

Ceftazidime / NXL104, an alternative to the
use of carbapenems in the treatment of
Gram-negative infections

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novexel
novel therapies for infectious disease



The challenge of Gram-negative resistance



- **Gram-negative pathogens are significant causes of serious infections**
- **High level of resistance and multi- or pan-resistant strains of some species**
 - **Plasmids can harbor several genes conferring resistance to different classes of antibiotics: β -lactams, quinolones, aminoglycosides, sulfonamides**
 - **Changes in permeability may affect several classes of antibiotics**
- **Limited therapeutic options**
 - **β -lactams, quinolones, aminoglycosides**
 - **Old drugs (colistine)**
- **Inappropriate empiric antimicrobial treatment contribute to significantly greater mortality rates**

Key factors in resistance



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- **Gene mutations causing resistance or decreased susceptibility**
 - **Presence of resistance genes**
 - **Activation of efflux pumps**
 - **Altered permeability due to loss or changes in porins**
 - **Any combination of above mechanisms**

 - **Exchange of resistance genes**
 - **Proliferation and spread of resistant clones**
 - **Selective pressure of antibiotic use**

Resistance in Gram-negatives (1)



- **β -lactams have been workhorse agents for the treatment of Gram-negative infections**
- **Gram-negative resistance to β -lactams is typically caused by production of β -lactamases, that demonstrated an extremely potential for evolution and spreading**
- **β -lactamases now compromise the utility of penicillins and cephalosporins**
NNIS report on resistance rates to 3rd generation cephalosporins in isolates from patients of US ICUs in 2003:
 - *K. pneumonia* : 20.6% non-susceptible (47% increase compared to 1998-2002)
 - *Enterobacter spp*: 31.1% non-susceptible
 - *E. coli*: 5.8% non-susceptible

Resistance in Gram-negatives (2)



- For the present, carbapenems remain reliable antimicrobial agents against Gram-negatives
 - CSLI recommends that ESBL producers be considered resistant to all penicillins, all cephalosporins and aztreonam
- Increasing prevalence of serine carbapenemases may compromise this treatment option
 - KPC enzymes, transferable plasmids
 - OXA enzymes (*Pseudomonas*, *Acinetobacter*)

Overview of β -lactamases (1)



Class A β -lactamases

- The most abundant class; TEM and SHV types have been the most investigated
- ESBL variants (mutations that broaden specificity)
- Inhibited by clavulanate, sulbactam, tazobactam
- CTX-M has spread in community and hospital settings
- KPCs are emerging (*Klebsiella*)

Class B β -lactamases

- Metallo-enzymes
- Still relatively rare (*Pseudomonas* mainly)
- Large substrate spectrum
- No clinically useful inhibitor

Overview of β -lactamases (2)



Class C β -lactamases

- Widely distributed in gram-negatives, chromosomal or plasmidic
- Plasmid-encoded variants on the increase
- Broad substrate specificity, high levels of enzyme
- No inhibitor in the clinic

Class D β -lactamases

- Relatively rare and of minor importance until recently (*Pseudomonas*, *Acinetobacter*)
- Heterogenous group of enzyme in terms of substrate specificity
- Carbapenemase variants emerging
- No clinically useful inhibitor

Strategies to overcome β -lactamases



Find or synthesize β -lactam resistant to hydrolysis

- 3rd generation cephalosporins: cefotaxime, ceftazidime, ceftriaxone
- Carbapenems
- Extension of antibacterial spectrum to MRSA may be difficult to combine with stability to β -lactamases (ceftobiprole, ceftaroline)

Co-administrate an inhibitor of β -lactamases

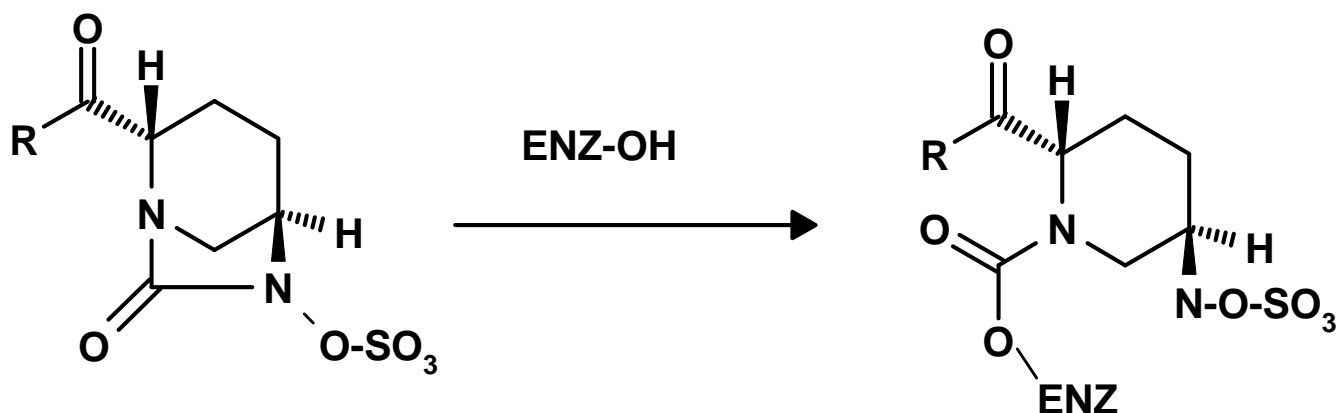
- Inhibitors in clinical use: Clavulanate, Tazobactam, Sulbactam
- β -lactam compounds
- Class A inhibitors
- Do not respond anymore to current β -lactamase epidemiology

The success story of combinations β -lactam / β -lactamase inhibitor



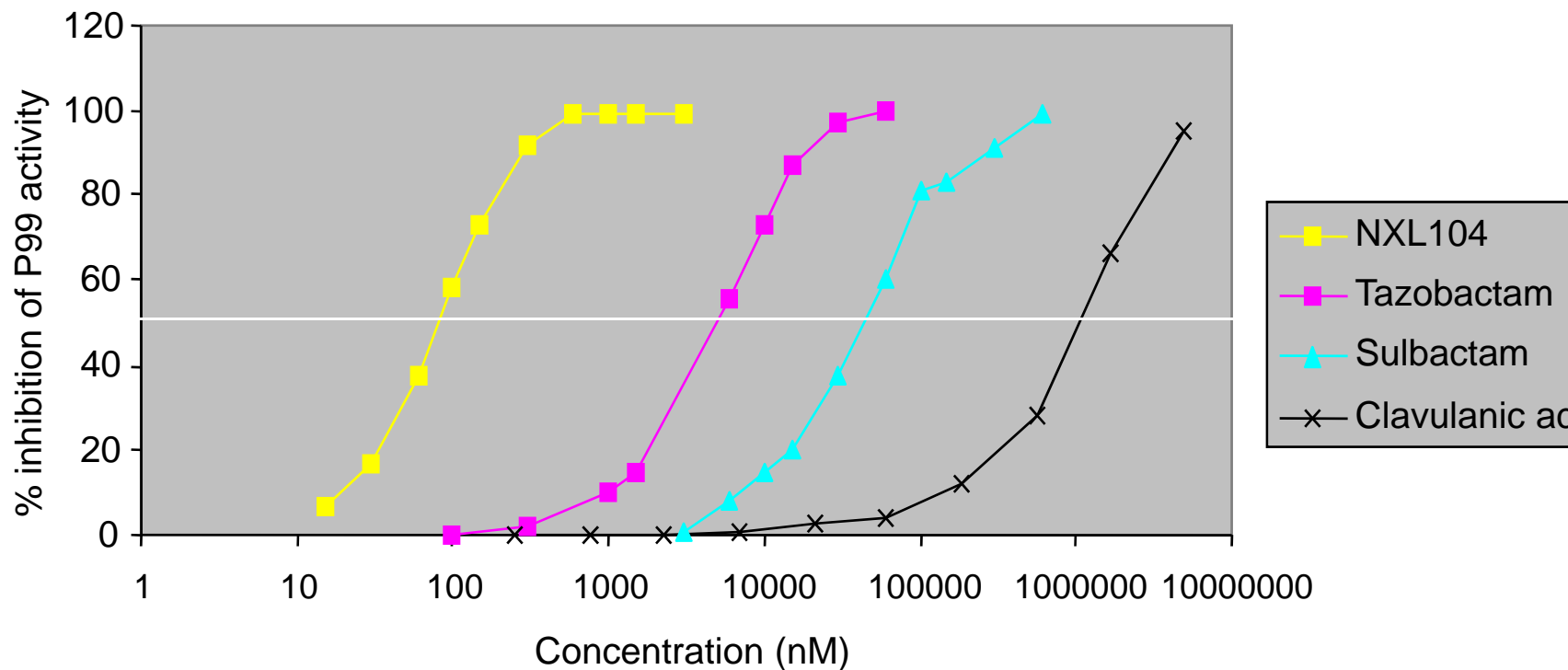
- **Safety of the compounds**
- **Suicide inhibitors**
 - Inhibitors are also substrates of the enzyme
 - Long half-life covalent intermediates
- **Inhibitor-resistant mutant enzymes**
 - They have emerged at an extremely low frequency
 - Substrate spectrum is often reduced due to lower catalytic efficiency
- **Drawbacks essentially reflects the evolutionary adaptation of b-lactamases**
 - Enzymes with broaden b-lactam spectrum: ESBLs, carbapenemases
 - New enzymes: mobilization from environmental pool for some OXAs
 - Intra- and inter-species spread: mobilization of class C on plasmids
 - Production of multiple enzymes

Mechanism-based inhibition of serine β -lactamases



**Inhibition of β -lactamase by acylation of the catalytic serine : formation of a very stable carbamoyl linkage in the enzyme-inhibitor complex
(Irreversible suicide inhibitor)**

NXL104 - Class C P99 inhibition



P99	NXL104	Tazobactam	Sulbactam	Clavulanic acid
IC ₅₀ (nM)	80	5,000	42,000	10 ⁶

NXL104 activity against purified β -lactamases



	Class	Enzyme	Clavulanate	Tazobactam	NXL104
IC ₅₀ (nM)	A	TEM-1	130	40	8
	A	SHV-4	5	120	1.5
	A	KPC-2	6,500	80,000	38
	C	P99	100,000	5,000	80
Turnover number	A	TEM-1	214	nd	2
	A	KPC-2	nd	nd	4
	C	P99	nd	55	5
Deacylation half time	A	TEM-1	~7 min	nd	~7 days
	C	P99	nd	~290 min	~7 days

Broader spectrum activity: Active on Class A and C β -lactamases

Lower turnover number: Active at lower concentration

Longer half-life of the covalent intermediate : Longer inactivation of enzyme

In vitro spectrum of β -lactamase inhibitors



		Clavulanate	Tazobactam	NXL104
Class A	TEM, SHV, and ESBLs	✓	✓	✓
	CTX-M and ESBLs	✓	✓	✓
	PER, VEB, GES	✓	✓	✓
	KPC	✗	✗	✓
Class B	IMP, VIM	✗	✗	✗
Class C	Chromosomal <i>Enterobacteriaceae</i> AmpC	✗	✗	✓
	Chromosomal <i>Pseudomonas</i> AmpC	✗	✗	✓
	Plasmidic ACC, DHA, CMY, FOX, LAT, MOX, MIR, ACT	✗	✗	✓
Class D	Penicillinase-type OXA-1, -31, -10, -13	Variable OXA-1, -10	Variable	Variable OXA-1, -31
	Carbapenemase-type OXA-23, -40, 48, -58	Variable	Variable OXA-23, -48	Variable OXA-48

CAZ/NXL104 *in vitro* antibacterial activity



Comparative anticipated spectrum of activity against β -lactamase producing pathogens

	CAZ	CAZ/104	PIP/TAZ	FEP	IPM	MER	ETP
<i>K. pneumoniae</i> (ESBL)	Variable susceptibility	Most isolates susceptible	Variable susceptibility	Variable susceptibility	Most isolates susceptible	Most isolates susceptible	Most isolates susceptible
<i>K. pneumoniae</i> (KPC)	Most isolates resistant	Most isolates susceptible	Most isolates resistant	Most isolates resistant	Most isolates resistant	Most isolates resistant	Most isolates resistant
<i>E. coli</i> (ESBL)	Variable susceptibility	Most isolates susceptible	Variable susceptibility	Variable susceptibility	Most isolates susceptible	Most isolates susceptible	Most isolates susceptible
<i>Enterobacter spp</i> (AmpC)	Most isolates resistant	Most isolates susceptible	Most isolates resistant	Variable susceptibility	Most isolates susceptible	Most isolates susceptible	Most isolates susceptible
<i>Citrobacter spp</i> (AmpC)	Most isolates resistant	Most isolates susceptible	Most isolates resistant	Variable susceptibility	Most isolates susceptible	Most isolates susceptible	Most isolates susceptible
<i>P. aeruginosa</i> (AmpC)	Most isolates resistant	Most isolates susceptible	Most isolates resistant	Variable susceptibility	Most isolates susceptible	Most isolates susceptible	Most isolates resistant
<i>P. aeruginosa</i> (MBL)	Most isolates resistant	Most isolates resistant	Most isolates resistant	Most isolates resistant	Most isolates resistant	Most isolates resistant	Most isolates resistant
<i>A. baumannii</i>	Most isolates resistant	Most isolates resistant					

	Most isolates susceptible
	Variable susceptibility
	Most isolates resistant

CAZ/NXL104 activity against KPC producers (1)



Organism	ID	Mechanism	CAZ	CRO	IPM	CAZ + NXL104 4 mg/L	CRO + NXL104 4 mg/L	IPM + NXL104 4 mg/L
<i>K. pneumonia</i>	YC	KPC-2	>128	>128	32	2	0,25	<=0.125
<i>E. coli</i>	2138	KPC-2	128	>128	16	0,25	<=0.125	0,25
<i>E. cloacae</i>	7506	KPC-2	>128	>128	128	8	0,5	0,25
<i>E. cloacae</i>	MAC	KPC-3 TEM-1, OXA-9	>128	>128	64	8	1	2
<i>K. pneumonia</i>	VA8	KPC-3	>128	32	0.25	1	<=0.125	0.25
<i>K. pneumonia</i>	VAKP	KPC-2	128	>128	32	1	<=0.125	2

CAZ/NXL104 activity against KPC producers (2)



Organism	ID	Mechanism	IPM	CAZ	CAZ + NXL104 4 mg/L	CTX	CTX + NXL104 4 mg/L	PIP + TAZO 4 mg/L
<i>E. coli</i>	DH5α	KPC-3	8	128	0.25	>128	≤0.015	>128
<i>K. pneumoniae</i>	CL-5761	KPC-3*	>32	>128	≤0.015	>128	≤0.015	>128
<i>K. pneumoniae</i>	CL-5762A	KPC-3*	32	>128	≤0.015	>128	≤0.015	>128
<i>K. pneumoniae</i>	CL-5762B	KPC-3*	32	>128	≤0.015	>128	≤0.015	>128
<i>K. pneumoniae</i>	CL-5763	KPC-3*	>32	>128	≤0.015	>128	≤0.015	>128
<i>Enterobacter</i>	E624	KPC-4	>32	>128	32	>128	1	>128

•: in addition to loss of OmpK35 (Woodford, AAC 2004)

E264: in addition to derepressed AmpC and porin loss; susceptible to cefotaxime

Isolates with combined mechanisms of resistance

ID	Organism	Mechanisms	ERT	IPM	PIP + TAZO 4 mg/L	CAZ	CAZ + NXL104 4 mg/L
H044161	<i>E. aerogenes</i>	AmpC	R	1	128	>128	0.5
H043100	<i>E. aerogenes</i>	AmpC	R	>32	>128	>128	64
H043100	<i>Enterobacter</i>	AmpC	R	0.5	16	32	1
H060340	<i>E. aerogenes</i>	AmpC	R	8	>128	>128	2
H053520	<i>E. cloacae</i>	AmpC	R	4	>128	>128	2
H045100	<i>E. cloacae</i>	CTX-M-1 & 9	R	1	64	128	0.5
H050980	<i>E. cloacae</i>	ESBL	R	1	128	>128	2
H051000	<i>E. cloacae</i>	ESBL	R	8	>128	>128	2
H042640	<i>E. aerogenes</i>	ESBL	R	16	128	>128	1
H053720	<i>Klebsiella sp</i>	CTX-M-1 group	R	0.5	>128	>128	1
H054000	<i>Klebsiella sp</i>	CTX-M-1 group	R	1	>128	128	1
H054120	<i>Klebsiella sp</i>	CTX-M-1 group	R	2	>128	>128	2
H054120	<i>K. pneumoniae</i>	CTX-M-1 group	R	1	>128	>128	2
H054200	<i>Klebsiella sp</i>	CTX-M-1 group	R	0.5	>128	>128	0.5
H055120	<i>Klebsiella sp</i>	CTX-M-1 group	R	4	>128	>128	4
H061260	<i>Klebsiella sp</i>	CTX-M-1 group	R	0.25	>128	>128	0.03
H051880	<i>E. cloacae</i>	CTX-M-9 group	R	2	128	128	2

CAZ/NXL104 is active against isolates with CTX-M or AmpC enzymes together with permeability alterations (Ertapenem-R)

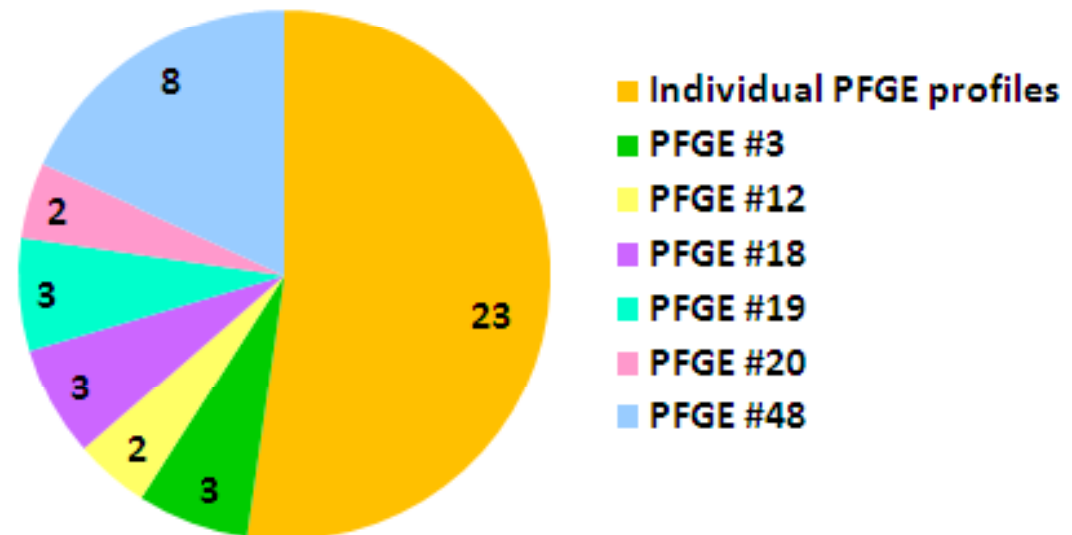
ESBL multiproducers

<i>K. pneumoniae</i> #	β -lactamase	CAZ	CAZ + NXL104 (4 mg/L)	PIP + TAZO (4 mg/L)	IPM
236	SHV-5, TEM-10	>128	2	>128	2
238	SHV-2, TEM-12, CTX-M-2	>128	2	>128	0.25
243	SHV-5, TEM-63	>128	1	>128	1
181	SHV-5, TEM-10	>128	2	>128	4
253	SHV-2, TEM-12, CTX-M-2	>128	1	>128	0.5
465	TEM-1B, CTX-M-2	64	4	64	1
427	SHV-1, TEM-1B, CTX-M-3	>128	1	>128	0.5
60	SHV-2, TEM-2, PER	64	4	>128	0.25
441	SHV-1, TEM-2, PER	>128	8	>128	0.25
444	SHV-5, TEM-2, PER	>128	16	>128	0.5
449	SHV-1, TEM-2, PER	>128	8	>128	0.25
157	SHV-5, TEM-26	>128	1	>128	0.25

CAZ/NXL104 combination is active against isolates producing multiple enzymes

CAZ/NXL104 activity against *P. aeruginosa*

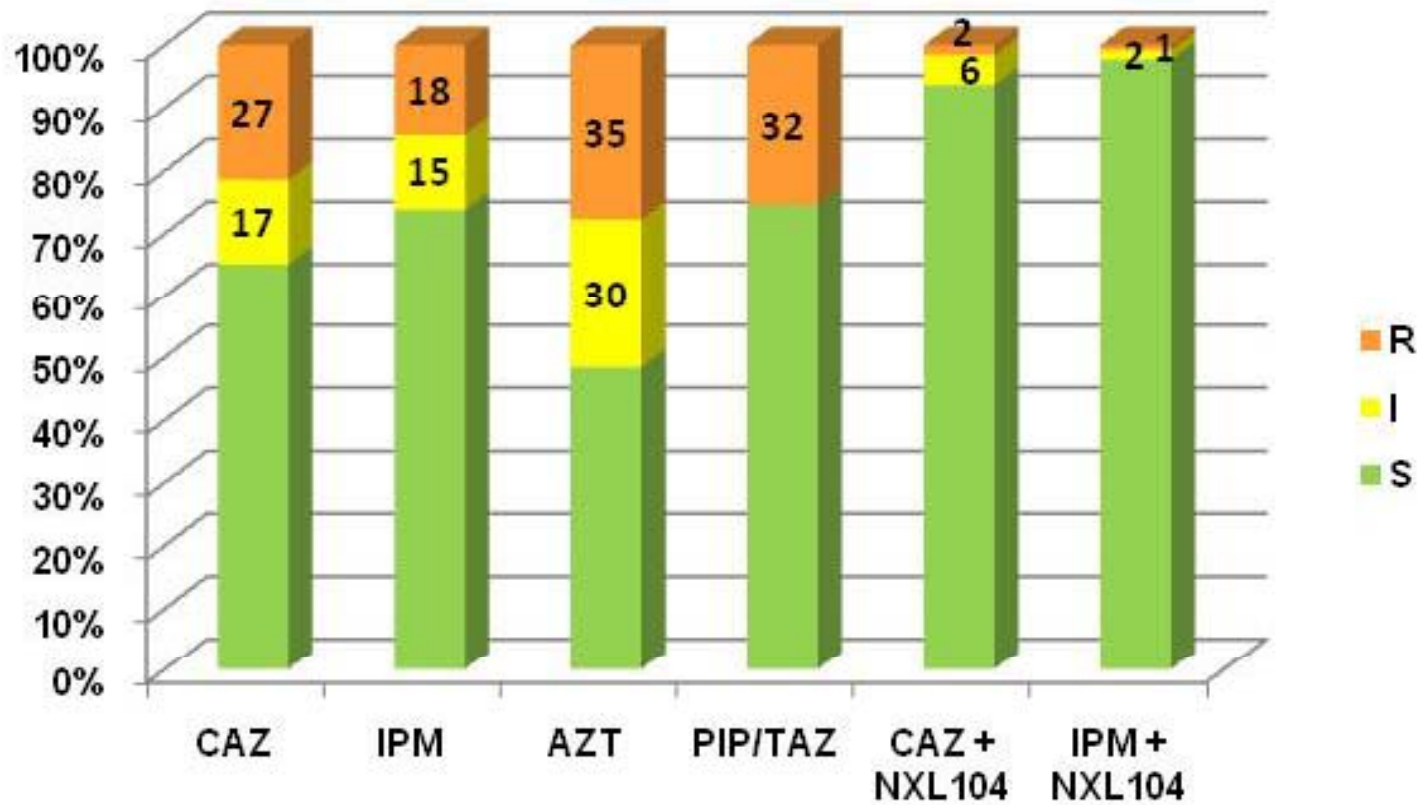
- Consecutive 126 *P. aeruginosa* strains isolated in Paris-South hospital
- Strains collected between December 2006 and April 2007
- 35% non-susceptible to ceftazidime (MIC \geq 16 $\mu\text{g}/\text{mL}$)
- 16% non-susceptible to imipenem (MIC \geq 8 $\mu\text{g}/\text{mL}$)
- High clonal diversity in the 44 isolates non-susceptible to ceftazidime (PFGE using *SpeI*)



CAZ/NXL104 activity against *P. aeruginosa*



Panel of 126 consecutive *P. aeruginosa* clinical isolates



94% of isolates susceptible to CAZ / NXL104 combination

NXL104 and Tazobactam at 4 µg/mL

in vitro time-kill activity of CAZ / NXL104



Bactericidal activity of CAZ/NXL104 combination at concentration close to MIC value

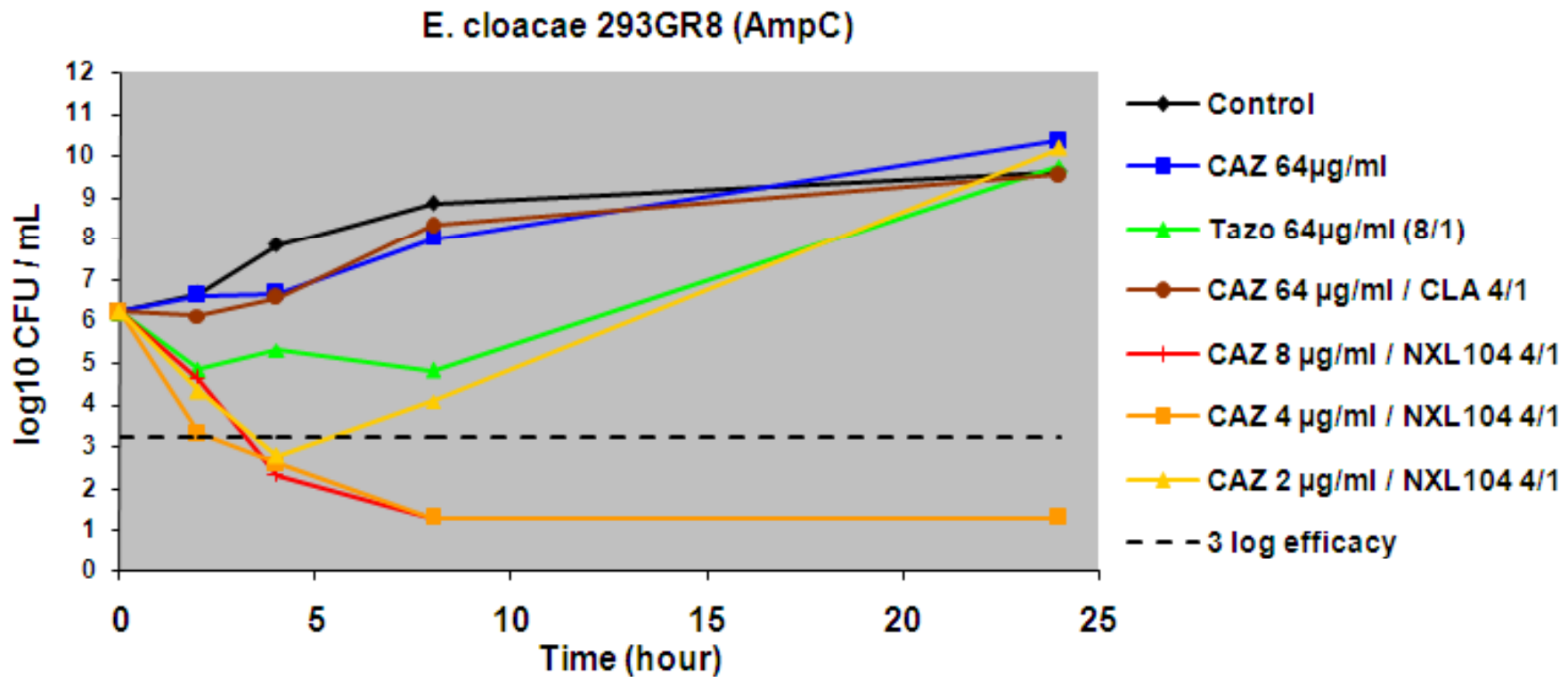
MICs at high inoculum

Tazo : 64

CAZ : >64

CAZ/CLA (4/1) : >64

CAZ/NXL104 (4/1) : 4



NXL104 – no potential for AmpC induction



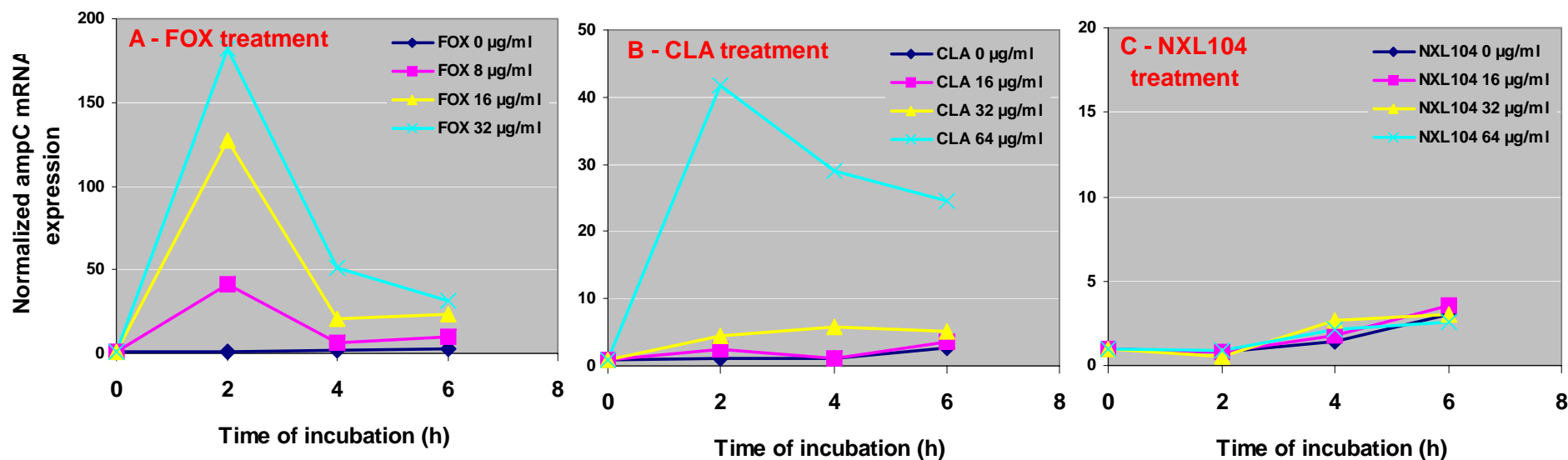
Level of *ampC* expression evaluated by RT-PCR in *E. cloacae* 293HT107 strain

Cefoxitin : 64 µg/mL

Ceftazidime: 0.5 µg/mL

Ceftazidime /CLA : 2 µg/mL

Ceftazidime / NXL104: 0.125 µg/mL



NXL104 demonstrated no potential for *ampC* induction in *E. cloacae* species, implying that it does not further compromise the activity of co-administered β -lactam antibiotics.

Resistance studies



Frequency of resistance to CAZ/NXL104 in single step

ID	Species	Mechanism	CAZ	CAZ + NXL104 (4 µg/mL)	Resistance frequency*
#1	<i>K. pneumoniae</i>	SHV, TEM, KPC OmpK35-, OmpK36 +/-	64	1	$< 1.99 \times 10^{-9}$
#2	<i>E. coli</i>	CMY-6	128	≤ 0.25	$< 1.22 \times 10^{-9}$
#3	<i>E. cloacae</i>	SHV-5a, TEM-1	64	≤ 0.25	1.7×10^{-9}
#4	<i>P. aeruginosa</i>	AmpC OprD-	64	8	4.39×10^{-8}

* : agar plates at 2xMIC of CAZ/NXL104
(i.e. 8 µg/mL NXL104)

CAZ/NXL104 – Summary of *in vivo* activity



in vivo efficacy of Ceftazidime/NXL104 combination demonstrated against CAZ-R enterobacterial strains, Class A and Class C β -lactamase producers. (s.c. dosing, tid, relevant comparators included)

Mouse septicemia model (*K. pneumoniae*, *E. cloacae*, *E. coli*, *C. freundii*)

Significant survival improvement : ED₅₀ 5-29 mg/kg (>90 with CAZ alone)

Mouse pneumonia model (*K. pneumoniae*)

3-6 log reduction in lung bacterial counts

Mouse pyelonephritis model (*K. pneumoniae*, *E. cloacae*, *E. coli*, *C. freundii*, *M. morgannii*)

2-4 log reduction in kidney bacterial counts

Rabbit meningitis model (*K. pneumoniae*)

Significant reduction of CSF bacterial load

NXL104 clinical development

Physicochemistry :

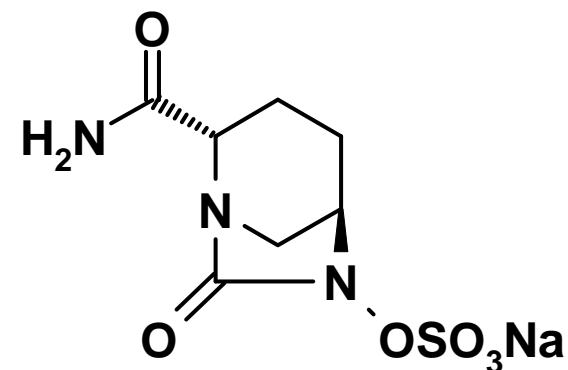
- MW 287.23
- sodium salt

Solubility and stability

- compatible with parenteral administration
- no interaction with ceftazidime

Preclinical and clinical development

- in combination with ceftazidime
- at a CAZ/NXL104 ratio of 4/1



NXL104 (active enantiomer)
(trans-7-oxo-6-(sulfoxy)-1,6-diazabicyclo[3.2.1]octan-2-carboxamide sodium salt)

Ceftazidime

Usual adult dosage : 3 x 1000 mg / day
Difficult to treat infections : 3 x 2000 mg / day

CAZ/NXL104
4/1 ratio

NXL104

3 x 250 mg / day
3 x 500 mg / day

NXL104 - Phase I Studies



Objective: evaluate the safety, pharmacokinetics and pharmacodynamics of NXL104 doses in healthy male volunteers, alone or in combination with ceftazidime

- Randomized, double-blind, placebo-controlled (8 A, 2 PI)
- Regimen of infusion: 30' IV infusion q8h
- Single ascending dose study
 - NXL104: 50, 100, 250, 500, 1000, 1500, 2000 mg
 - NXL104/CAZ: 250/1000 mg, 500/2000 mg
- Multiple ascending dose study
 - NXL104: 500, 750, 1000 mg, q8h for 5 days
 - NXL104/CAZ: 500/2000 mg, q8h for 10 days

Additional studies - ongoing

- Effect of age and gender
- Effect of renal impairment

NXL104 – Clinical PK



PK linearity

- Linearity up to 1000 mg (therapeutic range)
- Cmax and AUC slightly overproportional beyond 1000 mg (2.1 for 2)

Low variability between individuals

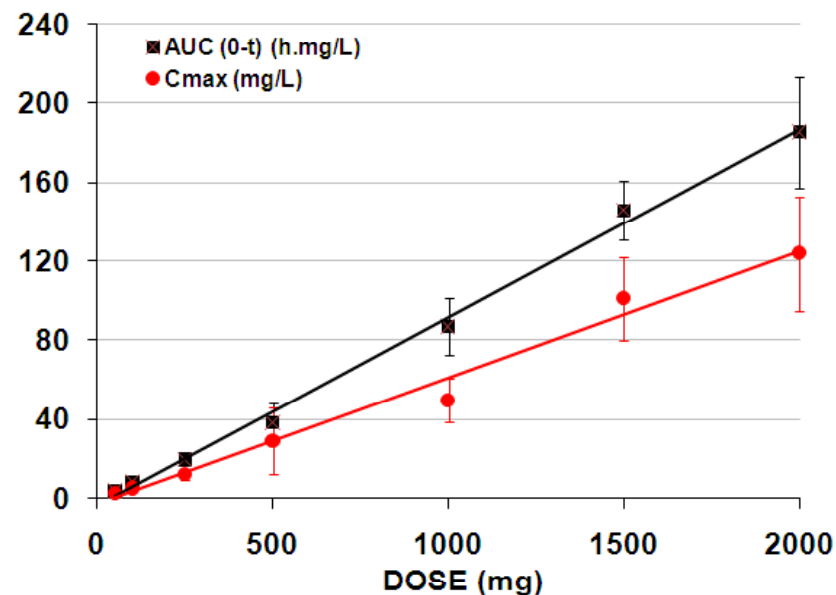
PK parameters are similar across doses

CL: 10.4 – 13.8 L/h

Vss : 19.4 – 26.2 L

$T_{1/2, z}$: 2.2– 2.7 h

>85% found unchanged in urine

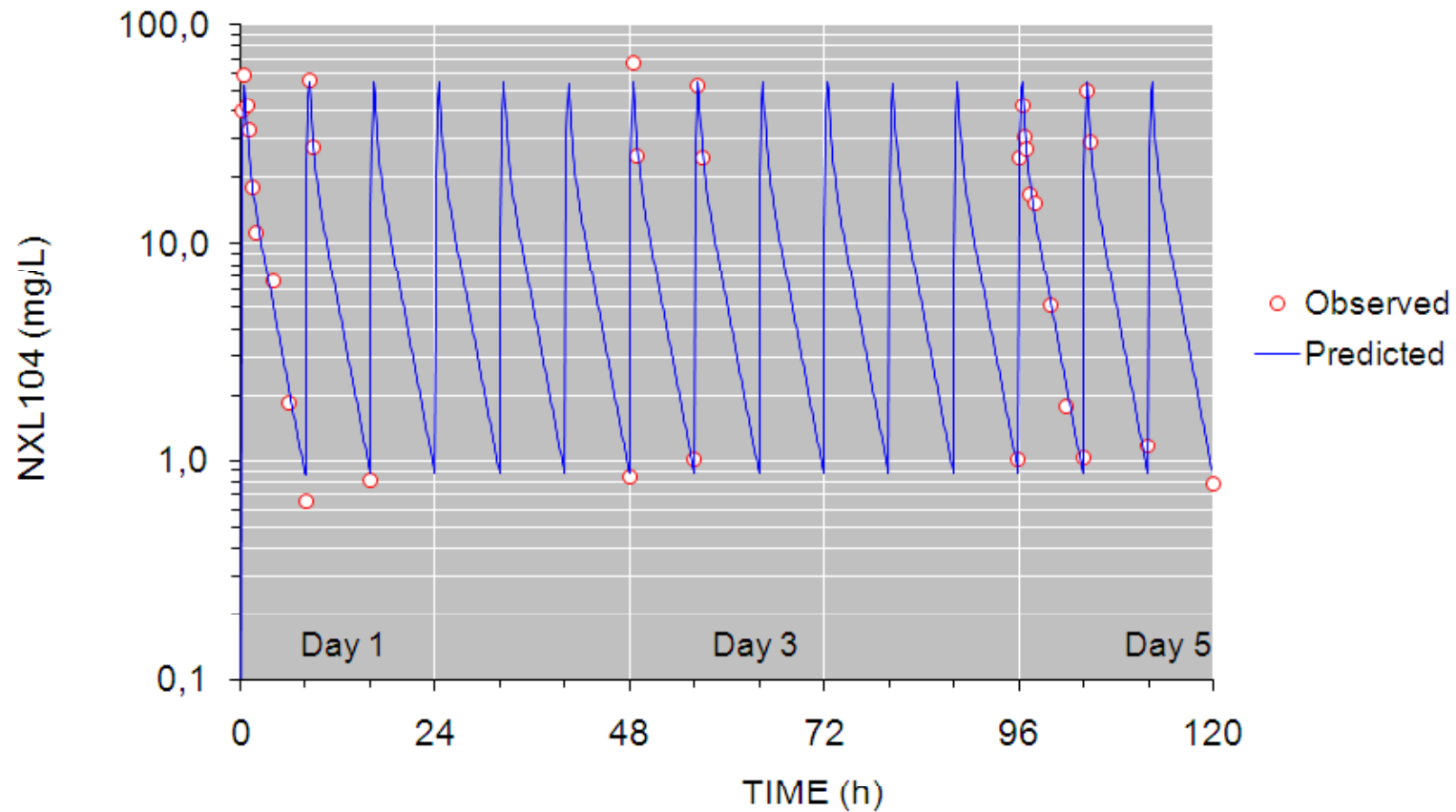


No significant influence of CAZ (1g or 2g) on NXL104 PK (250 mg or 500 mg)

NXL104 – Multiple dose study



Plasma PK profile from a subject dosed q8h with 1000 mg NXL104 for 5 days



NXL104 PK at steady-state is predictable from a single dose

NXL104 - ADME summary



Low volume of distribution

Plasma protein binding is low (<10% in vitro)

Good metabolic stability

No significant inhibition / induction of human CYP enzymes

> 85% of the dose excreted as unchanged in the urine

Rapid elimination in the urine

Most of the dose excreted within 6 h

CL = 12.3 L/h

T_{1/2,z} ~ 2.5h

In therapeutic range, exposure is proportional to the dose

No accumulation upon repeated dosing

NXL104 - Clinical safety (1)



A total of 88 subjects were exposed to NXL104

- **Single dose: 56 subjects, of whom 16 were also exposed to CAZ**
- **Repeated doses: 32 subjects, of whom 8 were also exposed to CAZ**

Clinical observations

No significant abnormality in vital signs or ECG parameters

Hematology and blood chemistry

No clinically significant abnormal values

Local tolerance :

- **Local intolerance related to continuous perfusion in the Multiple Dose Study (1 in 750mg cohort, 2 in 500/2000 combo cohort, 1 in placebo)**

NXL104 - Clinical safety (2)



Adverse Events following single dosing:

- No serious or severe Adverse Events reported
- 6 treatment-emergent AEs reported from 4 subjects dosed with 250, 500 or 2000 mg doses
 - 5 of mild intensity (anxiety-linked symptoms)
 - one of moderate intensity (orthostatic hypotension)
- No treatment-emergent AEs reported in the combination cohorts
- No relationship of reported AEs with the dose, no trends

Adverse Events following repeated dosing: None reported

NXL104 – *in vitro* PD

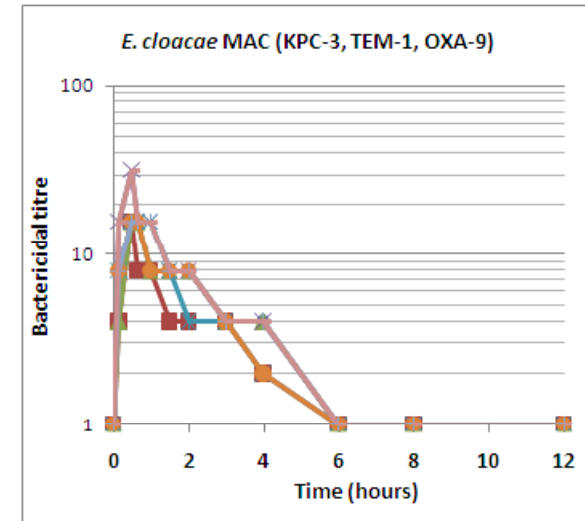
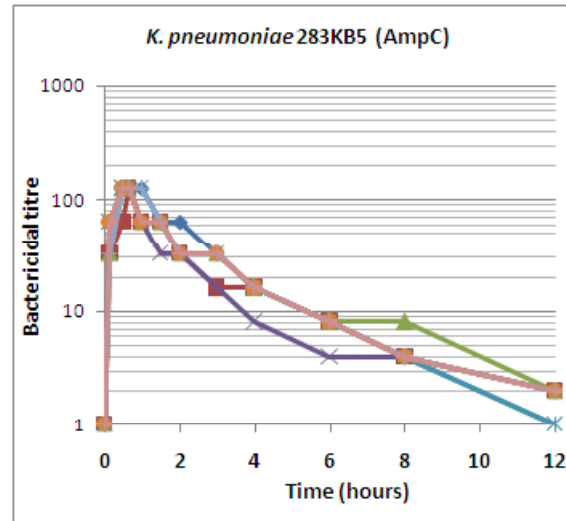
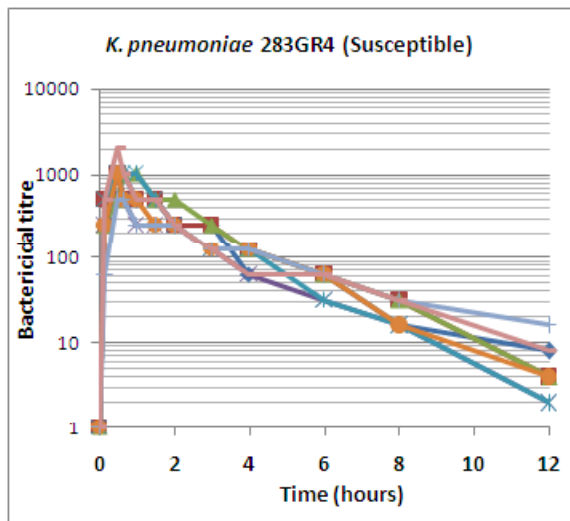


- Plasma samples from single dose study
- Inhibitory activity and bactericidal titer determined for cohorts having received CAZ + NXL104
- 3 strains used for titration of antibacterial activity

ID	Species	Mechanism	CAZ		CAZ /NXL104 (4/1)	
			MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)
283GR4	<i>K. pneumoniae</i>		≤0.125	0.25	≤0.125	0.5
283KB5	<i>K. pneumoniae</i>	AmpC	64	64	1	1
MAC	<i>E. cloacae</i>	KPC-3, TEM-1, OXA-9	>128	>128	4	8

Plasma bactericidal activity

Cohort dosed with 2g CAZ + 0.5 g NXL104



High bactericidal titres achieved for all donors

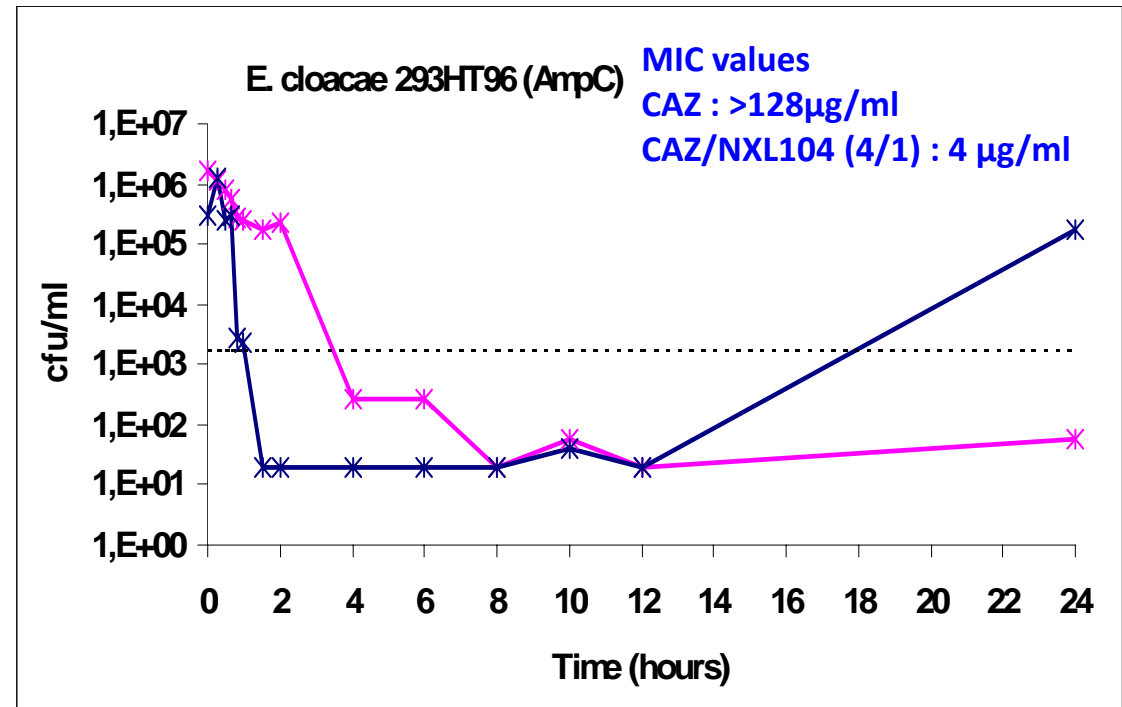
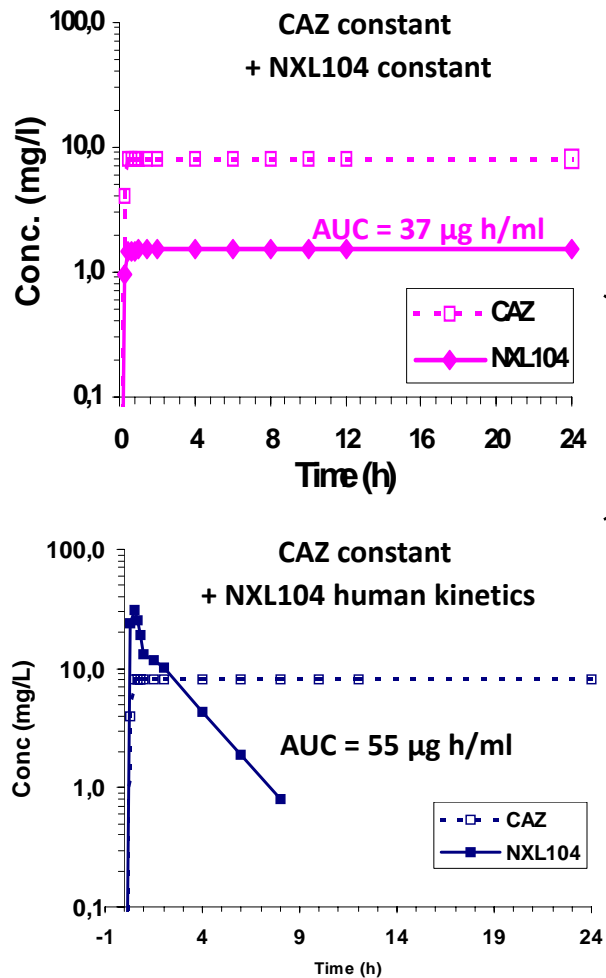
- highest in plasma samples taken at 30 min

Persistence of bactericidal activity: for all donors,

- at least 12h, 6h and 2h for the 3 strains, respectively (cohort dosed 1g CAZ/250 mg NXL104)
- at least 12h, 8h and 4h for the 3 strains, respectively (cohort dosed 2g CAZ/500 mg NXL104)

PK/PD *in vitro* model

Same constant CAZ concentration,
two different NXL104 profiles



Time-dependent activity of NXL104

Similar profile with two *K. pneumoniae* strains

CAZ/NXL104 key attributes



Medical need : increasing β -lactamase resistance is becoming a major concern, primarily in the hospital

Cephalosporin use in the clinic is mainly compromised by the spread of Class A and Class C β -lactamase mediated resistance

Strengths of the **ceftazidime/ NXL104 combination** :

- Broad spectrum activity against Class A/C β -lactamases (ESBLs, AmpC)
- Activity against β -lactam resistant Gram-negatives, particularly on *E. coli*, *Enterobacter spp.*, *C. freundii*, *K. pneumoniae*, which cause difficult to treat infections
- Activity against serine carbapenemases producing isolates
- Excellent safety and tolerability profile

CAZ /NXL104 as a first line agent for the treatment of serious Gram-negative infections, as an alternative to carbapenems