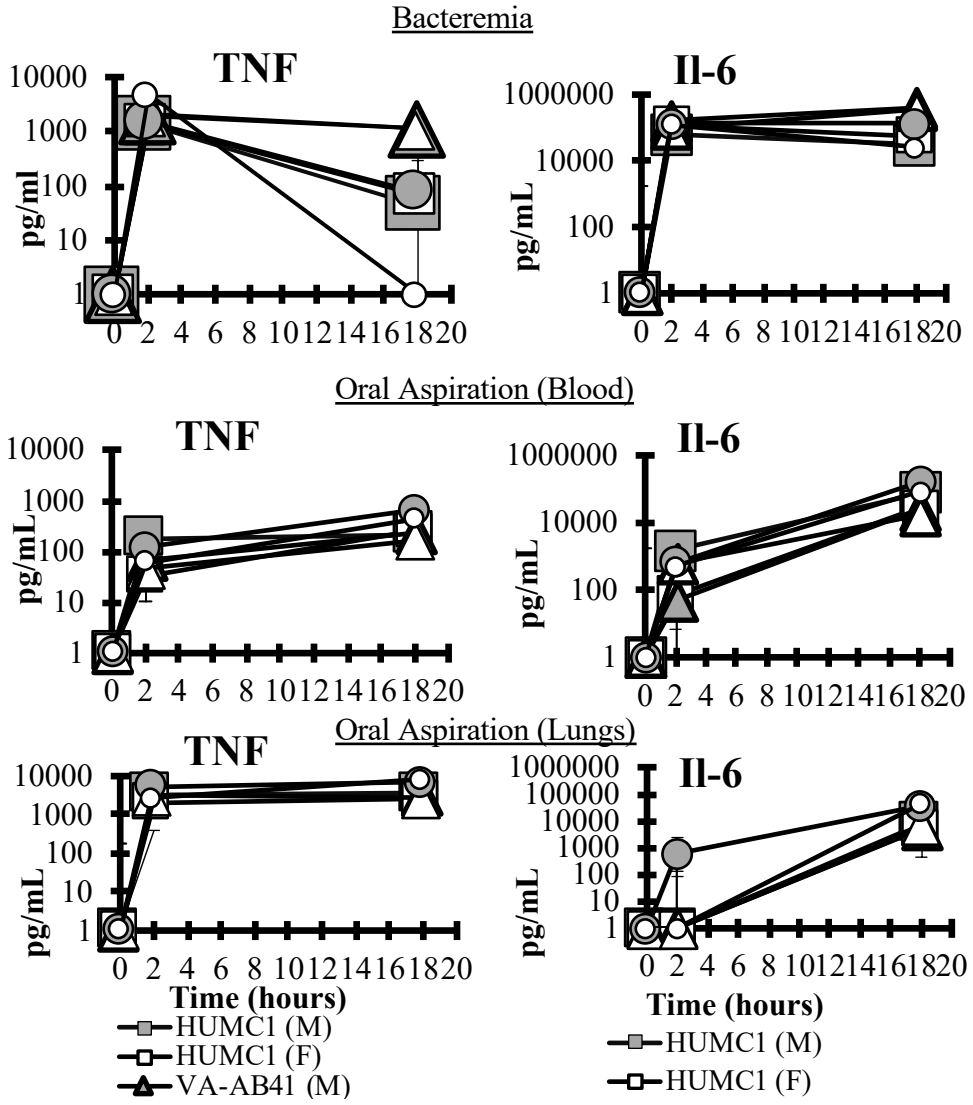
- Blood infection model: The physiology and host response indicates that the mice die due to sepsis.
- Oral aspiration pneumonia: The physiology and host response indicates that the mice die due to respiratory failure.



Cytokines

- Blood infection model: The physiology and host response indicates that the mice die due to sepsis.
- Oral aspiration pneumonia: The physiology and host response indicates that the mice die due to respiratory failure.

- Mice in the bacteremia model tend to be sicker than those in the OA model.



Mouse model summary

- *A. baumannii* Isolates
  - Available upon request.
  - Isolates will be deposited to CDC & FDA Antibiotic Resistance Isolate Bank
- Trigger to treat
  - Treatment is initiated 2 hrs post infection.
    - Decrease in activity score.
    - Increase in proinflammatory cytokines

Mouse model summary

To validate the mouse models by testing the efficacy of FDA-approved antibiotics, which have been confirmed by FDA to be effective for treating clinical infections based on adequate and well-controlled clinical trials.

Our goal is to further characterize their validity for predicting efficacy of proven antibiotics that will or will not be effective at treating *A. baumannii* infections.

Projected use of animal model in future drug development

- Useful for testing novel therapeutic agents or novel therapeutic combinations
  - Academia and Industry
- No other validated mouse model for the study of *A. baumannii* infection
  - Immune competent infection models
  - Characterized host/pathogen interaction
  - Humanized PK regimens
    - PK modeling in sick mice
  - Validated clinical endpoints

Projected use of animal model in future drug development (cont.)

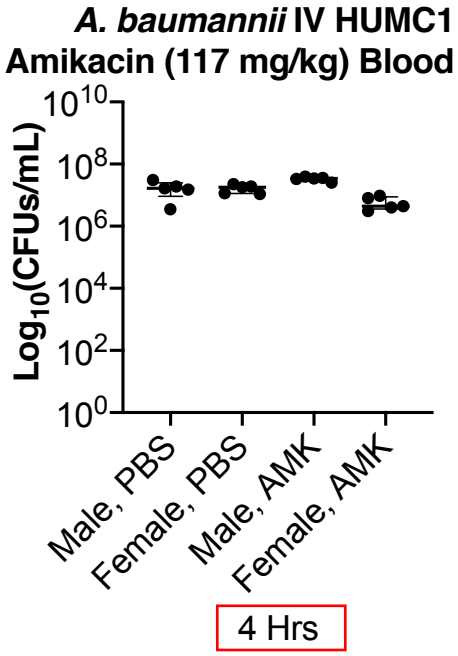
- Mouse ≠ Human
- Limitations
  - Comparatively higher inoculum to cause disease
  - Not all human clinical isolates are virulent in the mice (for *A. baumannii*, hypervirulent strains differ from non-hypervirulent strains; this is important to consider for PK/PD studies)
  - Differences in mouse vs human PK

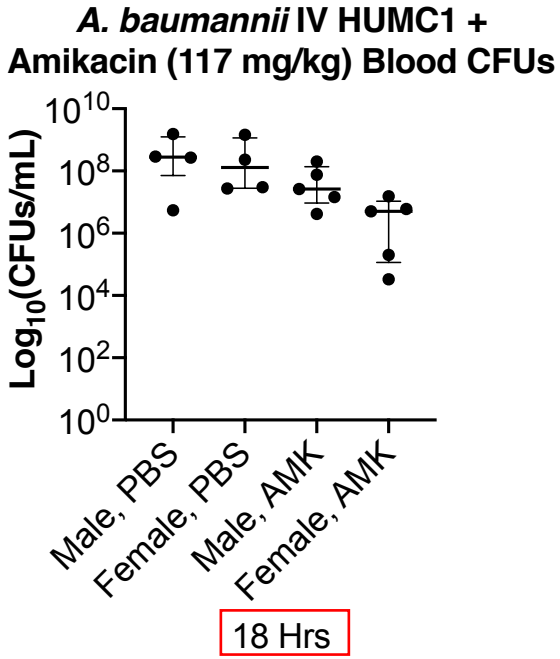
Efficacy (IV Model)

- HUMC1 (AMK^R)
- VA-AB41 (AMK^S)

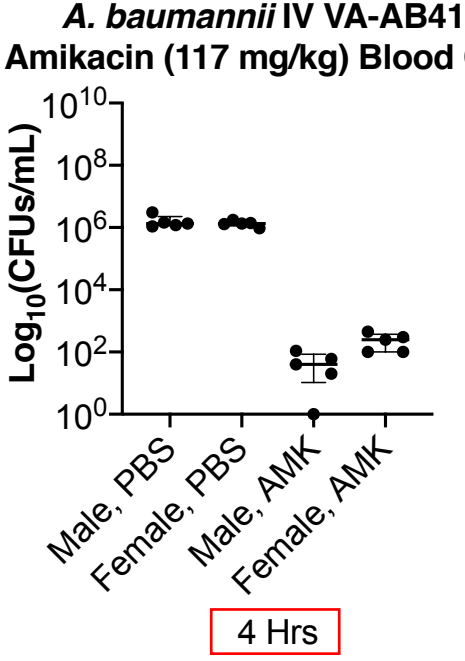
- Response to therapy (humanized PK) reflects predicted clinical outcomes

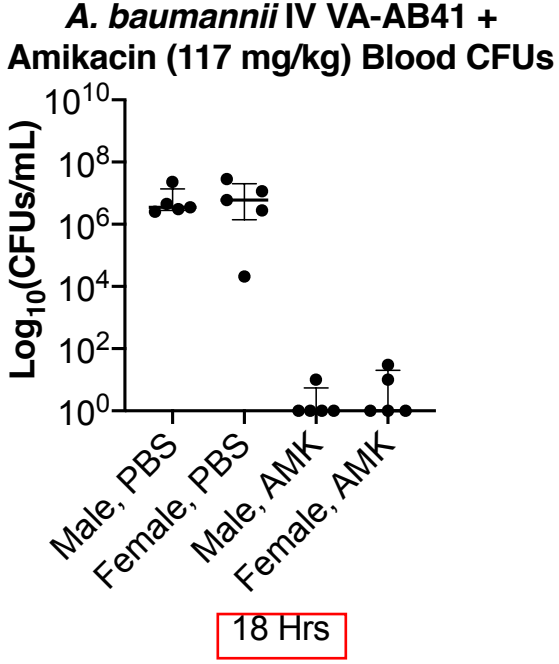
In vitro susceptibility: Resistant





In vitro susceptibility: Sensitive





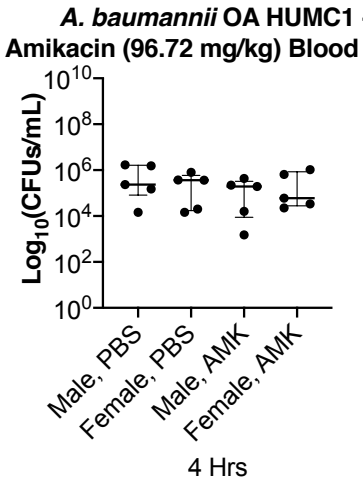
Efficacy (Lung Model)

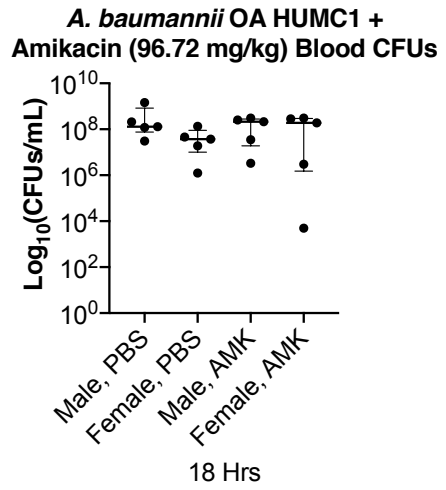
- HUMC1 (AMK^R)
- VA-AB41 (AMK^S)

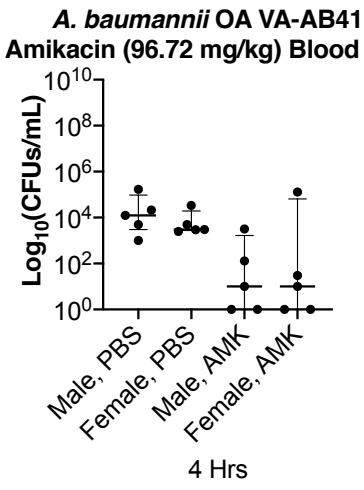
- Response to therapy (humanized PK) reflects predicted clinical outcomes

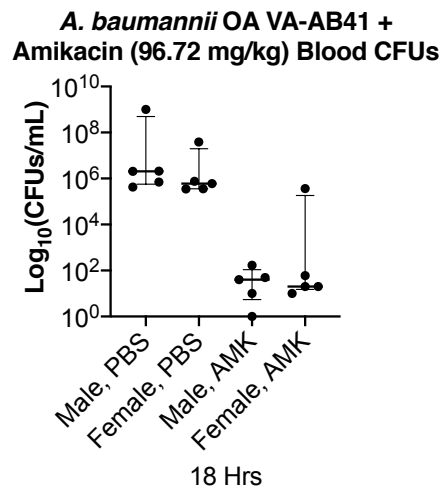
In vitro susceptibility: Resistant

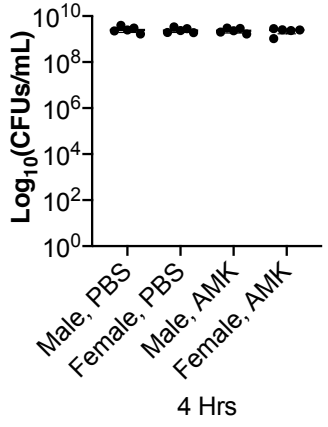
In vitro susceptibility: Sensitive

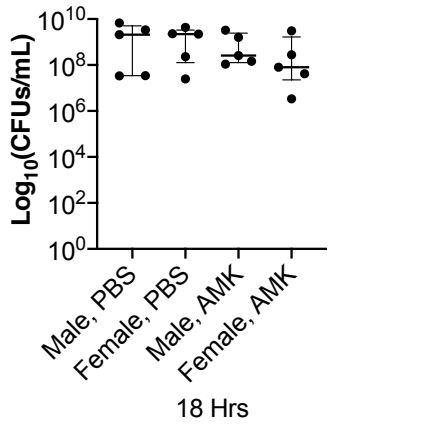


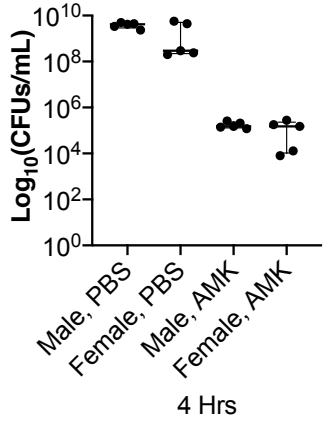


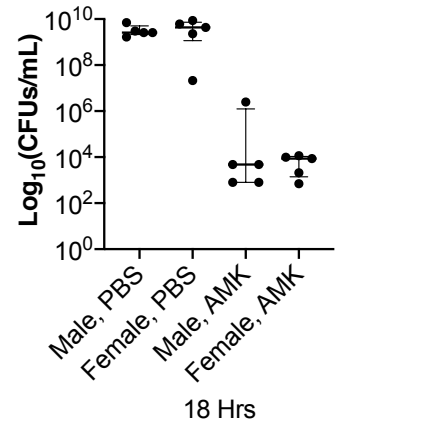






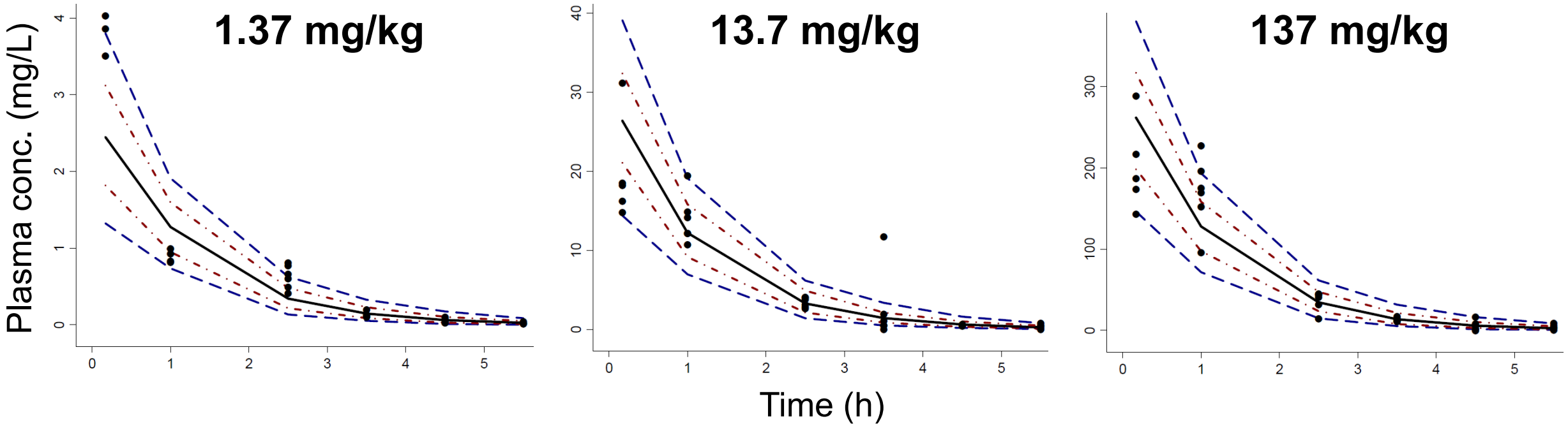




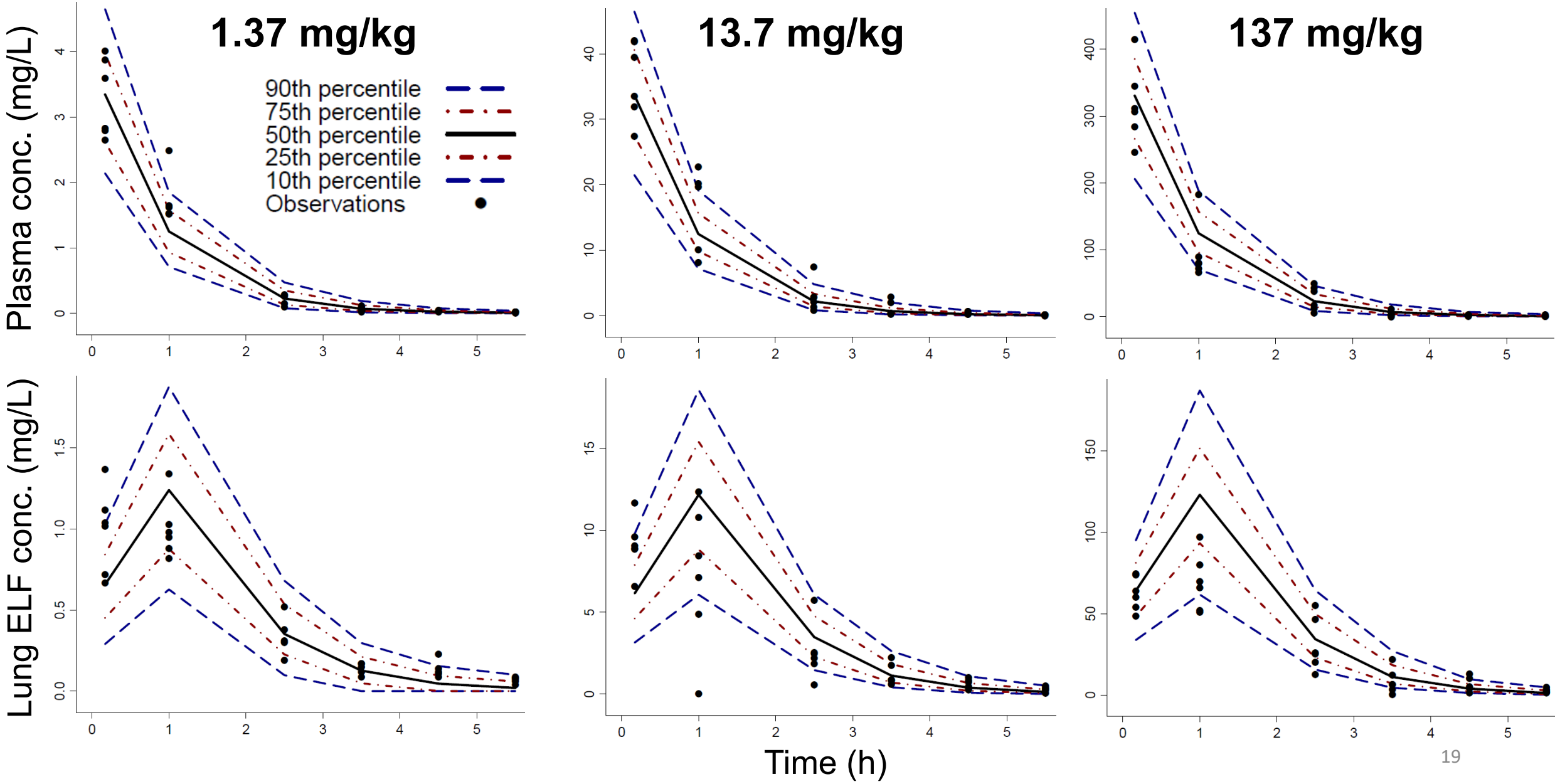


PK- Amikacin

Amikacin dose range study: visual predictive check – IV challenge



Amikacin dose range study: visual predictive check – OA challenge



Population PK estimates for amikacin dose range studies

	IV Model - Amikacin				OA Model - Amikacin			
Parameter	**Symbol**	**Unit**	**Mean (SE)**	**BSV (SE)**	**Symbol**	**Unit**	**Mean (SE)**	**BSV (SE)**
Clearance	CL	mL/h	9.93 (7.9%)	0.185 (54.8%)	CL	mL/h	9.93 (7.9%)	0.185 (54.8%)
Absorption half-life	T1/2abs	min	1.75 (12.1%)	0.1 (fixed)	T1/2abs	min	1.01 (34.9%)	0.1 (fixed)
Volume of central compartment	V1	mL	11.4 (14.1%)	0.1 (fixed)	V1	mL	8.56 (10.6%)	0.1 (fixed)
Ratio of AUC(elf) by AUC(plasma)					Felf	-	0.727 (16.9%)	0.1 (fixed)
ELF to plasma equil. half-life					T1/2elf	min	21.9 (8.2%)	0.1 (fixed)
Volume of ELF					Velf	mL	0.1 (fixed)	

- Same clearance after IV and OA challenge.
- Slightly larger volume of distribution for IV vs. OA.
- Terminal half-life: 48 min for IV and 36 min for OA.
- AUC(ELF) 72.7% of the AUC(plasma).

Clinical datasets used as input for the humanization

Eur J Clin Pharmacol (2015) 71:75–83
DOI 10.1007/s00228-014-1766-y

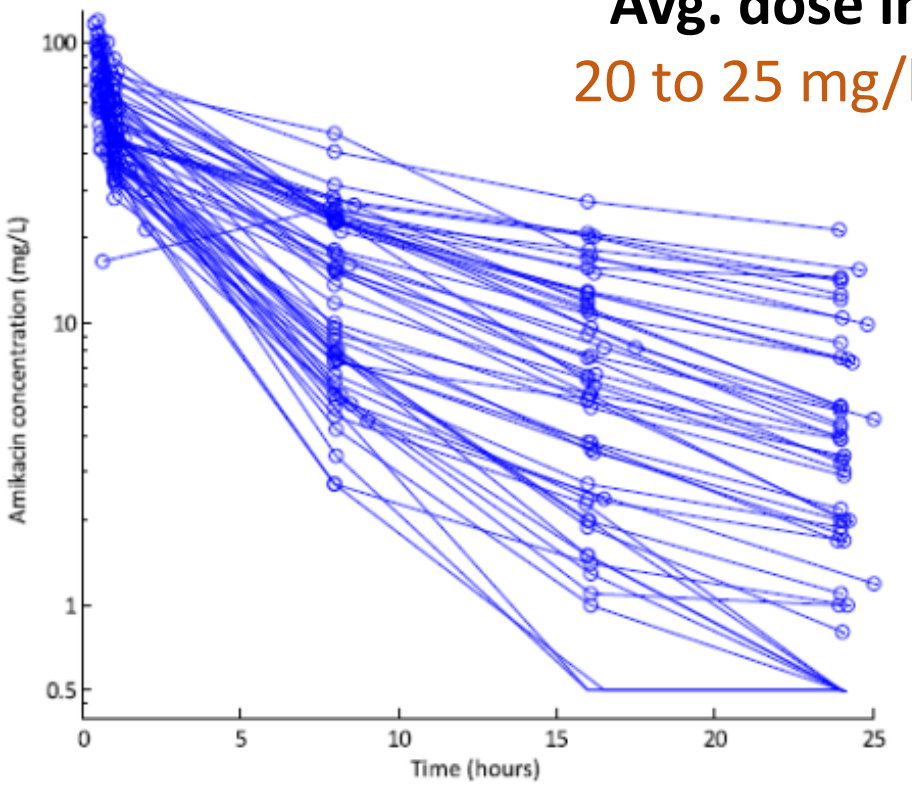
PHARMACOKINETICS AND DISPOSITION

Population pharmacokinetics of single-dose amikacin in critically ill patients with suspected ventilator-associated pneumonia

C. Burdet · O. Pajot · C. Couffignal · L. Armand-Lefèvre · A. Foucrier · C. Laouénan · M. Wolff · L. Massias · F. Mentré

Avg. dose in humans:

20 to 25 mg/kg amikacin



Population Pharmacokinetic Modeling and Optimal Sampling Strategy for Bayesian Estimation of Amikacin Exposure in Critically Ill Septic Patients

Isabelle K. Delattre, MSc, Flora T. Musuamba, PharmD,*† Joakim Nyberg, MSc,‡ Fabio S. Taccone, MD,§ Pierre-François Laterre, MD,‖ Roger K. Verbeeck, PhD,† Frédérique Jacobs, MD, PhD,§ and Pierre E. Wallemacq, PhD**

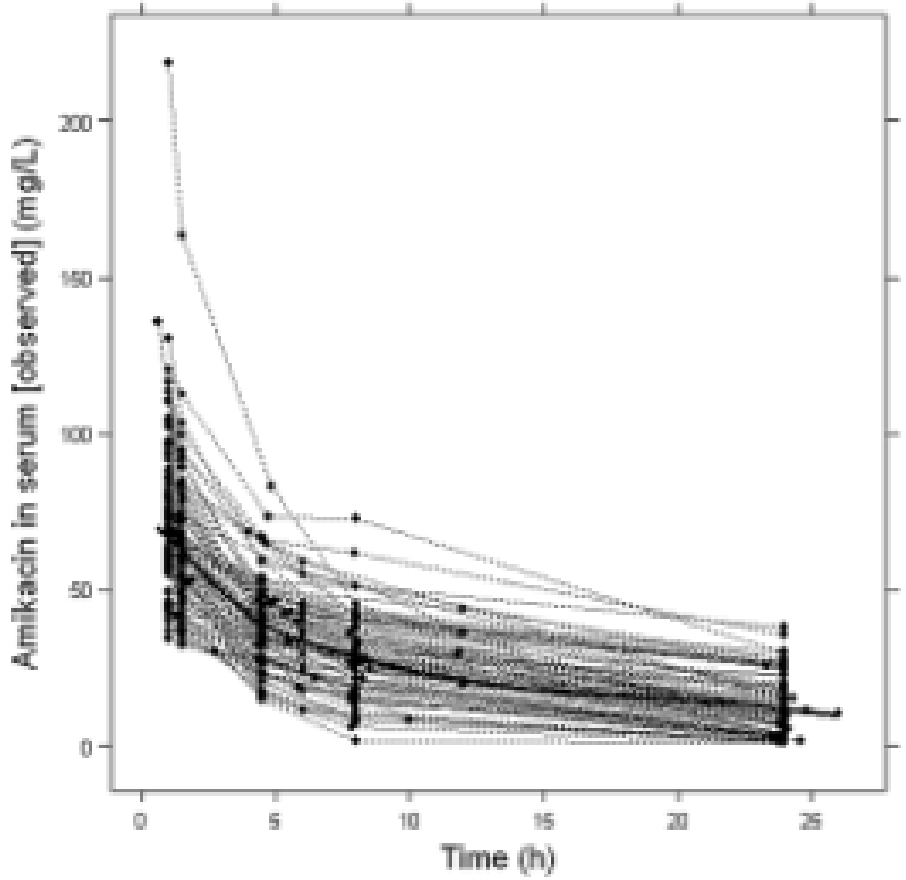
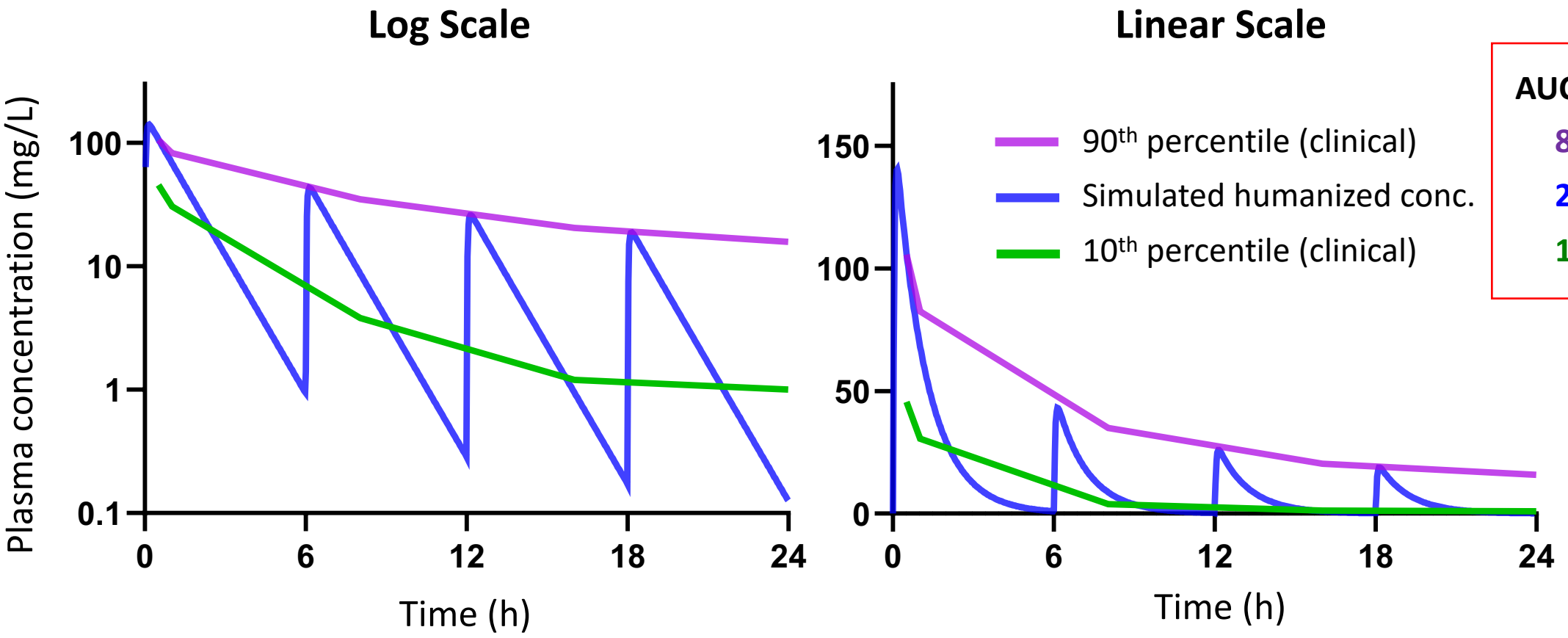


FIGURE 1. Individual concentration–time profiles in critically ill septic patients (n = 88) after administration of a first dose of amikacin (25 mg/kg) infused in 30 minutes.

Amikacin humanized dosing **IV method** (4 doses every 6 h) – Plasma

Daily Dose – 117 mg/kg/day

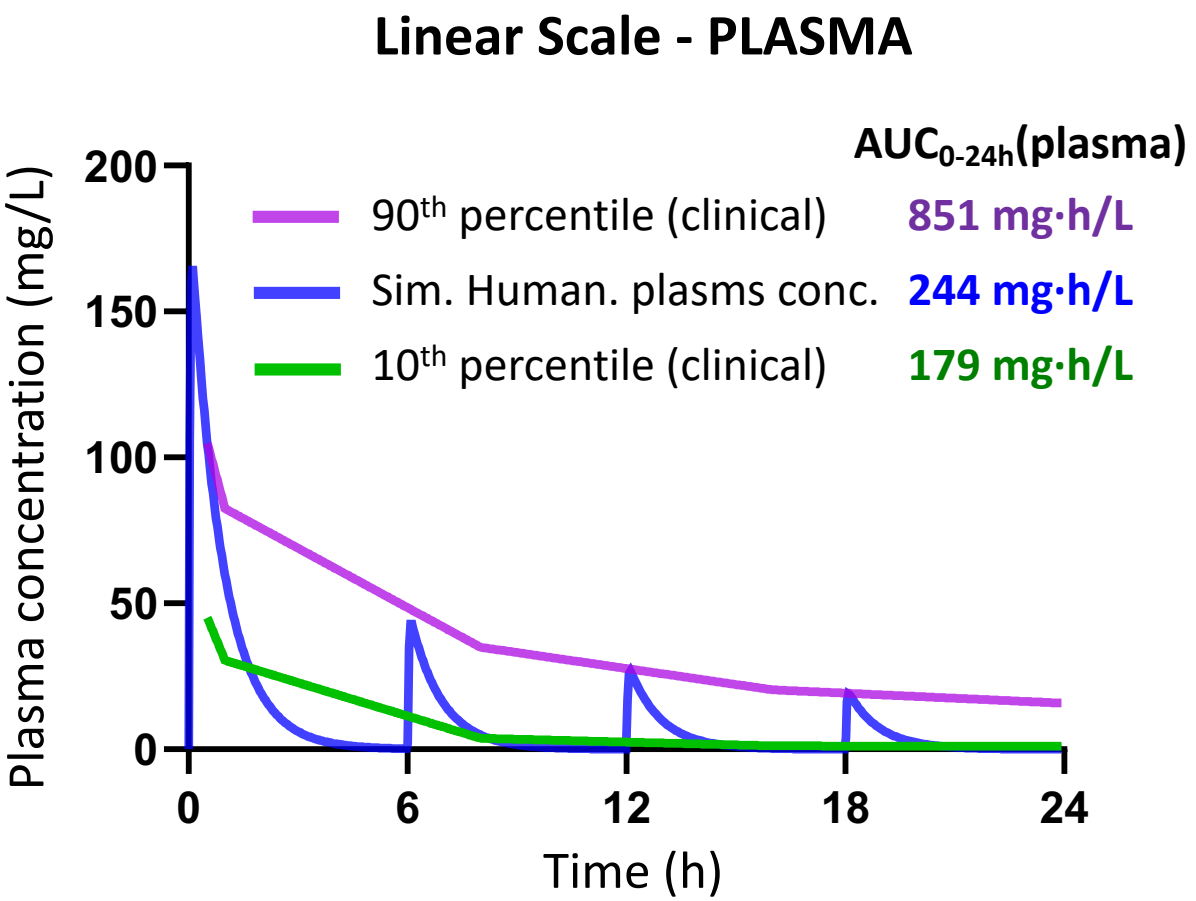
Fractions of daily doses 61.8% at 0 h, 18.6% at 6 h, 11.3% at 12 h, and 8.2% at 18 h.

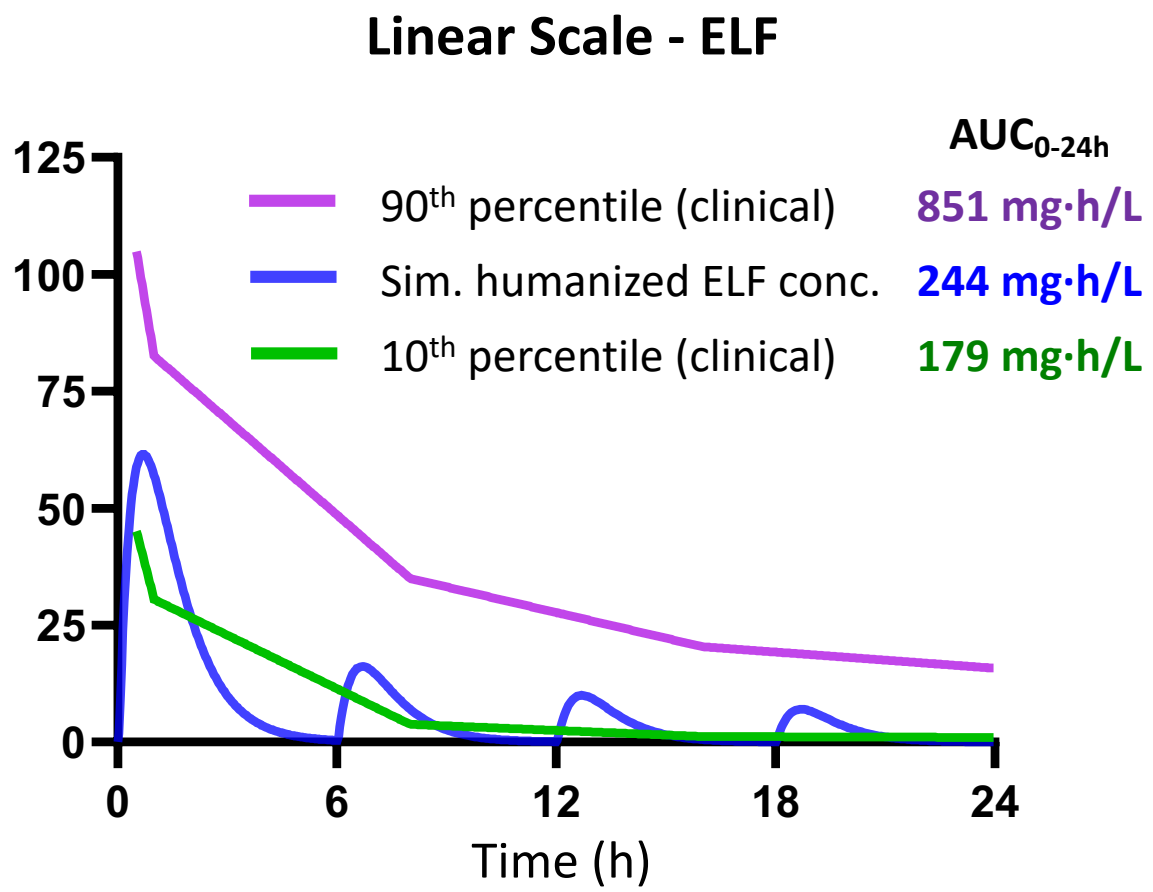


Amikacin humanized dosing **OA method** (4 doses every 6 h) – Plasma

Daily Dose – 96.7 mg/kg/day

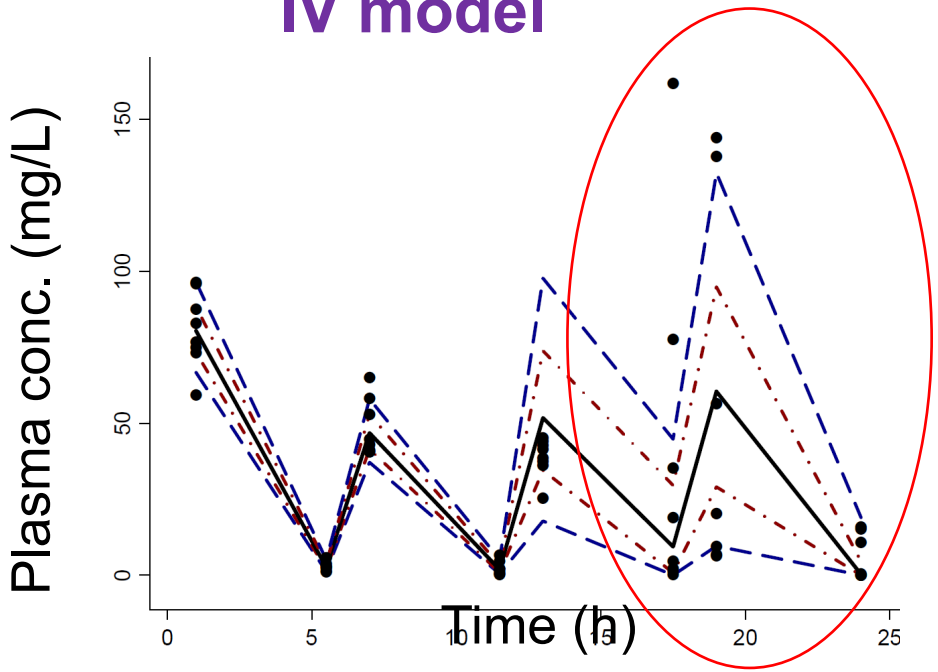
Fractions of daily doses 65.0% at 0 h, 16.9% at 6 h, 10.5% at 12 h, and 7.4% at 18 h.





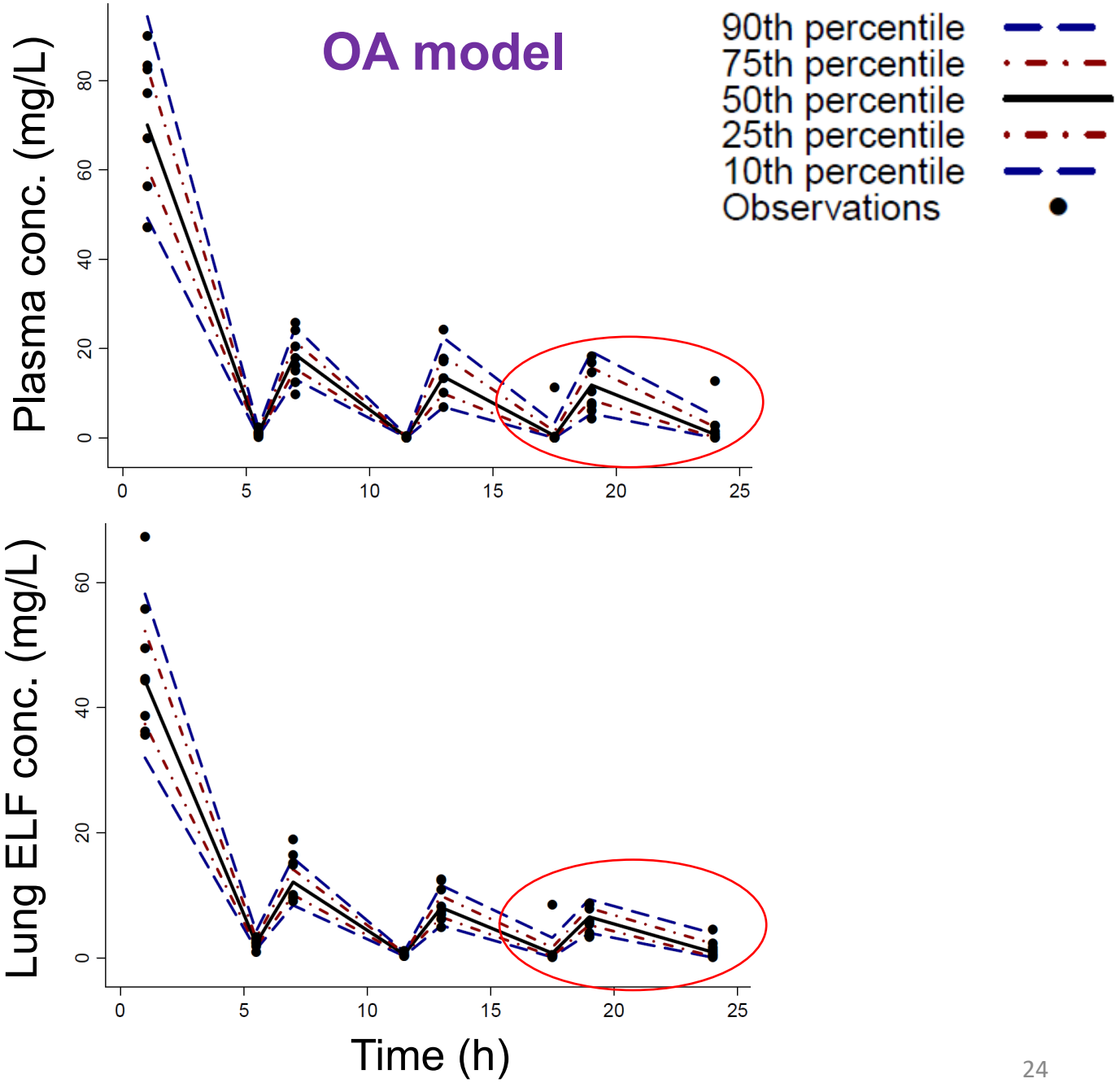
Amikacin PK validation: visual predictive checks

IV model

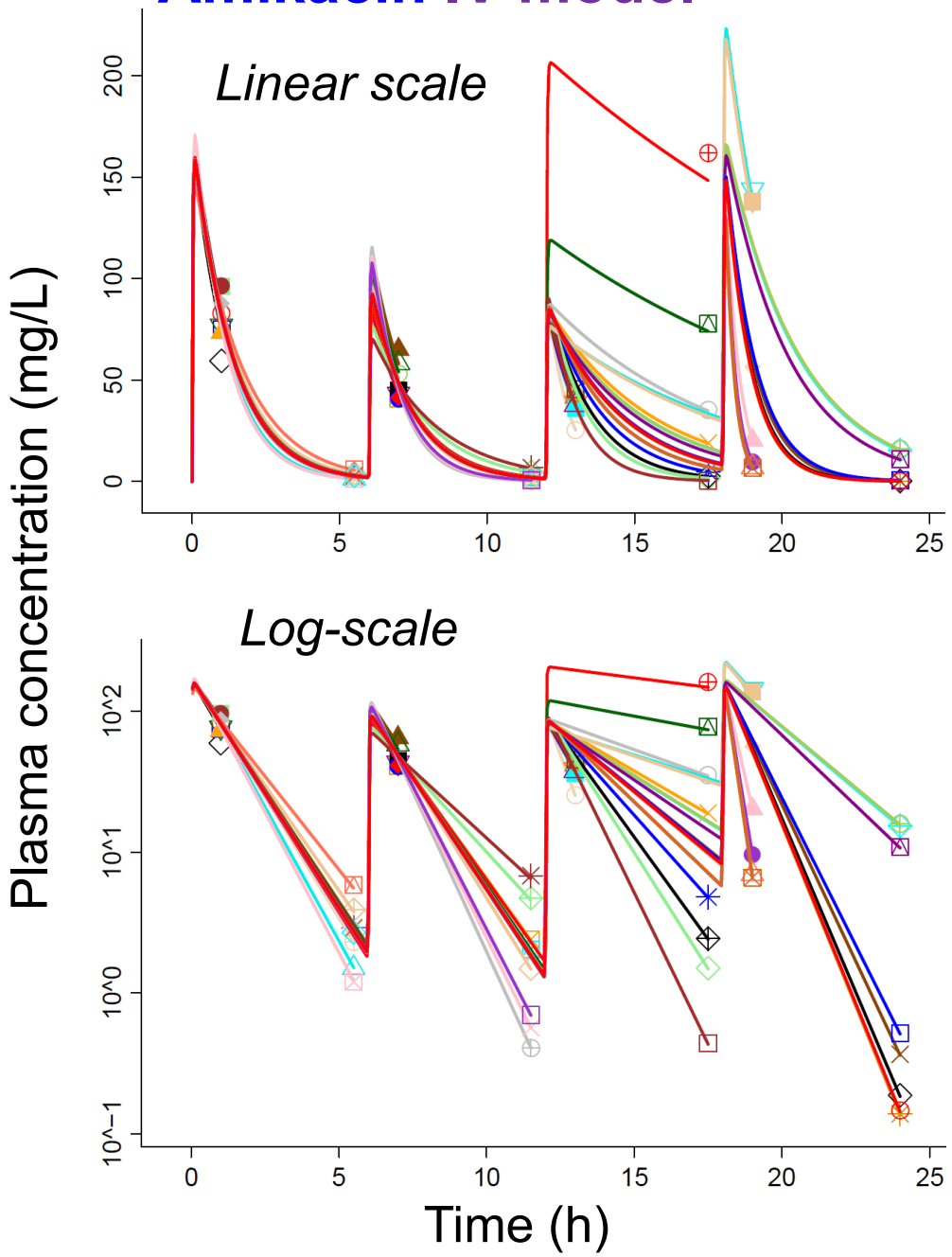


→ Large variability from 18 to 24 h

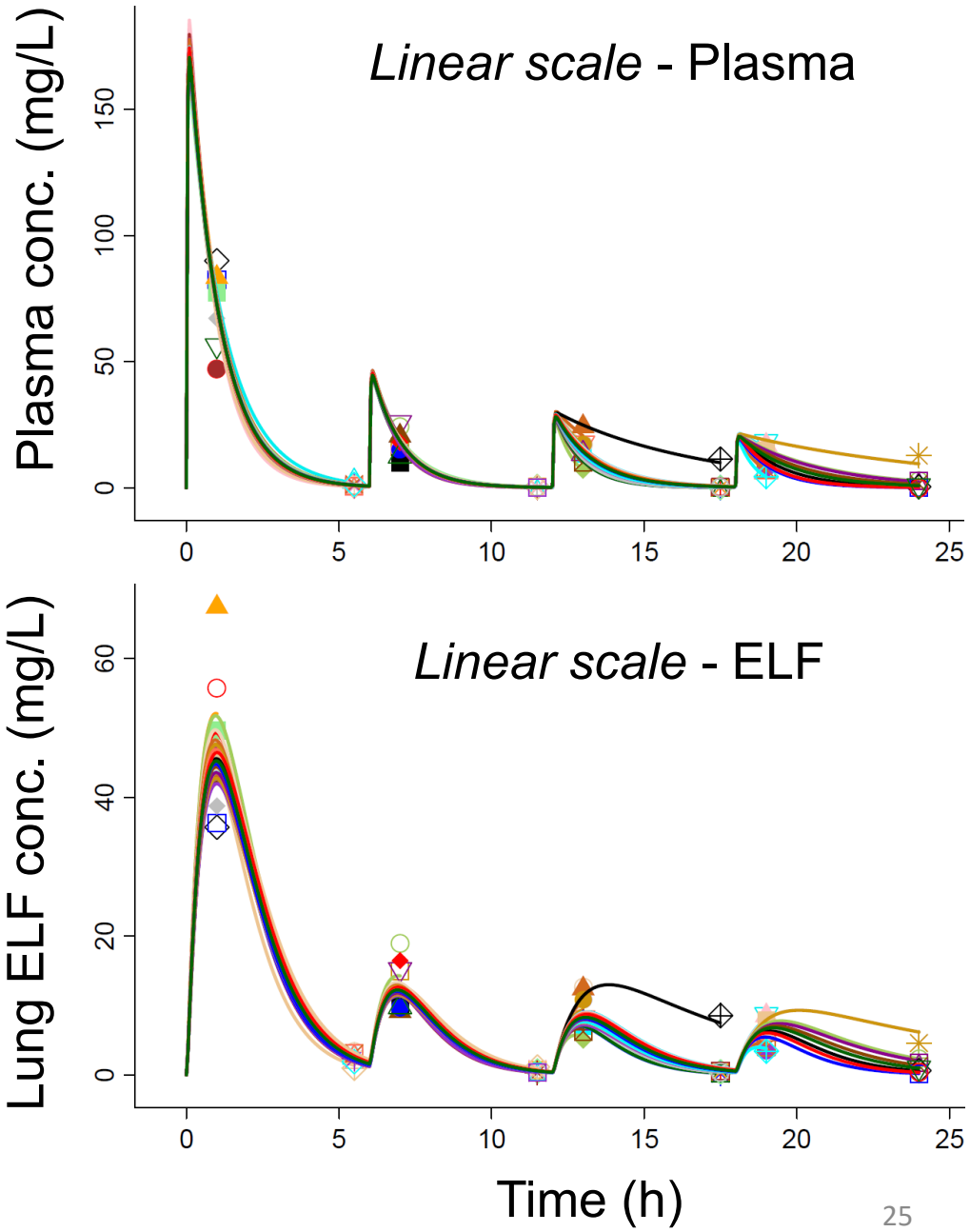
OA model



Amikacin IV model



Amikacin OA model



Population PK estimates for amikacin from humanized PK validation

IV Model - Amikacin

Parameter	Unit	Mean (SE)	BSV (SE)
CL_0-6h	mL/h	7.35 (6.8%)	0.1 (fixed)
CL_6-12h	mL/h	3.81 (6.5%)	0.155 (68.5%)
CL_12-18h	mL/h	1.39 (25.1%)	1.07 (29.9%)
CL_18-24h	mL/h	1.60 (23.5%)	0.779 (47.4%)
T1/2abs	min	1.00 (21.2%)	0.115 (146%)
V1_0-6h	mL	9.75 (14.7%)	0.1 (fixed)
V1_6-12h	mL	5.24 (10.4%)	0.250 (72.4%)
V1_12-18h	mL	3.50 (28.3%)	0.430 (80.9%)
V1_18-24h	mL	1.51 (18.6%)	0.195 (147%)

OA Model - Amikacin

Parameter	Unit	Mean (SE)	BSV (SE)
CL_0-6h	mL/h	7.44 (5.5%)	0.1 (fixed)
CL_6-12h	mL/h	7.69 (4.9%)	0.1 (fixed)
CL_12-18h	mL/h	5.92 (13.1%)	0.500 (42.3%)
CL_18-24h	mL/h	4.39 (17.6%)	0.656 (39.7%)
T1/2abs	min	1.12 (24.7%)	0.1 (fixed)
V1	mL	7.69 (6.4%)	0.1 (fixed)
Felf	-	0.604 (4.9%)	0.1 (fixed)
T1/2elf	min	36.4 (4.4%)	0.1 (fixed)
Velf	mL	0.10 (fixed)	

- Clearance decreased over time in both models (more pronounced for IV model).
- Volume of distribution also decrease for IV model.
- Large between subject variability (BSV) for clearance after 12 h.
- ELF penetration results comparable to those in the dose range study.

Conclusions of dosing simulations – Amikacin

- PK model in mice was used to simulate human-like drug exposures.
- We targeted the 90th percentile of concentrations in VABP patients.
- The AUCs for amikacin associated with the 10th and 90th percentiles were approximately **179** and **851** mg·h/L
- The AUC in patients for a dose of 30 mg/kg is ~**308** mg·h/L.
- Peaks in critically-ill patient are typically 60 to 100 mg/L (medians).
- Humanized concentrations were similar to the targeted conc. range.
- Clearances decreased and became more variable after 12 h, potentially due to bacterial damage to the kidneys.

PK- Polymyxin B

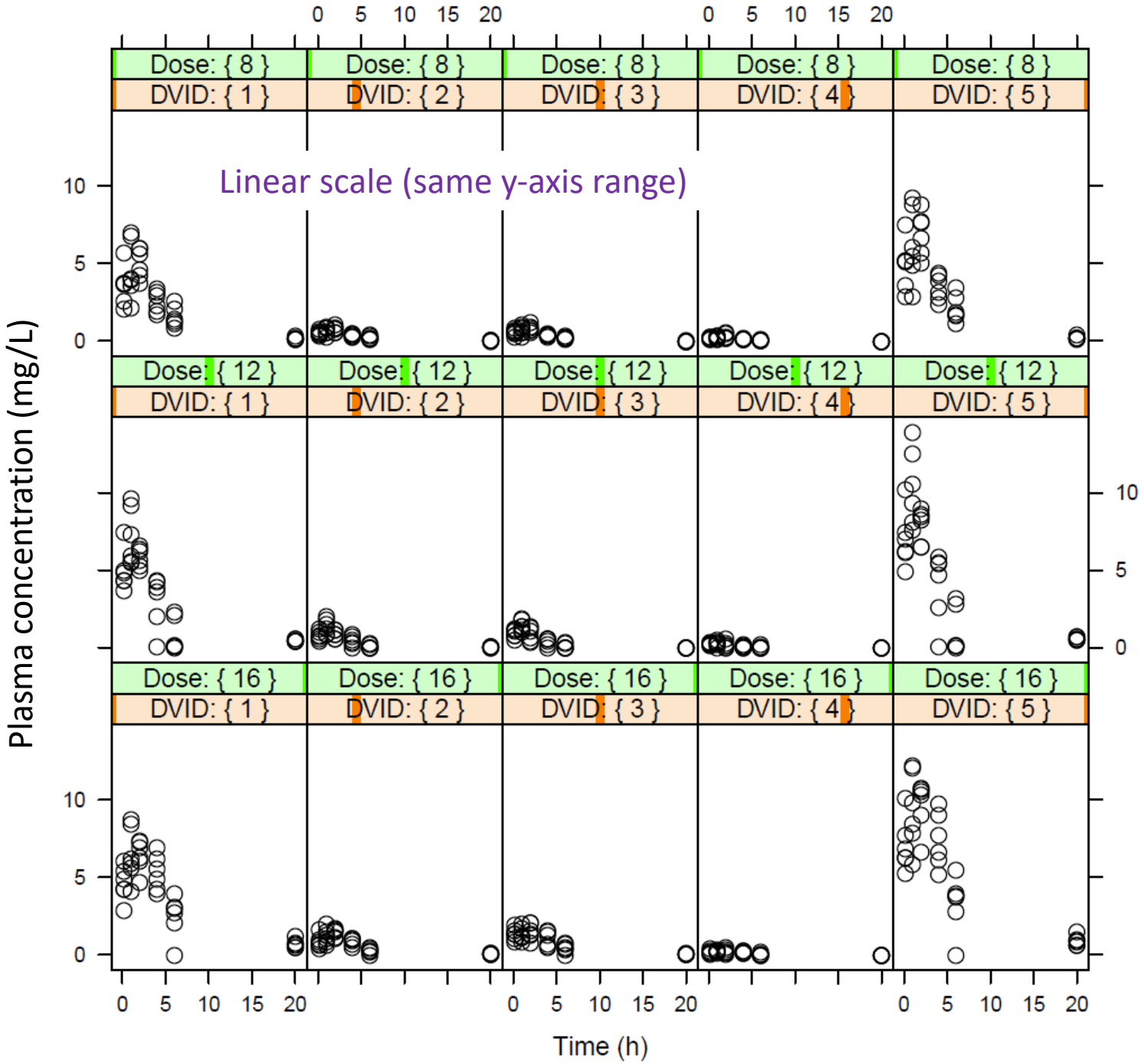
Polymyxin B concentrations in mice for IV challenge model

DVID 1 = polymyxin B1
DVID 2 = polymyxin B1-Ile
DVID 3 = polymyxin B2
DVID 4 = polymyxin B3
DVID 5 = All 4 components

Polymyxin B1 was the predominant component.

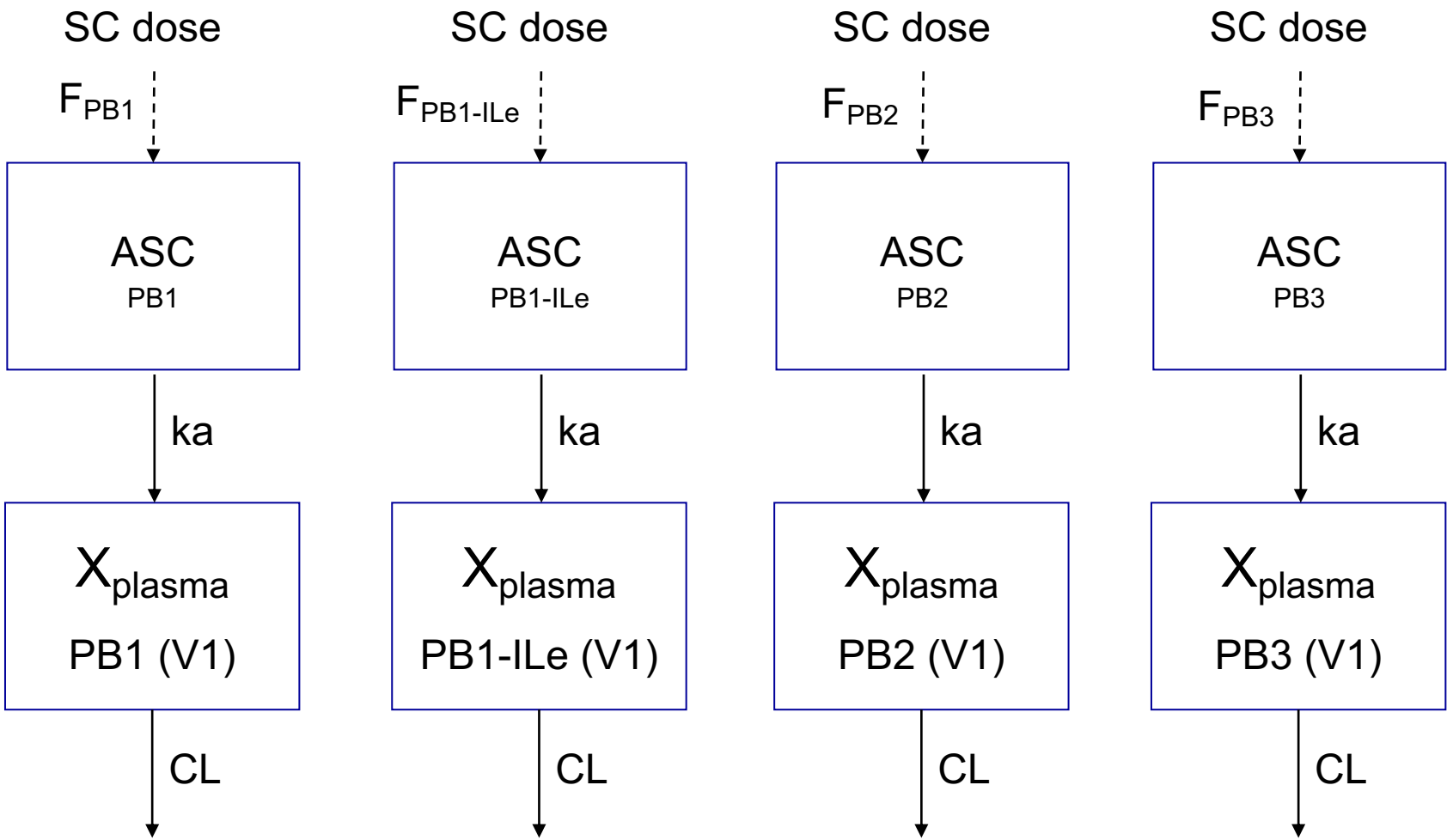
Polymyxin B3 was the lowest abundancy component.

Relative bioavailability (F) estimated during modeling.



Structural model for all 5 dependent variables in IV challenge mice

Structure: One-compartment model for each component, sum of four F equal to 1

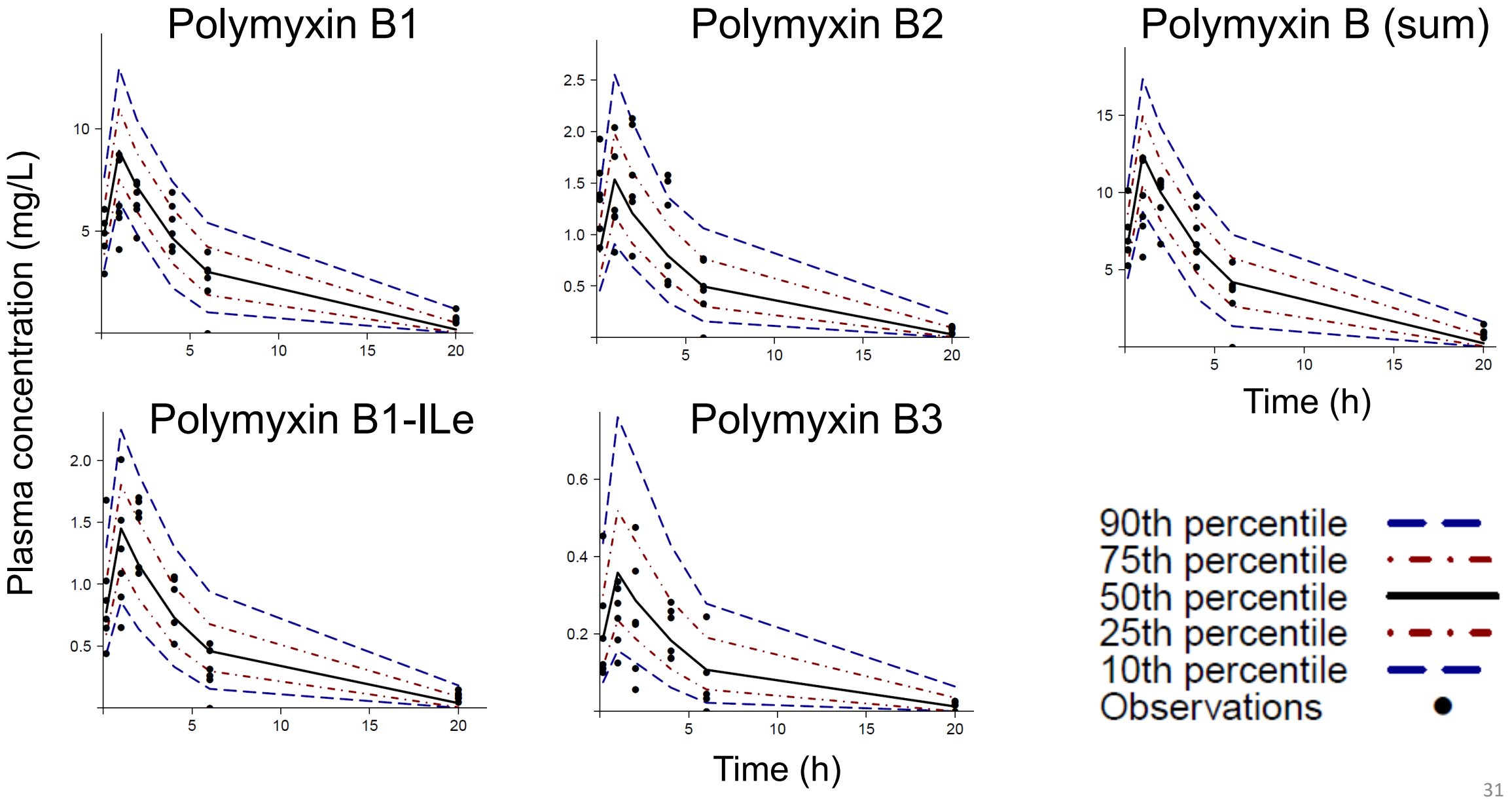


Total polymyxin B concentration modeled as the sum of all 4 components.

V1 and CL were shared across all four components of polymyxin B.

Other modeling choices and estimation algorithms were thoroughly tested at this stage.

Polymyxin B dose range study: VPC for 16 mg/kg – IV challenge



Parameter estimates for polymyxin B for IV model

(importance sampling in S-ADAPT; Luna et al. dataset)

Symbol	Parameter	Unit	Population Mean (relative standard error, SE%)	Between Subject Variability, CV (SE%)
T1/2abs(L)	Absorption half-life at 8 and 12 mg/kg	min	7.20 (9.2%)	0.109 (77.5%)
T1/2abs(H)	Absorption half-life at 16 mg/kg	min	11.7 (54.7%)	0.125 (105%)
CL	Clearance	mL/h	**5.60 (6.0%)**	0.471 (26.2%)
V1	Volume of distribution for central compartment	mL	**26.3 (6.2%)**	0.308 (26.1%)
F(PB1)	Bioavailability of PB1	-	72.4% (2.7%)	small
F(PB1-ILe)	Bioavailability of PB1-ILe	-	12.0% (3.3%)	small
F(PB2)	Bioavailability of PB2	-	12.8% (3.2%)	small
F(PB3)	Bioavailability of PB3	-	2.83%	small

Estimating the same volume of distribution for all components stabilized the model and allowed us to estimate the BSV for both CL and V1.

All additive residual error standard deviations were fixed to 0.025 mg/L.
All proportional residual errors were fixed to a 10% coefficient of variation.

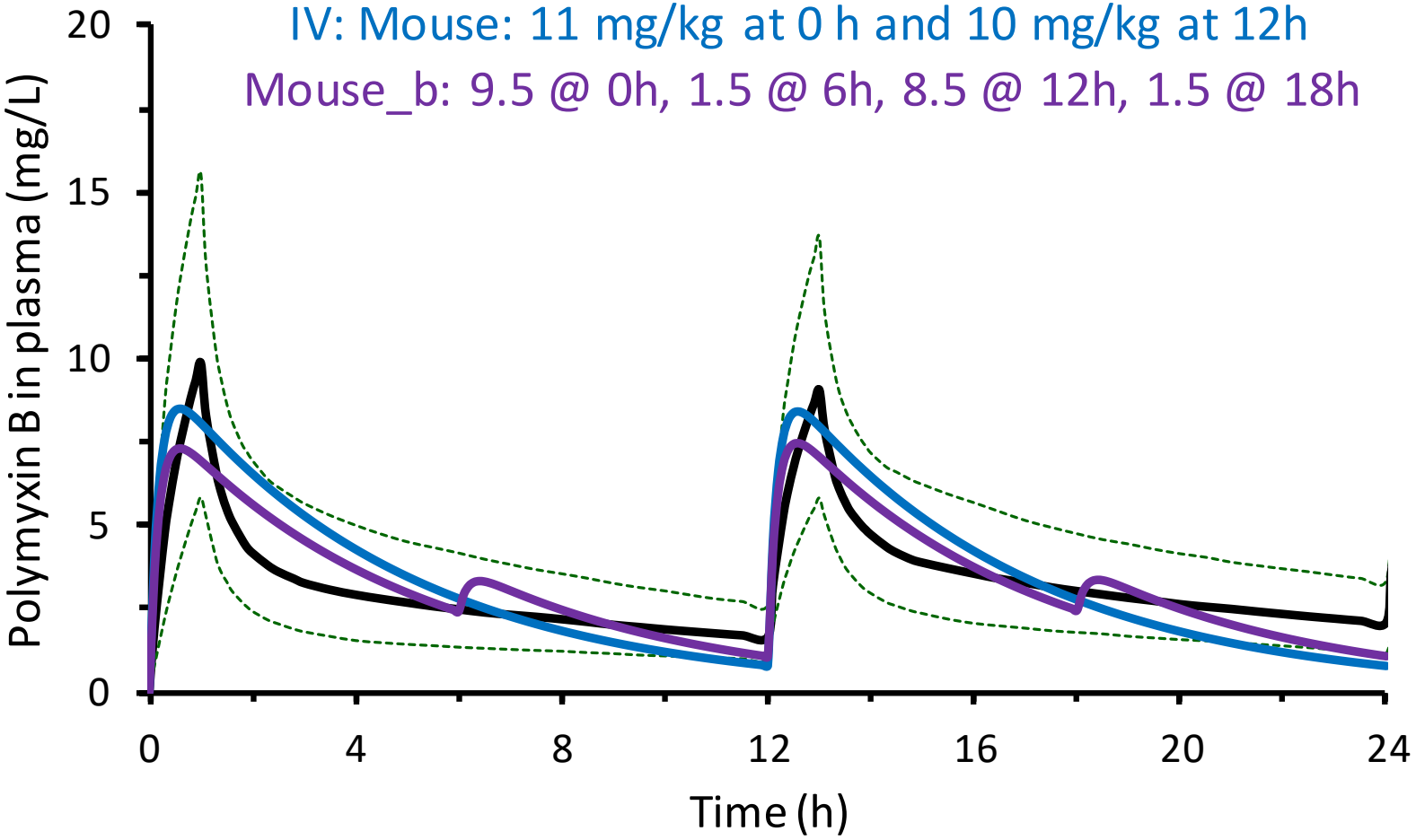
Humanized dosing for polymyxin B for IV model

Polymyxin B in critically ill patients

2 mg/kg load, 1.5 mg/kg Q12h maintenance dose;

IV: Mouse: 11 mg/kg at 0 h and 10 mg/kg at 12h

Mouse_b: 9.5 @ 0h, 1.5 @ 6h, 8.5 @ 12h, 1.5 @ 18h



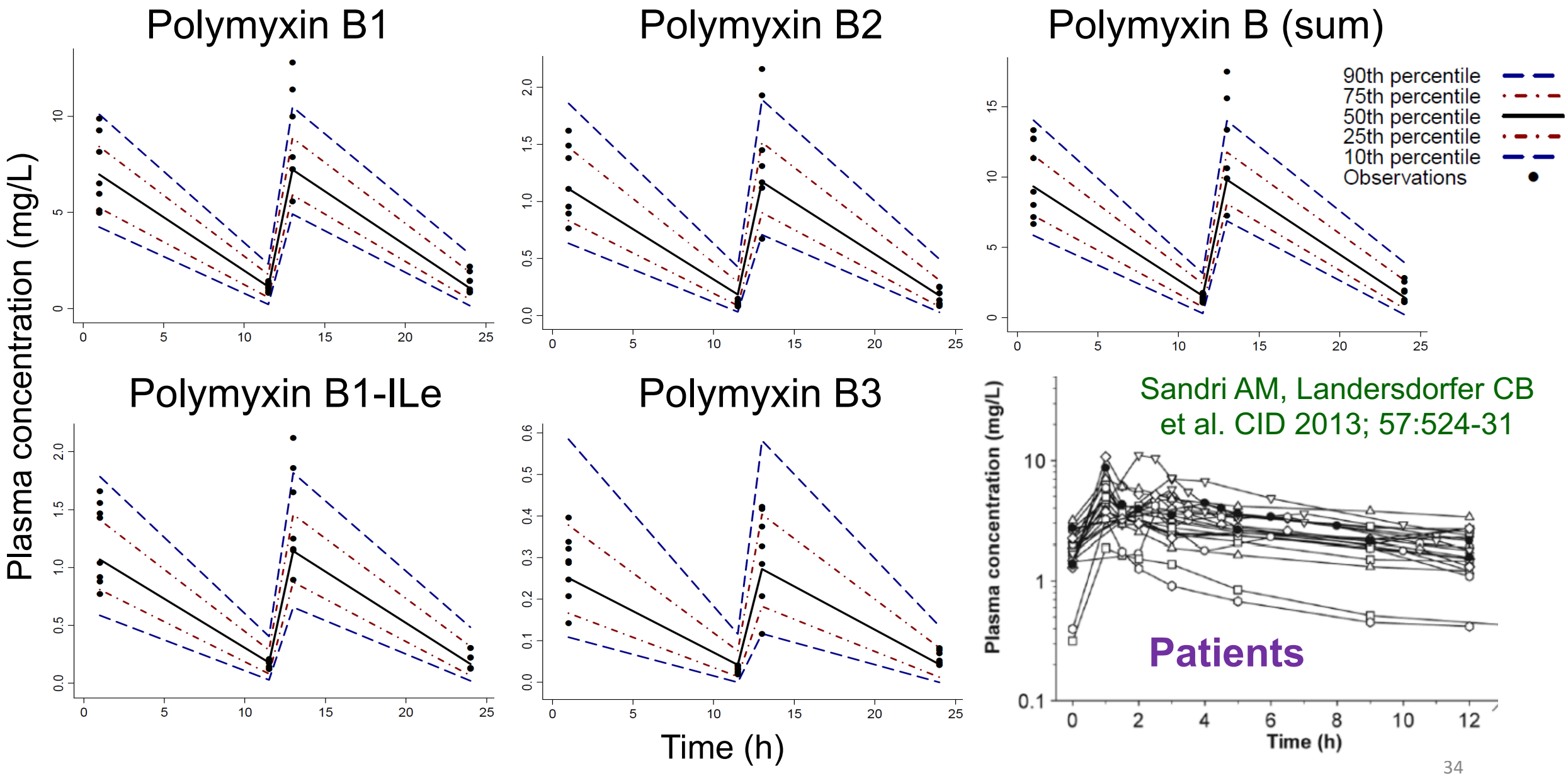
AUC (mg*h/L)

90%	116	Percentiles in Patients
50%	80	
10%	52	
Mouse	**83**	Mean predictions in mice
Mouse_b	**81**	

We recommend 11 mg/kg at 0 h and 10 mg/kg at 12 h option

Total concentrations were matched. This assumes that the protein binding in plasma was similar between infected mice and patients.

Polymyxin B dose range study: VPC for validation – IV challenge



Sandri AM, Landersdorfer CB et al. CID 2013; 57:524-31

Conclusions for humanizing PMB

- For the IV model: we recommend 11 mg/kg (0 h) & 10 mg/kg (12 h)
  - Match the total drug AUC in plasma from 0 to 24 h between patients and mice
  - Mouse values stay between the 10th and 90th percentile in humans

- For the OA model, we recommend 7 mg/kg (0 h) & 6 mg/kg (12 h)
  - This matches the total drug AUC from 0 to 24 h in patient plasma with the unbound AUC in mouse ELF. The mouse plasma AUC are slightly higher.

- The IV vs. OA challenge considerable affected total clearance.

- It seems important to validate the PK in the mouse model using the same isolates and pathogens that are used in efficacy studies.

PK- Meropenem

Meropenem peak and trough concentrations for bolus and short-term infusion regimens in critically ill patients

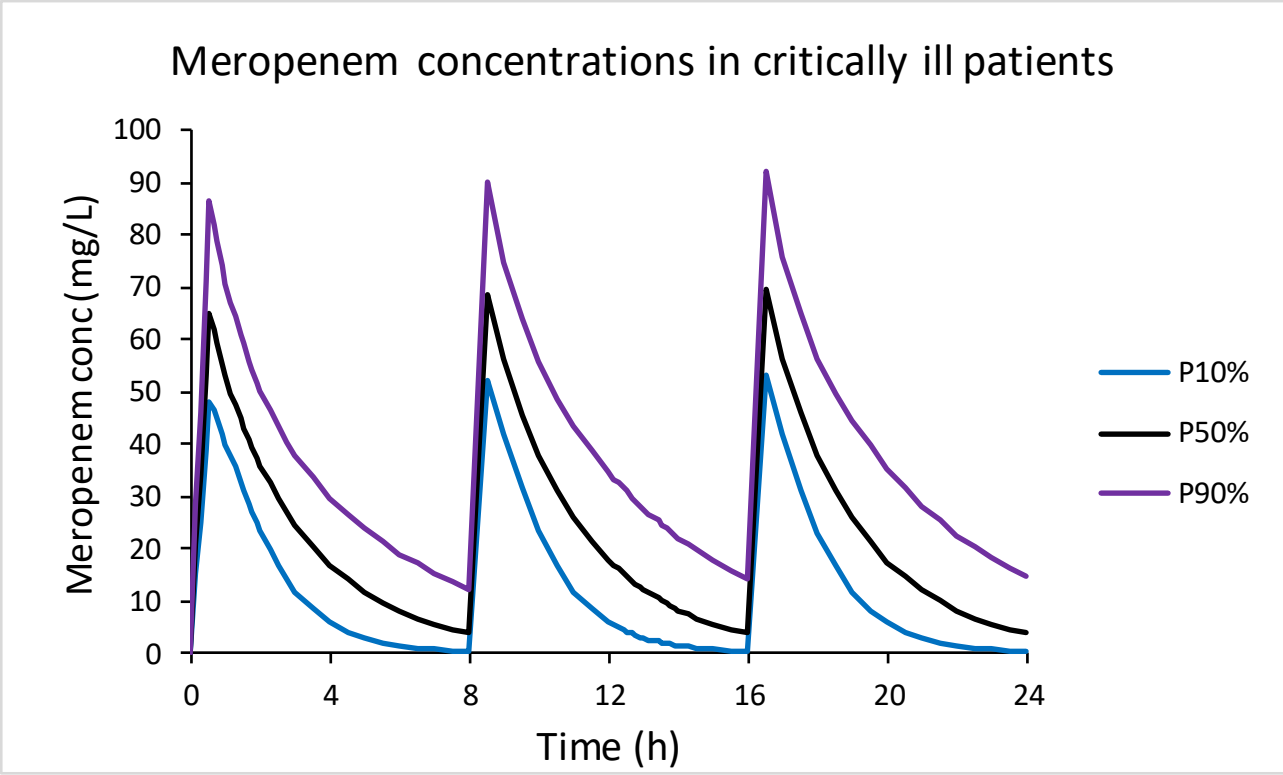
- We sought to mirror the PK in critically ill patients with normal renal function by humanizing the mouse dosage regimens.
- Targeted dosage regimen in humans: 2g short-term (30min) infusions every 8h.

For this dosage regimen:

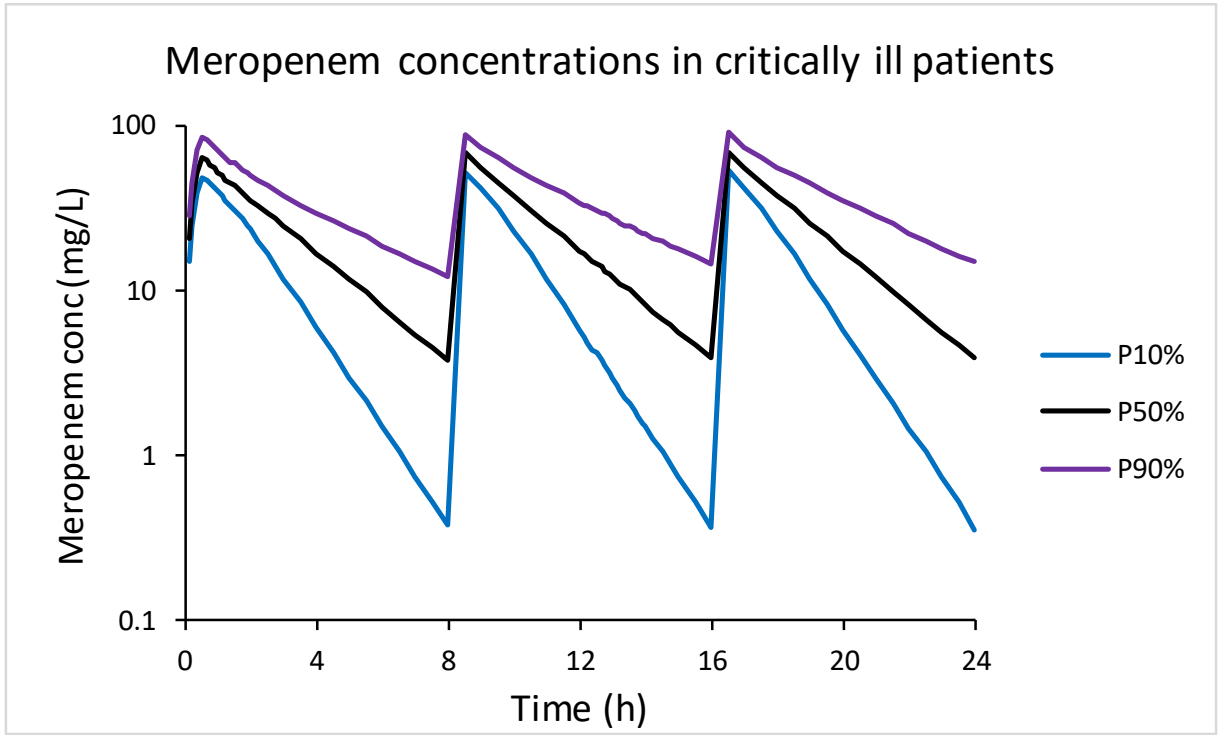
- The average peak concentrations ~60 to 100 mg/L.
- The average trough concentrations ~ 1 to 10 mg/L.
- Meropenem is minimally protein bound in human plasma.

Monte Carlo simulated meropenem concentrations in humans for 2g q8h as 30 min infusion in critically ill patients

Linear scale

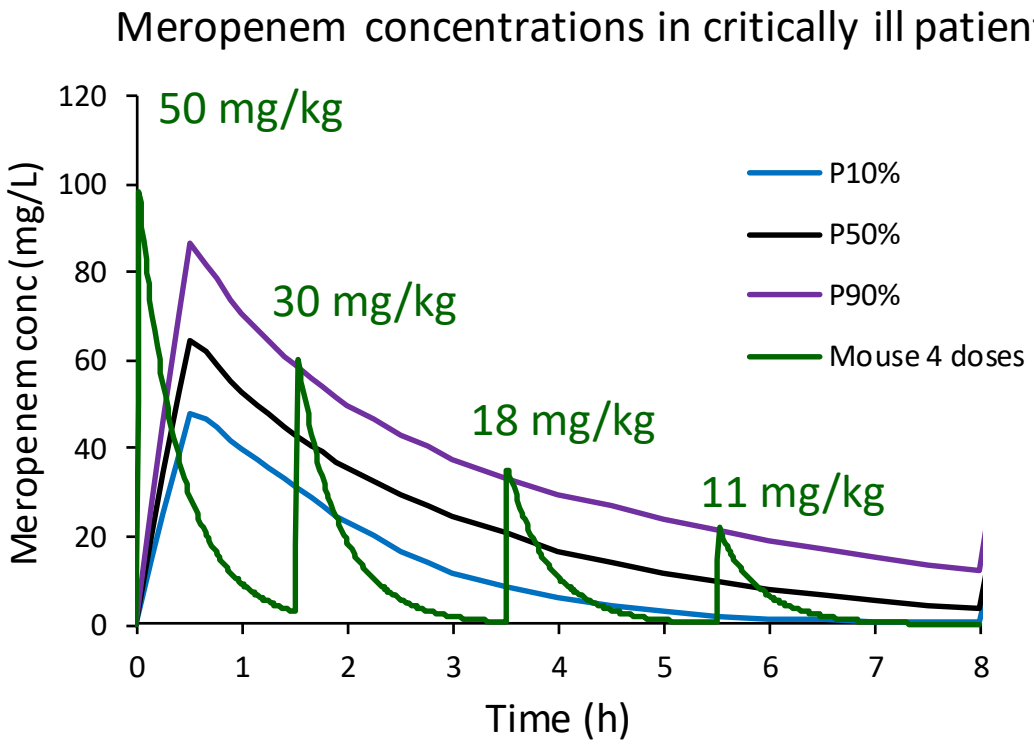


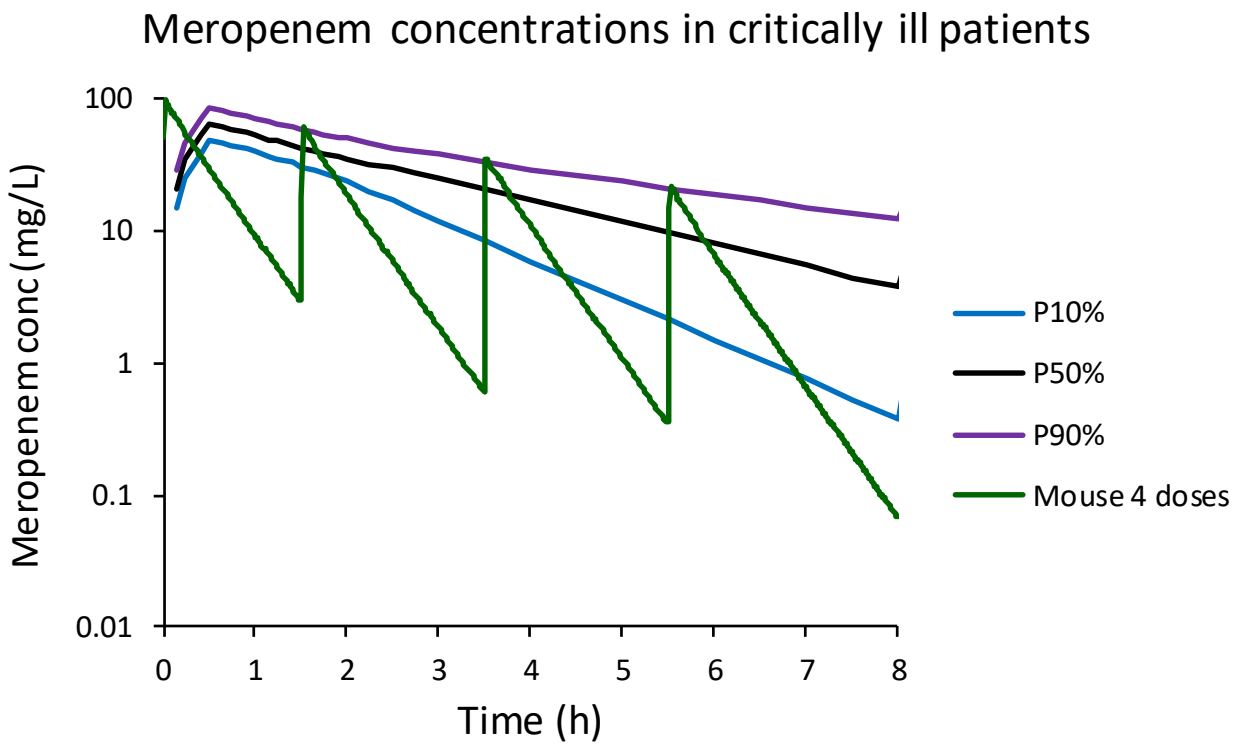
Log-scale



Monte Carlo simulated meropenem concentrations in humans for 2g q8h as 30 min infusion in critically ill patients

Linear scale **Humanized mouse regimen** **Log-scale**





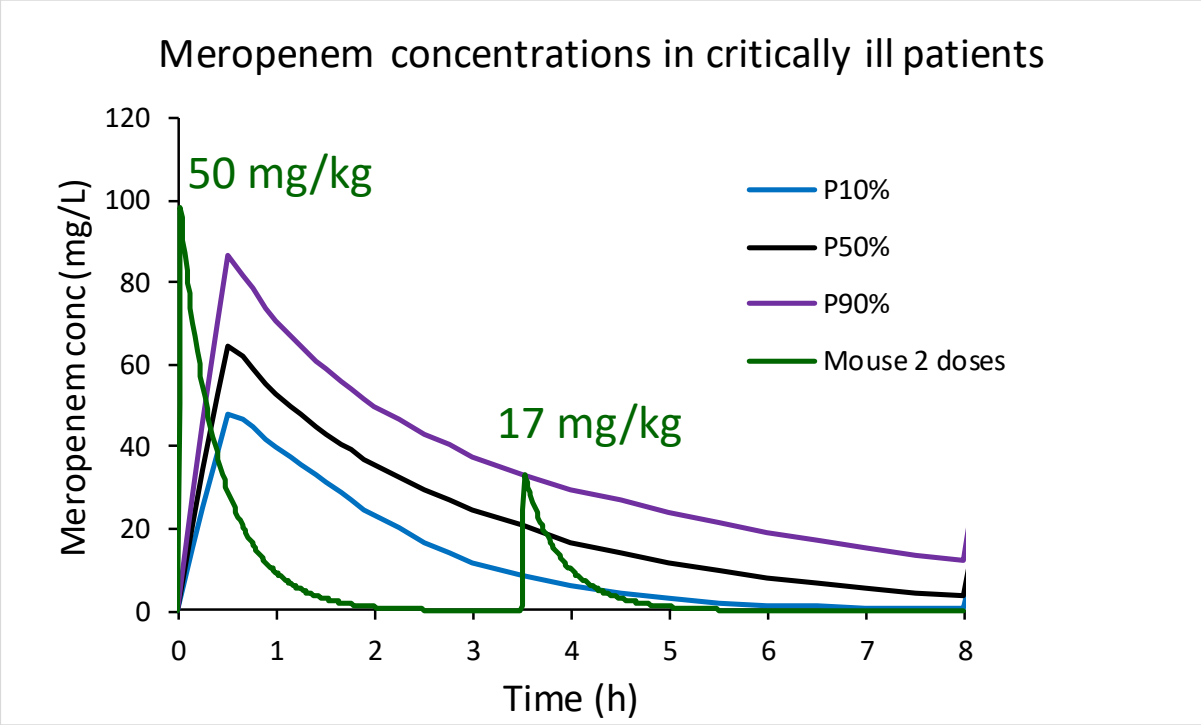
Best capture of the human concentration time profile. However, this would result in 12 (=4 x 3) doses per day.

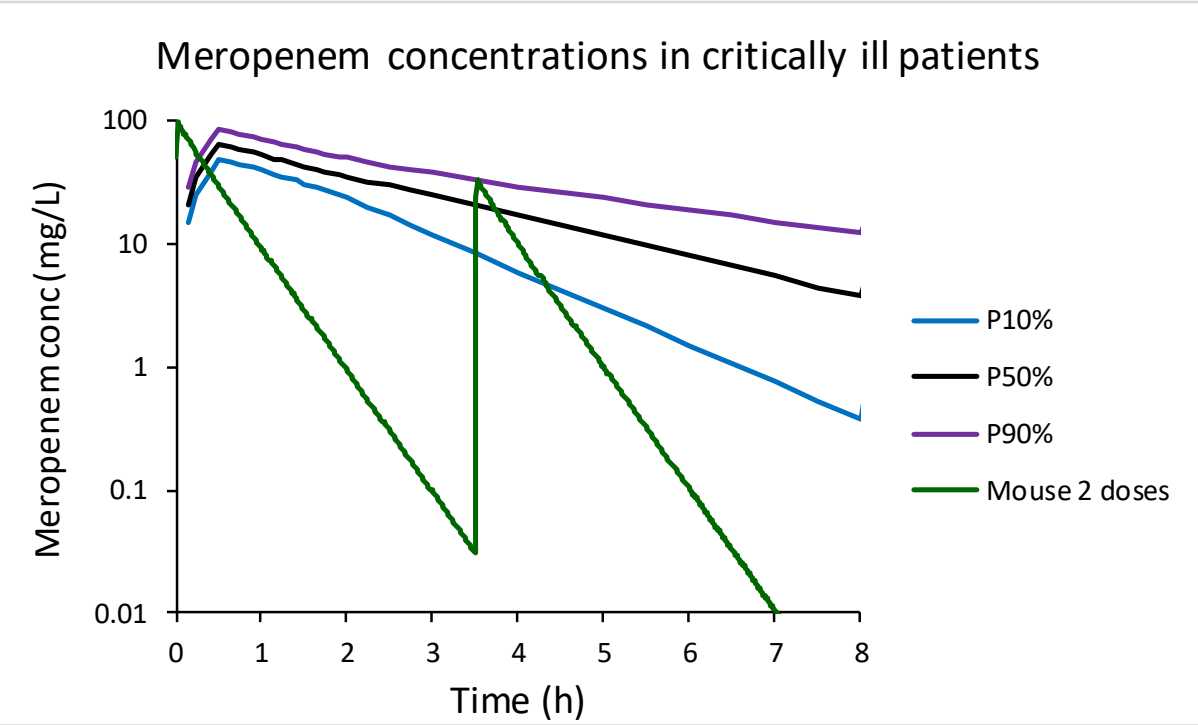
Monte Carlo simulated meropenem concentrations in humans for 2g q8h as 30 min infusion in critically ill patients

Linear scale

Humanized mouse regimen

Log-scale





If we use the criterion not to over-shoot the 90% percentile in patients, 2 doses every 8 h (i.e. 6 doses per day) do likely not well capture the time-course of plasma concentrations in humans.

Free time above MIC (fT>MIC) for different humanized regimens in mouse (OA model) using 4, 3 or 2 doses every 8 h

Carbapenem targets in mice: Stasis: ~20% *f*T>MIC
Near-max killing: ~40% *f*T>MIC

Regimen			MIC (mg/L) 0.5	1	2	4	8	16
4 doses Q8h	50 mg/kg @ 0h	**Plasma**	87%	76%	65%	52%	37%	22%
	30 mg/kg @ 1.5h	**ELF**	75%	63%	47%	25%	8%	0%
	18 mg/kg @ 3.5h							
	11 mg/kg @ 5.5h							
2 doses Q8h	50 mg/kg @ 0h	**Plasma**	51%	43%	36%	28%	21%	13%
	17 mg/kg @ 3.5h	**ELF**	43%	35%	26%	14%	7%	0%

2 doses Q8h would only achieve fT>MIC >35% for MICs up to 2 mg/L in plasma.
The covered MICs in ELF were approximately 2-fold lower than those in plasma.

Meropenem in mice

- Very short half-life relative to that in humans.
- Limited to 6 daily SC injections

- The addition of **cilastatin** or **probenecid** may reduce meropenem clearance and improve murine model.

- Uranyl nitrate likely no option, since this compound is toxic and is becoming increasingly hard to purchase.

Summary

- Immune competent mouse model
- Humanized PK
  - AMK and PMB
  - MERO in progress
- Therapeutic outcomes
  - Amikacin- outcomes reflect *in vitro* antimicrobial sensitivity data.
  - Polymyxin B & Meropenem- In progress
- Each drug to be studied has its own unique problems for developing a humanized dosing strategy

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