

Mouse Model for Testing Therapeutics Against Pulmonary *Pseudomonas* Infection

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The Food and Drug Administration

Contract: HHSF223201810171C

UNIVERSITY OF
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CENTER FOR
PREDICTIVE MEDICINE

Development of a Validated Platform for Preclinical Screening of Novel Drugs Against MDR *P. aeruginosa* Infection

Goal 1: Validate pre-existing model against a panel of *P. aeruginosa* with different antibiotic resistance profiles to represent the potential variety seen in the clinic (Dr. Lawrenz).

Goal 2: Establish human simulated dosing for two antibiotics that can be used as comparators in the model when testing novel INDs (Dr. Lepak).

Neutropenic Mouse Model of Pulmonary Infection

P. aeruginosa is an opportunistic pathogen that causes disease in immunocompromised individuals (e.g. cystic fibrosis, cancer, severe injuries)

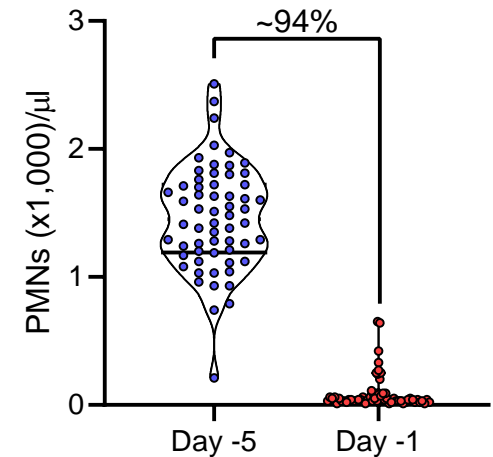
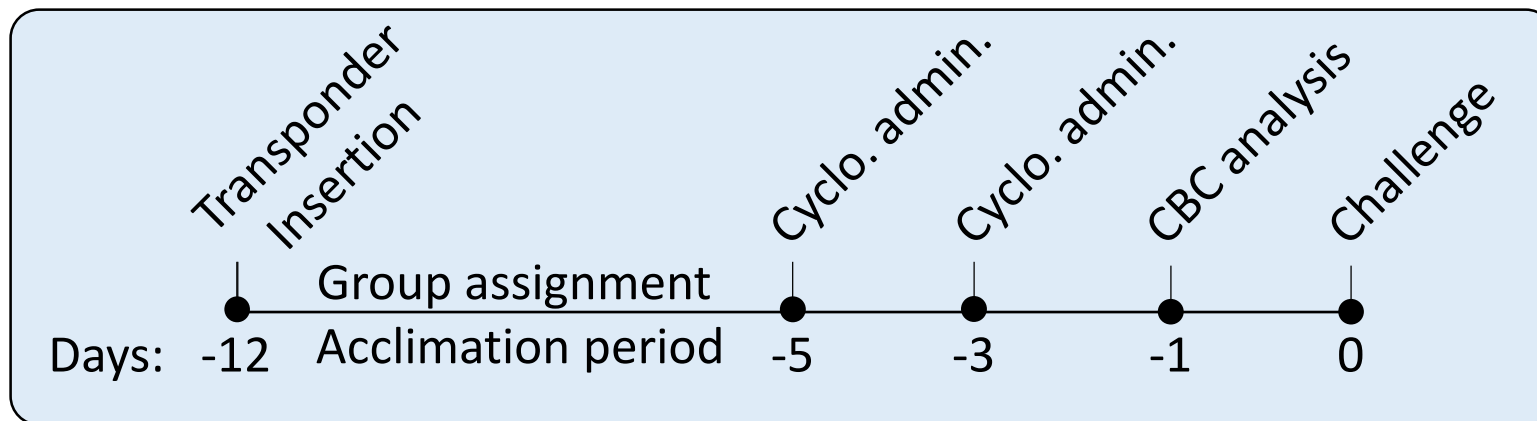
Immunocompetent mice are resistant to *P. aeruginosa* pulmonary infection
(require large numbers of bacteria)

Neutropenic Mouse Model of Pulmonary Infection

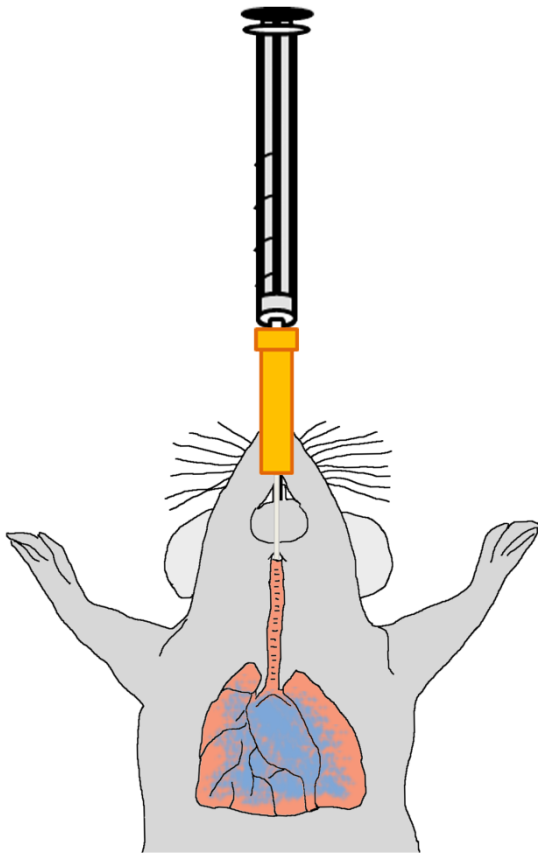
P. aeruginosa is an opportunistic pathogen that causes disease in immunocompromised individuals (e.g. cystic fibrosis, cancer, severe injuries)

Immunocompetent mice are resistant to *P. aeruginosa* pulmonary infection (require large numbers of bacteria)

To overcome this barrier, we use a transient neutropenic mouse model.

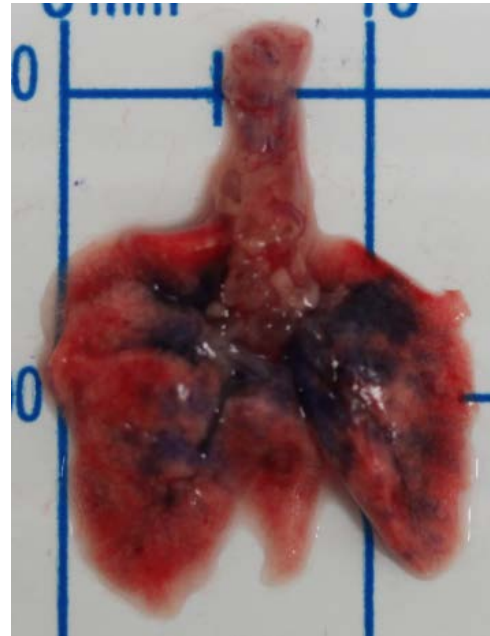


Intubation-Mediated Intratracheal (IMIT) Instillation



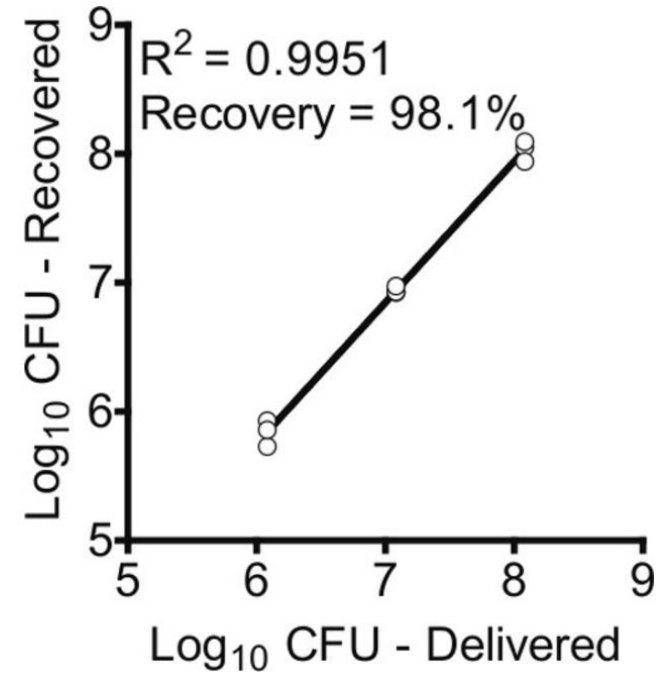
Insert blunt needle into catheter and administer bacteria

Dye distribution



Distribution throughout the lungs

Bacterial Recovery



1. ~98% efficient delivery of inoculum
2. Reproducible

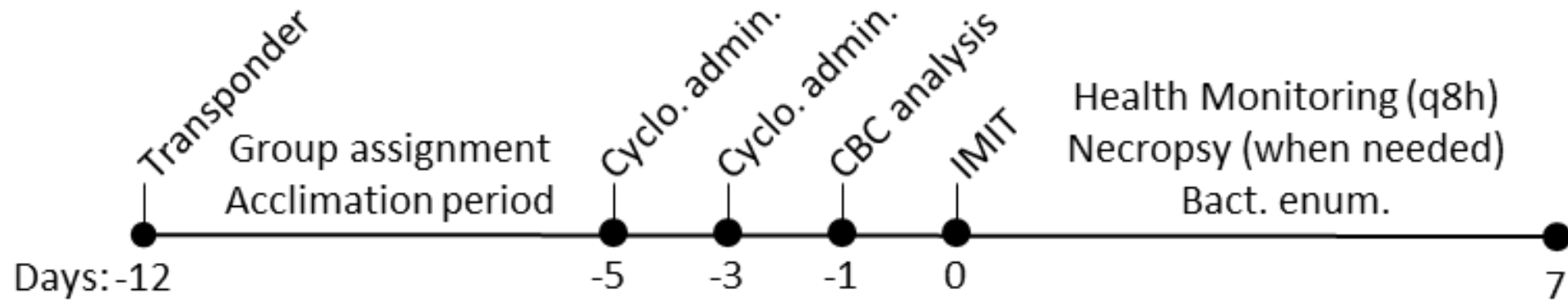
Selection of *P. aeruginosa* Strains

FDA-CDC Antimicrobial Resistance Isolate Bank *Pseudomonas aeruginosa* Panel

<i>P. aeruginosa</i> strains to be characterized in mouse model.						
Strain	Antibiotic Phenotype			Known Resistance	Virulence Determinants	Genome Accession #
	Resistant	Sensitive	Inter.			
UNC-D	Ceft, Imi, Mer, Pip, Tob	Azt, Col	--	N/A	exoS ⁺ /exoU ⁻	N/A
0230	Ami, Cefe, Ceft, Cip, Dor, Gen, Imi, Lev, Mer, Pip, Tob	Azt, Col, Pol	--	VIM	exoS ⁺ /exoU ⁻	SAMN04901620
0231	Azt, Cefe, Ceft, Cip, Dor, Gen, Imi, Lev, Mer, Pip, Tob	Ami, Col, Pol	--	KPC	exoS ⁺ /exoU ⁻	SAMN04901621
0241	Cefe, Ceft, Cip, Dor, Gen, Imi, Lev, Mer, Pip, Tob	Col, Pol	Ami, Azt	IMP	exoS ⁺ /exoU ⁻	SAMN04901631
0246	Ami, Azt, Cefe, Ceft, Cip, Dor, Gen, Imi, Lev, Mer, Pip, Tob	Col, Pol	--	NDM	exoS ⁻ /exoU ⁺	SAMN04901636

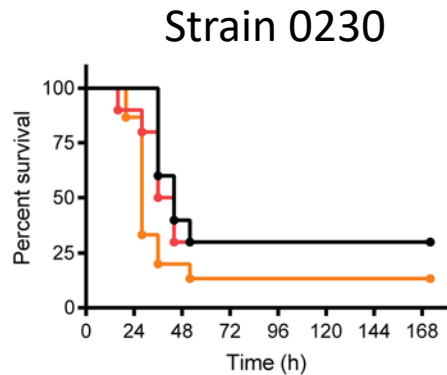
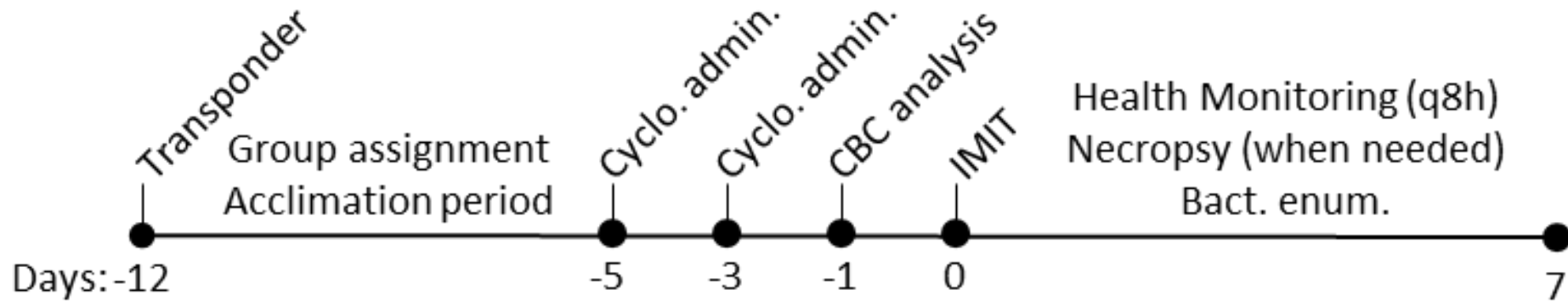
No data available on the virulence of these strains

Task 1: Determine the LD₅₀

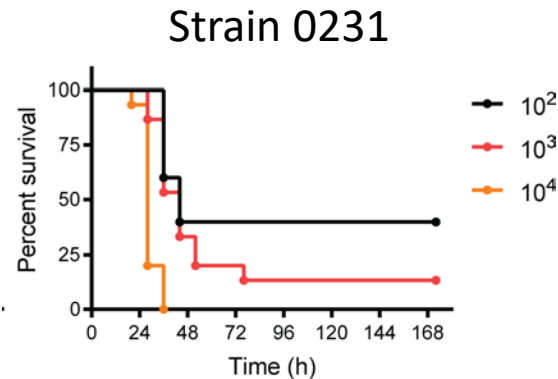


Male and female BALB/c mice (n=10) were infected with escalating doses of each strain

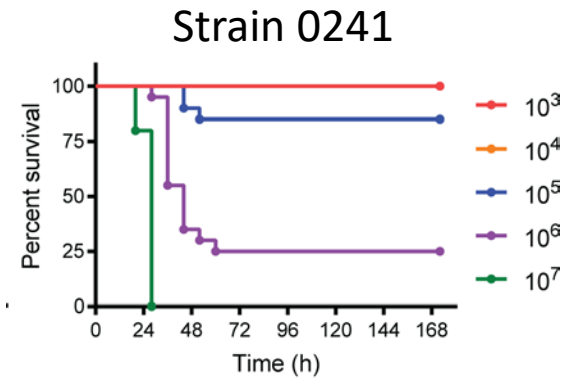
Task 1: Determine the LD₅₀



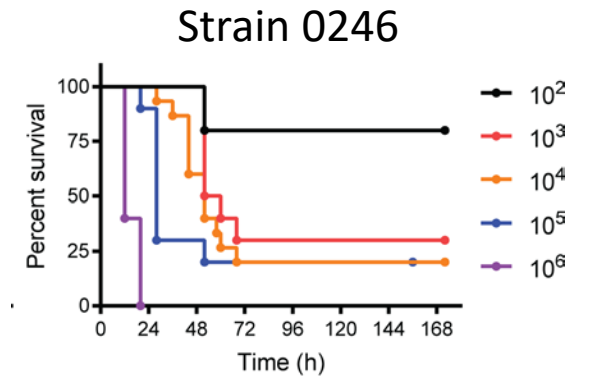
LD₅₀ = 20 CFU



LD₅₀ = 39 CFU



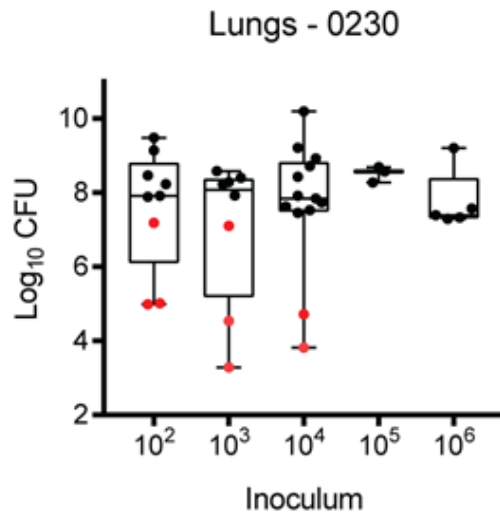
LD₅₀ = 4.1 x 10⁵ CFU



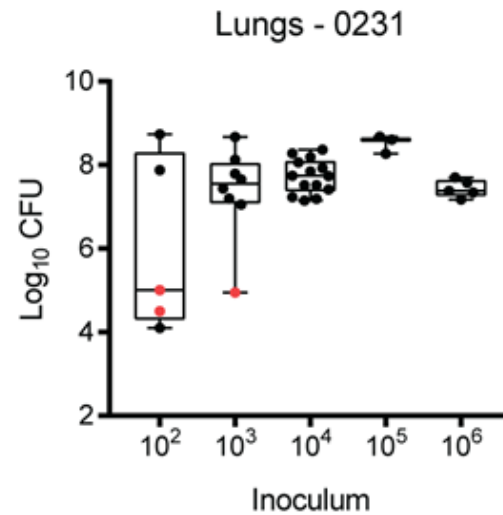
LD₅₀ = 604 CFU

Task 1: Determine the LD₅₀

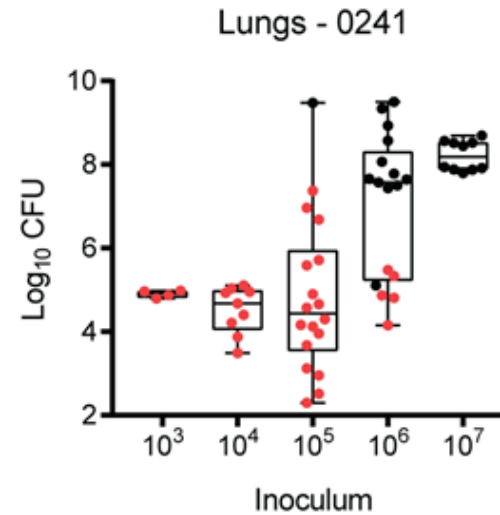
Strain 0230
LD₅₀ = 20 CFU



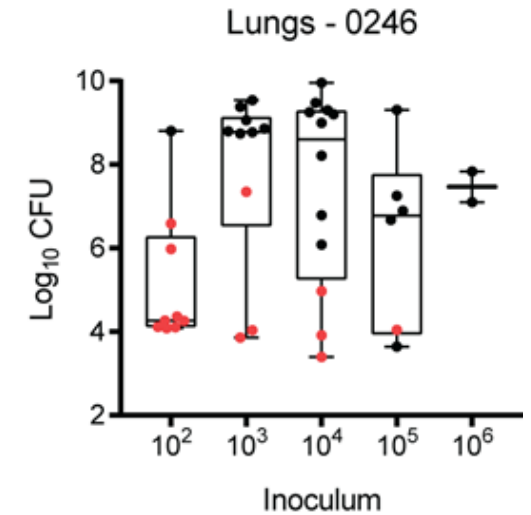
Strain 0231
LD₅₀ = 39 CFU



Strain 0241
LD₅₀ = 4.1 x 10⁵ CFU



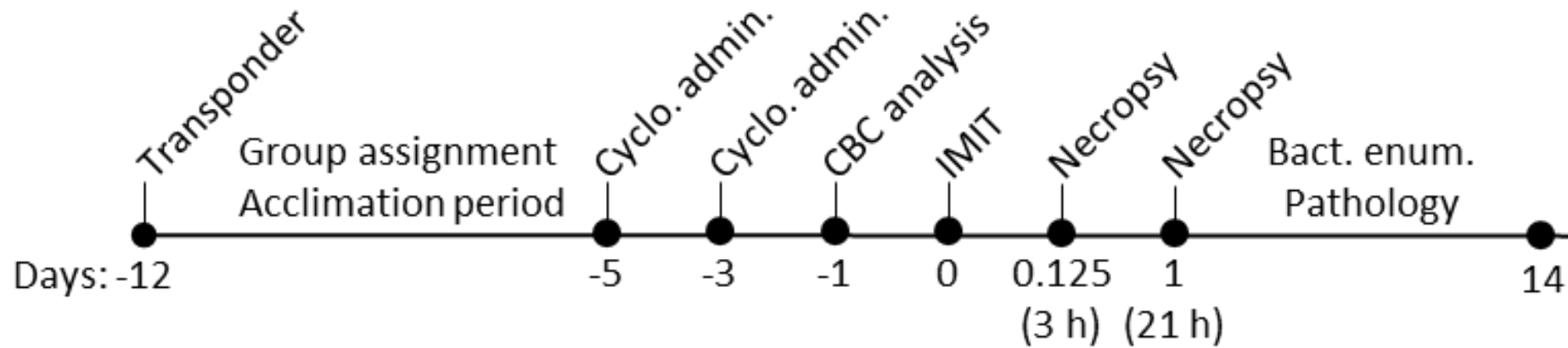
Strain 0246
LD₅₀ = 604 CFU



Mice that succumbed to infection showed bacterial proliferation in the lungs

CFU determined from animals developing moribund disease (black symbols) or from animals that survived to end of study (red symbols)

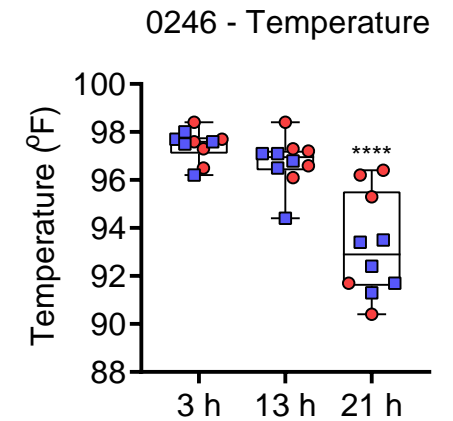
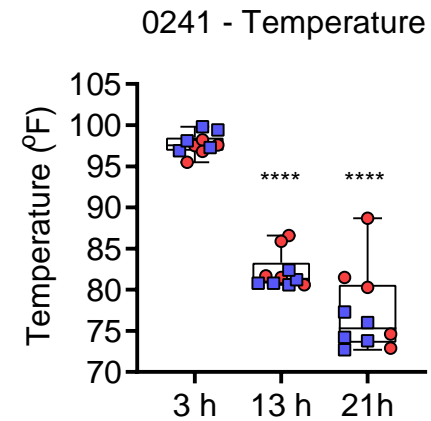
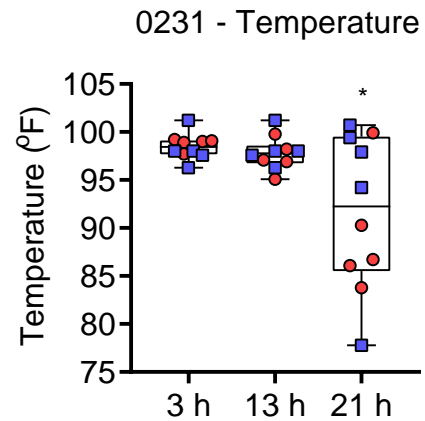
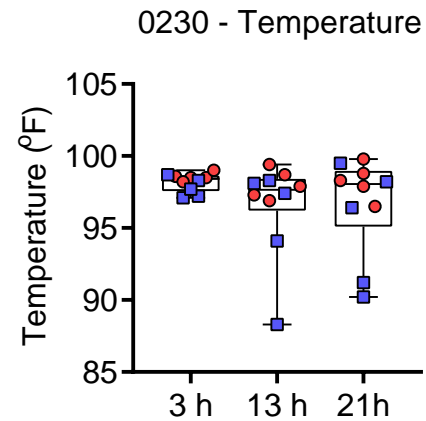
Task 2: Define the Natural History Over 21 h



Male and female BALB/c mice (n=10) were infected with 10x LD₅₀ of each strain

Task 2: Define the Natural History Over 21 h

Strain:	0230	0231	0241	0246
Target inoculum:	10^3 CFU	10^3 CFU	4×10^6 CFU	10^4 CFU



Male = blue squares; Female = red circles.

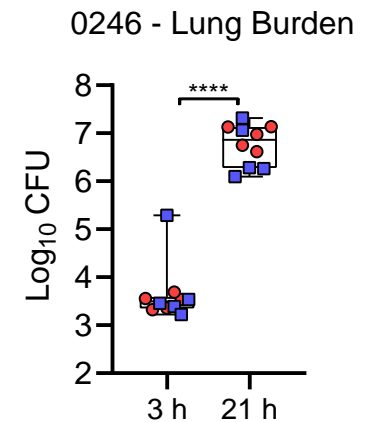
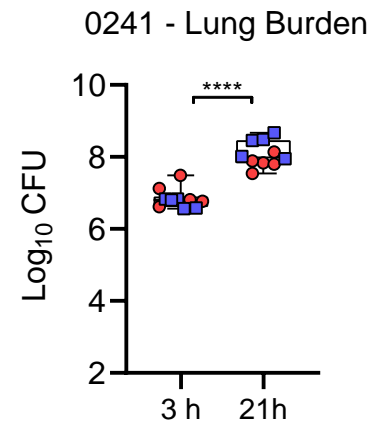
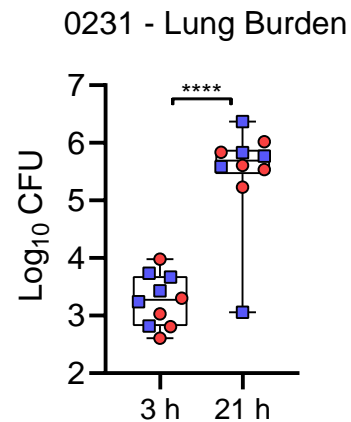
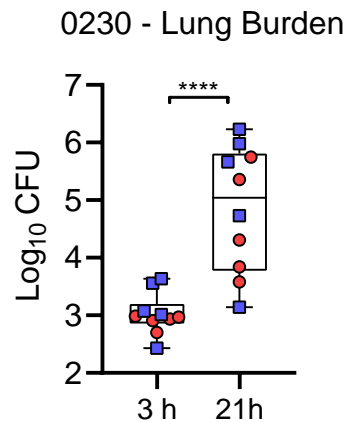
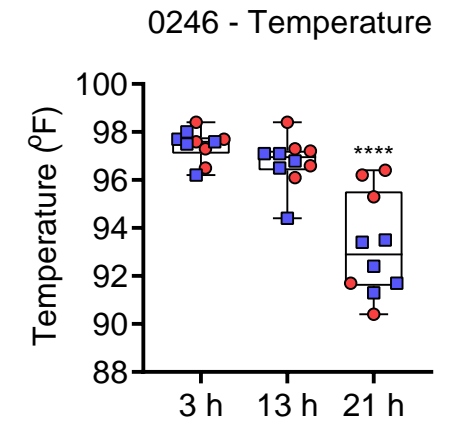
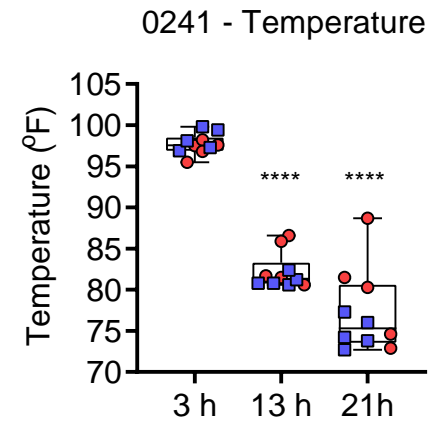
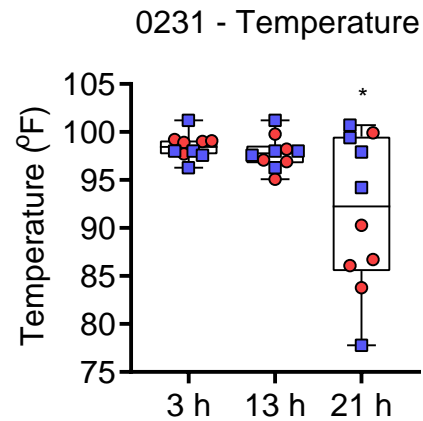
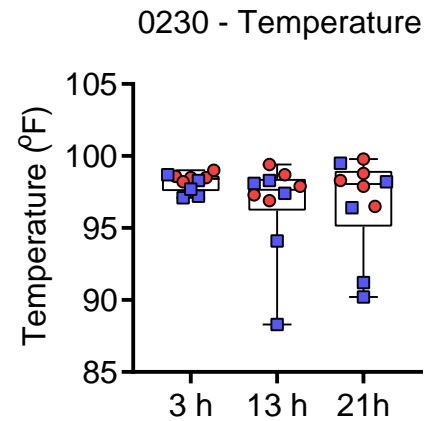
Task 2: Define the Natural History Over 21 h

Strain: 0230
Target inoculum: 10^3 CFU

0231
 10^3 CFU

0241
 4×10^6 CFU

0246
 10^4 CFU

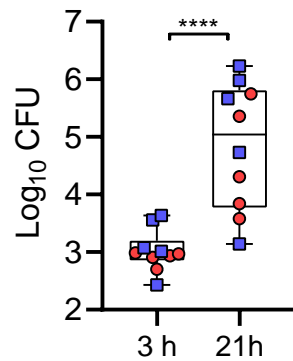


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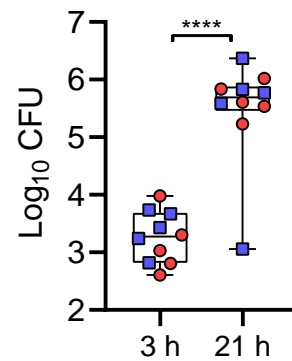
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Strain:	0230	0231	0241	0246
Target inoculum:	10^3 CFU	10^3 CFU	4×10^6 CFU	10^4 CFU

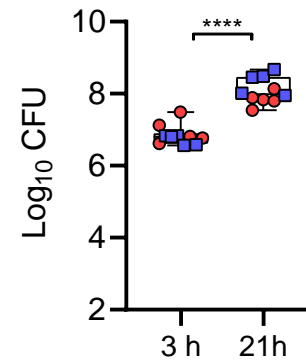
0230 - Lung Burden



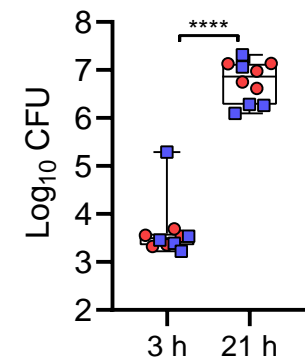
0231 - Lung Burden



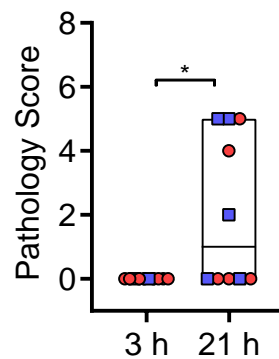
0241 - Lung Burden



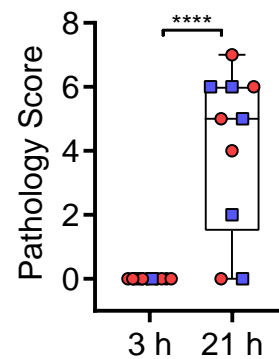
0246 - Lung Burden



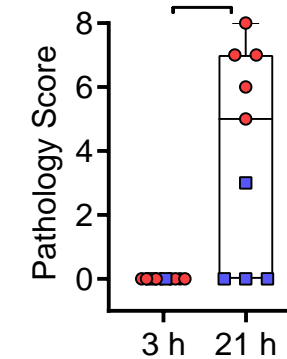
0230 - Pathology



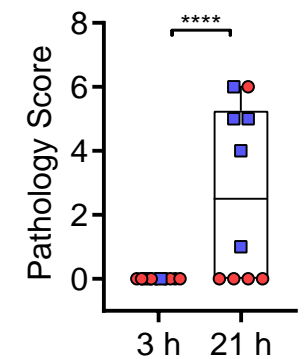
0231 - Pathology



0241 - Pathology



0246 - Pathology



Male = blue squares; Female = red circles. Pathology Score is based on four criteria and 12 point total scale.

Summary of Current Progress

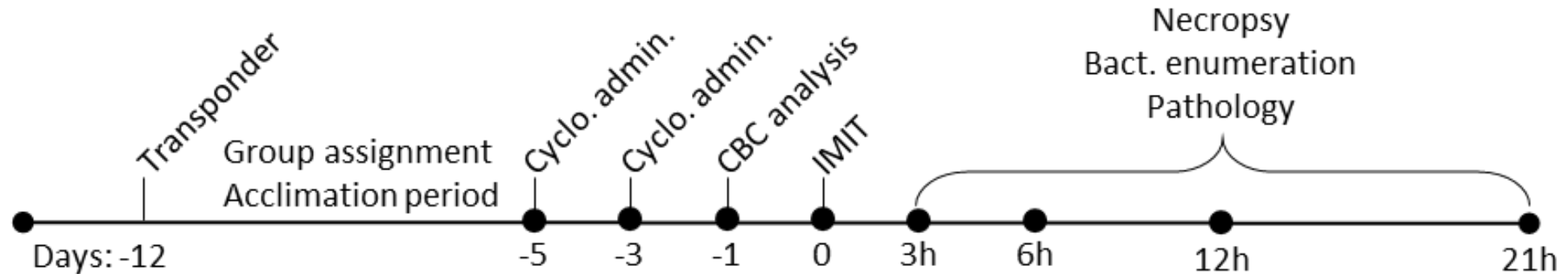
FDA-CDC Antimicrobial Resistance Isolate Bank *Pseudomonas aeruginosa* Panel

<i>P. aeruginosa</i> strains to be characterized in mouse model.										
Strain	Antibiotic Phenotype			Known Resistance	Virulence Determinants	LD ₅₀ CFU	Bacterial Load		Pathology	
	Resistant	Sensitive	Inter.				3 h	21 h	3 h	21 h
UNC-D	Ceft, Imi, Mer, Pip, Tob	Azt, Col	--	N/A	exoS ⁺ /exoU ⁻	3.6x10 ⁴	N/A	N/A	N/A	N/A
0230	Ami, Cefe, Ceft, Cip, Dor, Gen, Imi, Lev, Mer, Pip, Tob	Azt, Col, Pol	--	VIM	exoS ⁺ /exoU ⁻	20	1.1x10 ³	7.2x10 ⁴	0	4.2
0231	Azt, Cefe, Ceft, Cip, Dor, Gen, Imi, Lev, Mer, Pip, Tob	Ami, Col, Pol	--	KPC	exoS ⁺ /exoU ⁻	30	1.8x10 ³	3.0x10 ⁵	0	5.1
0241	Cefe, Ceft, Cip, Dor, Gen, Imi, Lev, Mer, Pip, Tob	Col, Pol	Ami, Azt	IMP	exoS ⁺ /exoU ⁻	4.1 x10 ⁵	6.9x10 ⁶	1.2x10 ⁸	0	6
0246	Ami, Azt, Cefe, Ceft, Cip, Dor, Gen, Imi, Lev, Mer, Pip, Tob	Col, Pol	--	NDM	exoS ⁻ /exoU ⁺	604	4.3x10 ³	5.8x10 ⁶	0	4.5

Future Directions: “Trigger-to-Treat” Criteria

Our original model began treatment at 3 h post-infection, but our data with these strains indicates that no pathology or symptoms are observed at 3 h post-infection.

Is it possible to identify disease-specific “Trigger-to-Treat” criteria?



Development of a Validated Platform for Preclinical Screening of Novel Drugs Against MDR *P. aeruginosa* Infection

Goal 1: Validate pre-existing model against a panel of *P. aeruginosa* with different antibiotic resistance profiles to represent the potential variety seen in the clinic (Dr. Lawrenz).

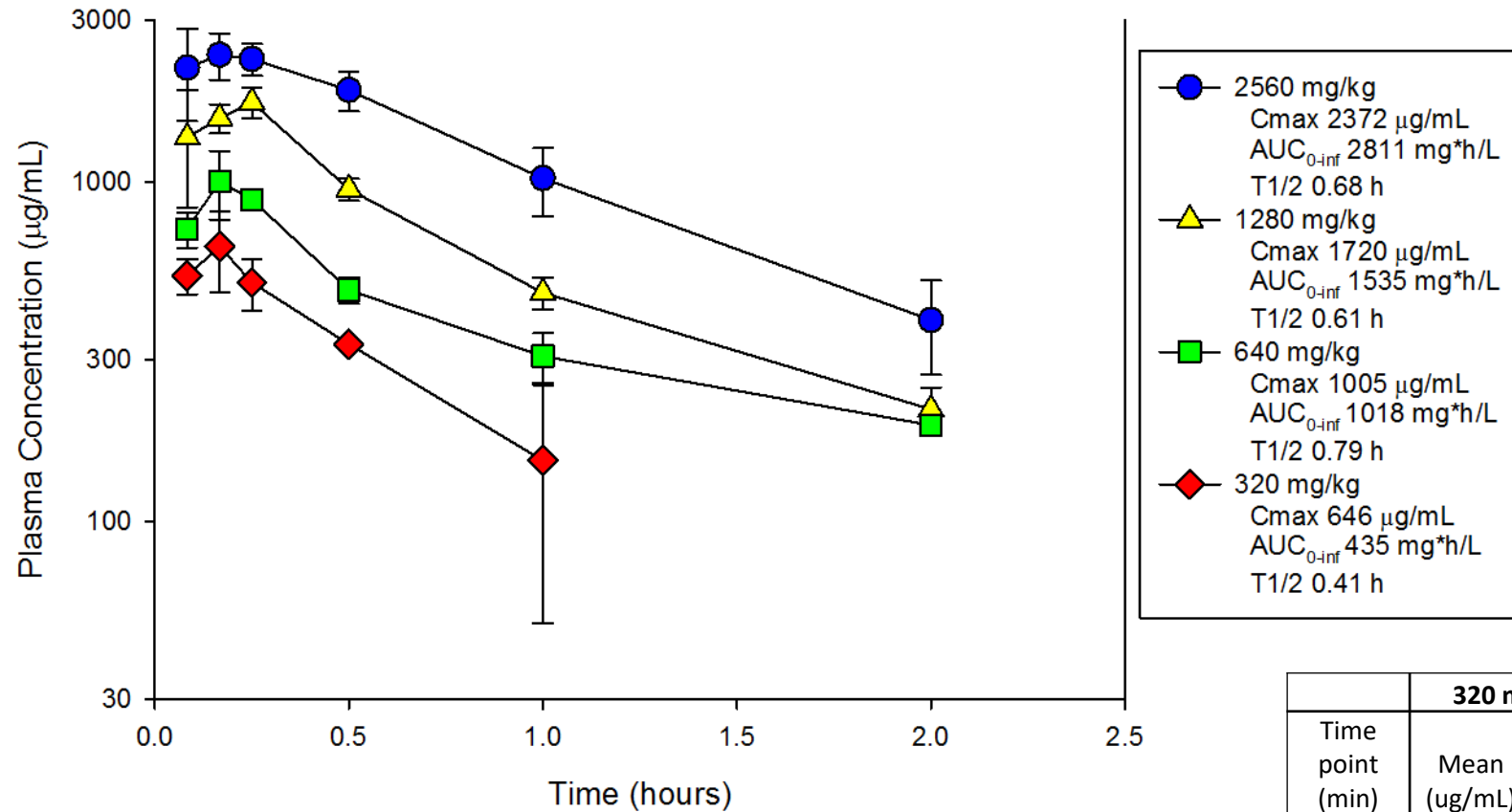
Goal 2: Establish human simulated dosing for two antibiotics that can be used as comparators in the model when testing novel INDs (Dr. Lepak).

Comparator Antibiotics

FDA-CDC Antimicrobial Resistance Isolate Bank *Pseudomonas aeruginosa* Panel

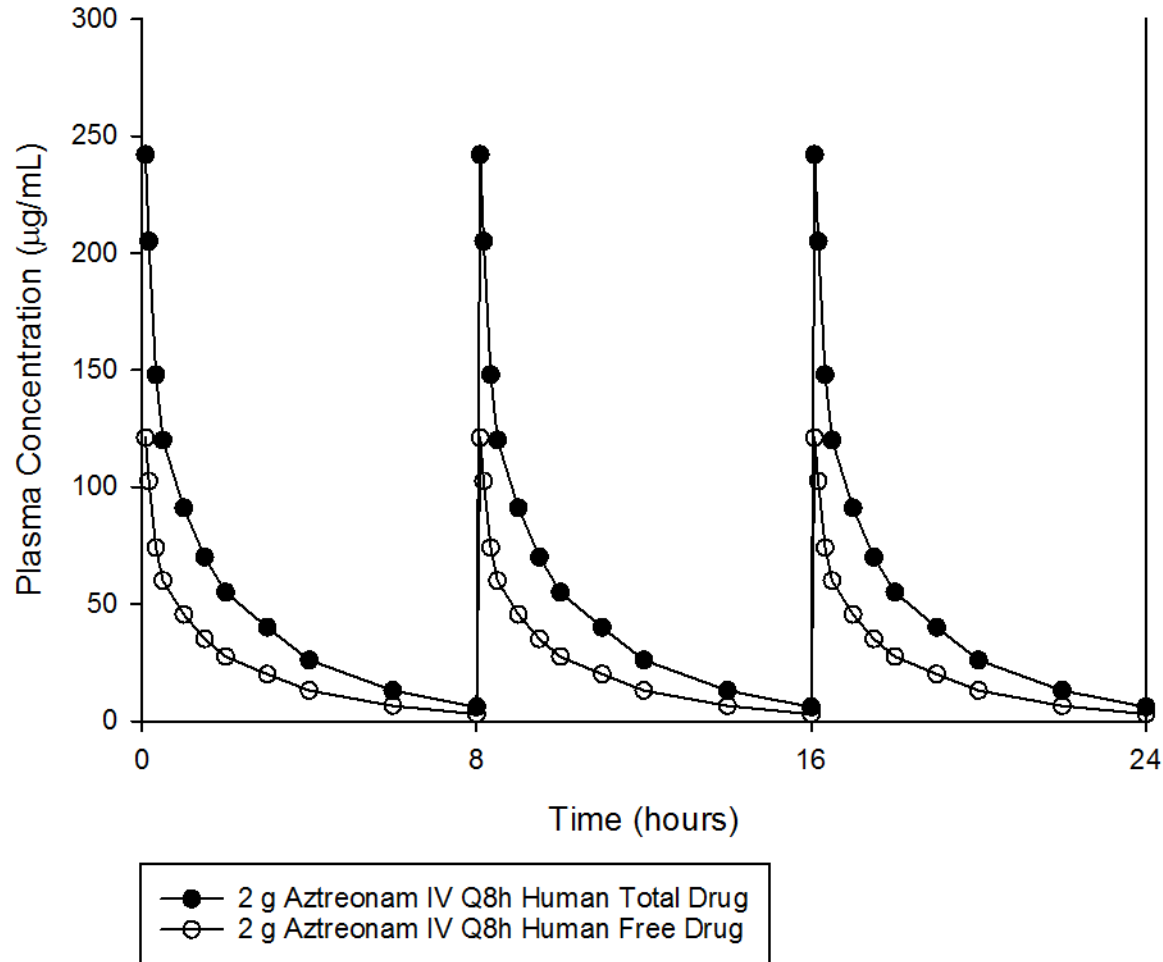
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	Resistant	Sensitive	Inter.				3 h	21 h	3 h	21 h
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0246	Ami, Azt, Cefe, Ceft, Cip, Dor, Gen, Imi, Lev, Mer, Pip, Tob	Col, Pol	--	NDM	exoS ⁻ /exoU ⁺	604	4.3x10 ³	5.8x10 ⁶	0	4.5

Aztreonam Plasma Pharmacokinetics - Mouse



	320 mg/kg SC		640 mg/kg SC		1280 mg/kg SC		2560 mg/kg SC	
Time point (min)	Mean (ug/mL)	Stdev	Mean (ug/mL)	Stdev	Mean (ug/mL)	Stdev	Mean (ug/mL)	Stdev
5	530.3	63	727.6	87.4	1353.8	511	2171	659
10	646.4	173	1005.2	229.5	1542	151	2372	374
15	506.2	87.5	889.3	45.7	1720	180	2303	249
30	333.3	9.8	481	43.1	953.1	70	1867	248
60	151.2	101.1	307.1	51.8	472.3	51.3	1026	233
120			191.9	7.5	213.7	34.5	392	121

Aztreonam Plasma Pharmacokinetics – Human (from literature)



Human Aztreonam PK

Dose (g)	C _{max} ($\mu\text{g/mL}$)	T _{1/2} (h)	AUC ($\text{mg}\cdot\text{h/L}$)
0.5	60	1.6	
1	120	2	230
2	240	1.9	415

Human Aztreonam protein binding ~50%

The Challenge – Picking a dose and regimen that is meaningful

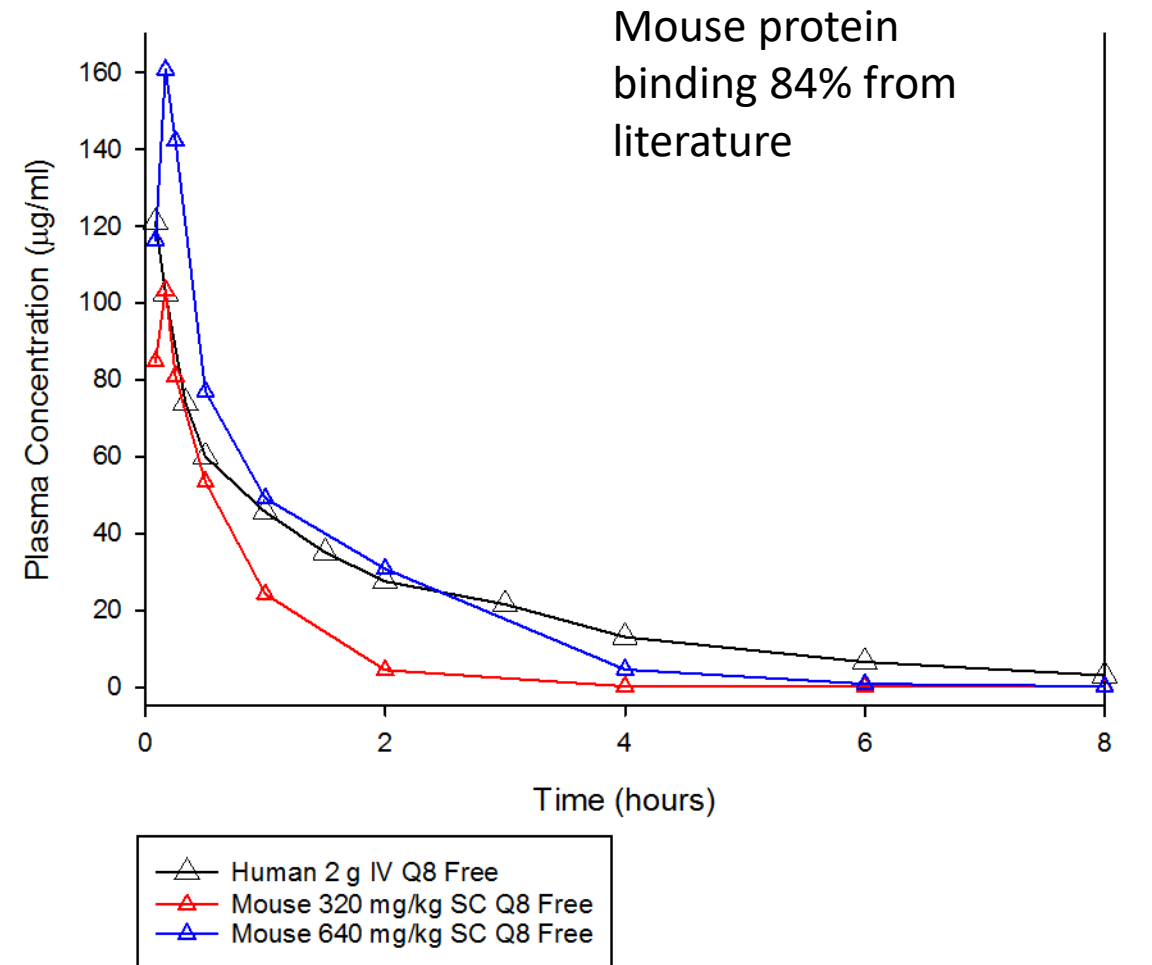
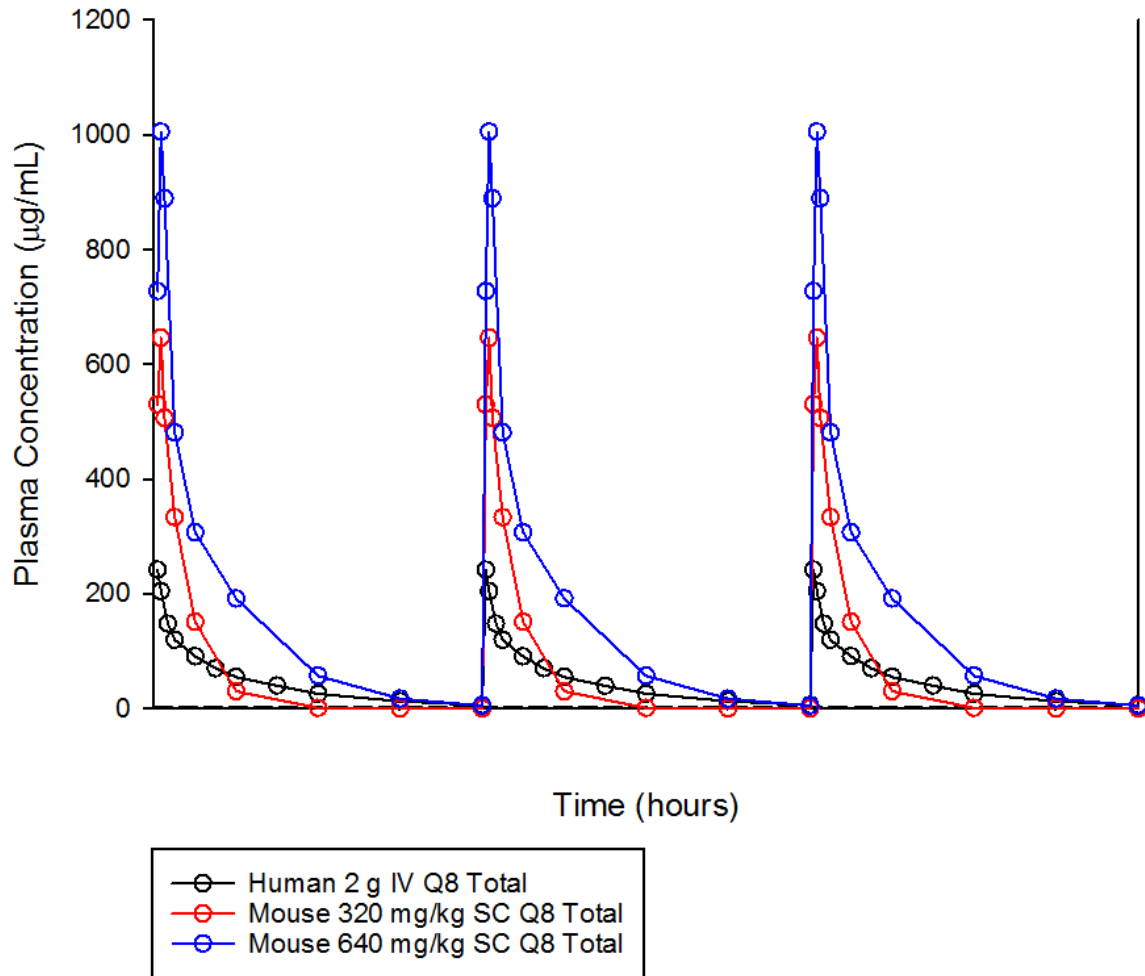
- Matching PK concentration-time curves and importantly PK/PD exposures depends on the underlying PK, MIC of organisms, and frequency of administration
- Due to differences in metabolism/elimination (common) noted in this study, it is impossible to exactly match the concentration-time curve in mice compared to humans with standard administration procedures

 • Prioritize dosing regimens in mice to mimic human exposure based on PK/PD driver

OR

- Match the shape of the curve using complex dosing regimens
 - Multiple doses (varying timing and drug amount) over the “usual” dosing interval
 - This is logistically very challenging and may lead to increased error/iatrogenic events
 - How closely do you need to “match”?

Comparison of Total and Free Aztreonam concentrations between Human and Mouse



Free Cmax is relatively well-matched between 320-640 mg/kg

Comparison free T>MIC Exposures based on regimen and MIC

Dose (mg/kg)	Dosing Interval (h)	Free Drug % Time above MIC (4 mg/L)	Free Drug % Time above MIC (32 mg/L)	Free Drug % Time above MIC (>64 mg/L)
320	4	51	21	0
640	4	100	45	5
1280	4	96	50	19
2560	4	100	66	32
320	6	34	14	0
640	6	69	30	3
1280	6	64	33	12
2560	6	78	44	22
320	8	26	10	0
640	8	52	22	2
1280	8	48	25	9
2560	8	59	33	16
320	12	17	7	0
640	12	32	15	1
1280	12	35	17	6
2560	12	39	22	11
HUMAN 2 g Dose	8	97	38	0

Good Coverage of T>MIC exposures

Aztreonam targets (prev. identified in mouse models):

- Stasis 40-50% free T>MIC
- 1- or 2 –log kill 50-60% free T>MIC

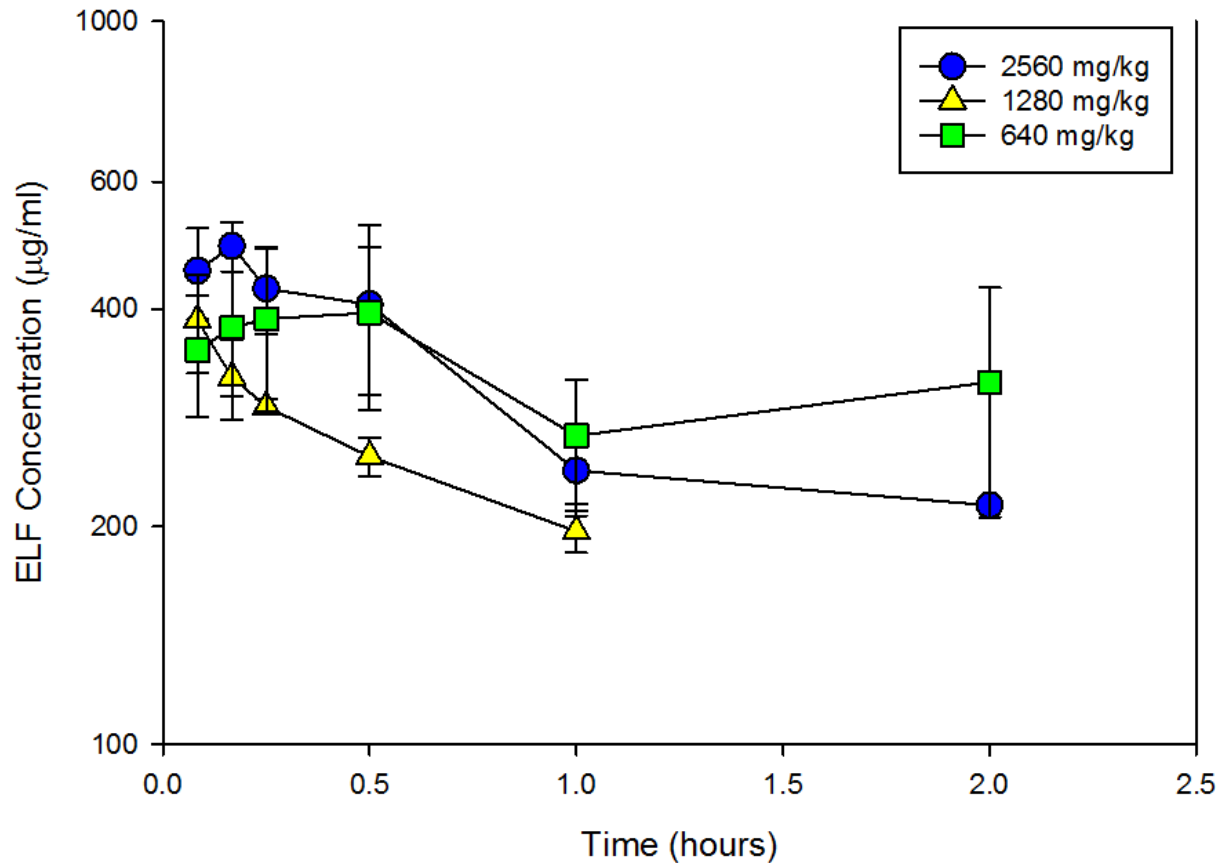
Human protein binding 50%, mouse protein binding 84%

Dosing Regimen to Achieve Study Goals

- Aztreonam 640 mg/kg/6h SC in the mouse will achieve a free Cmax slightly higher than humans (1.3-fold), but will achieve T>MIC exposures against organisms (susceptible and resistant) that would be predicted to be therapeutically successful for a susceptible organism (MIC=4 µg/ml), marginal for a resistant strain (MIC=32 µg/ml), and unsuccessful for a highly resistant strain (MIC>64 µg/ml) in order to demonstrate and distinguish differences in drug efficacy using this mouse model

Dose (mg/kg)	Dosing Interval (h)	Free Drug % Time above MIC (4 mg/L)	Free Drug % Time above MIC (32 mg/L)	Free Drug % Time above MIC (>64 mg/L)	Free Cmax (mg/L)
Mouse 640 SC	6	69	30	3	160
HUMAN 2 g Dose IV	8	97	38	0	121

Aztreonam ELF Pharmacokinetics - Mouse



Dose (mg/kg)	Penetration (% AUC ELF/plasma)
640	42.6
1280	18.7
2560	24.2

Robust human ELF Aztreonam data, in literature, is essentially non-existent

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