NXL104
β-lactamase inhibitor

21 March 2007 – San Diego
Challenge of Antibacterial Drug Development

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β-lactamase inhibition

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β-lactamases causing concern in the clinic

- Rapid evolution and spread of extended spectrum β-lactamases (ESBLs)
- Plasmid mediated spread of AmpC cephalosporinases
- Evolution of carbapenem hydrolysing enzymes : Class A carbapenemases and Class B metallo-enzymes
- ESBL or AmpC associated with permeability lesion or efflux

Marketed β-lactamase inhibitors

- Clavulanate, Tazobactam, Sulbactam
- Active against Class A β-lactamases

NXL104 compound : Class A / C inhibitor

Formerly AVE1330A

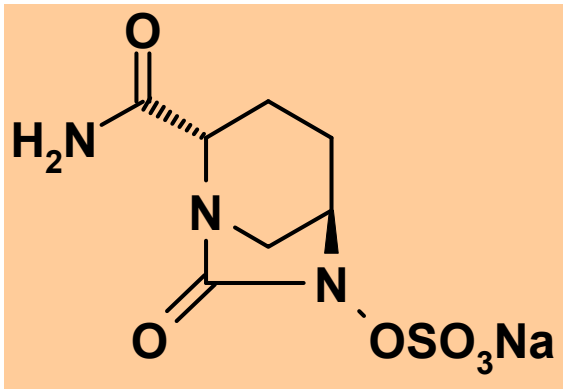
Physicochemistry :

- MW 287.23
- sodium salt
- soluble compound
- stability in solution at RT

Compatible with parenteral administration

Preclinical and clinical development

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



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

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NXL104

Inhibition of β-lactamases

***in vitro* antibacterial activity**

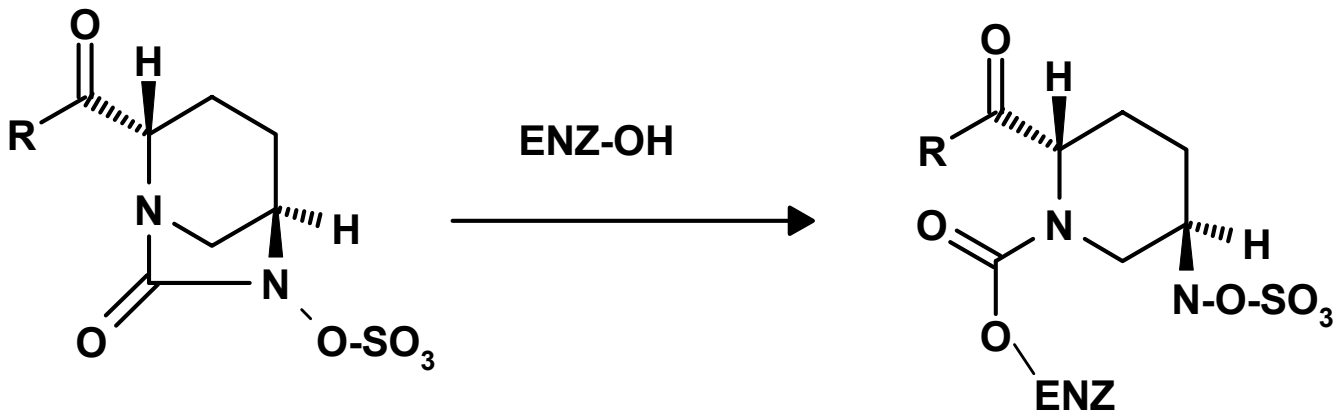
***in vivo* antibacterial activity**

DMPK and safety

Phase I clinical trial

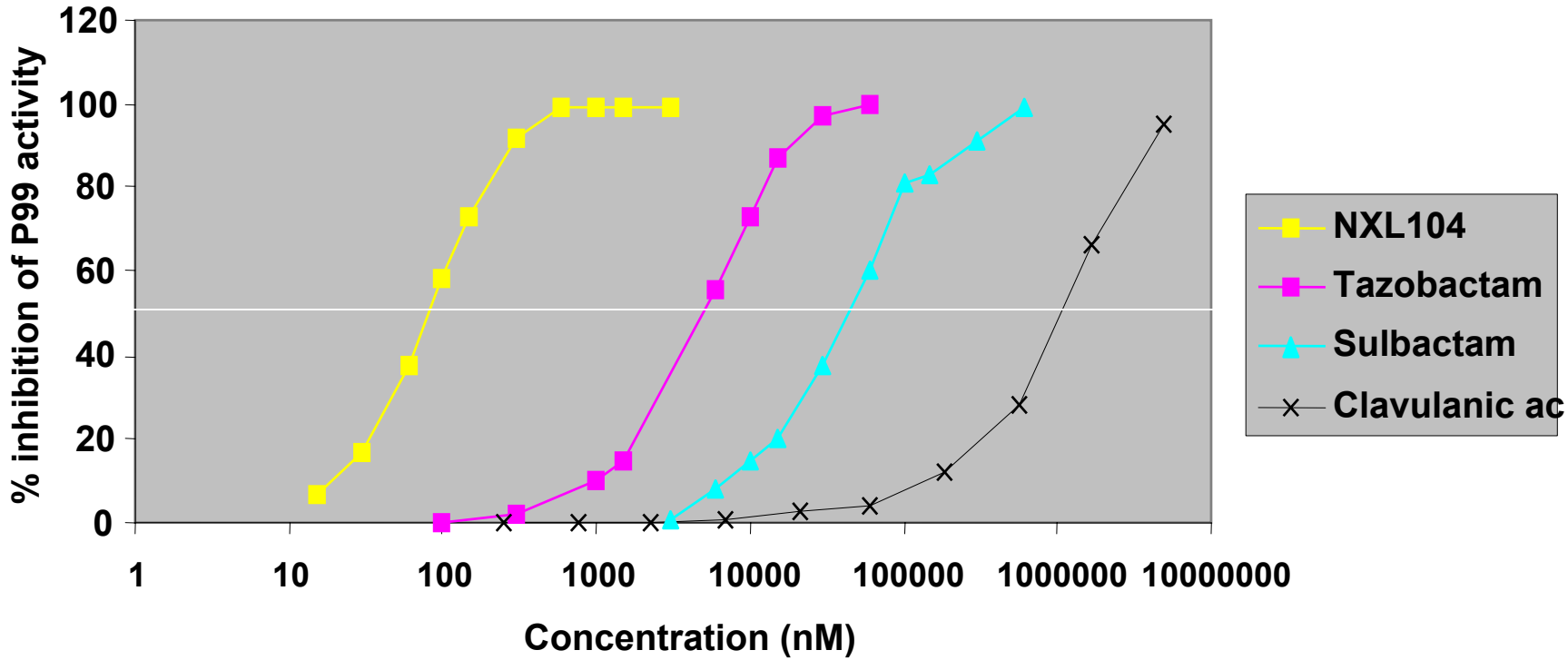
NXL104 – Mechanism of inhibition

Determination of 3D structure for P99 class C and TEM-1 class A β-lactamases complexed with an NXL104 close analogue



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

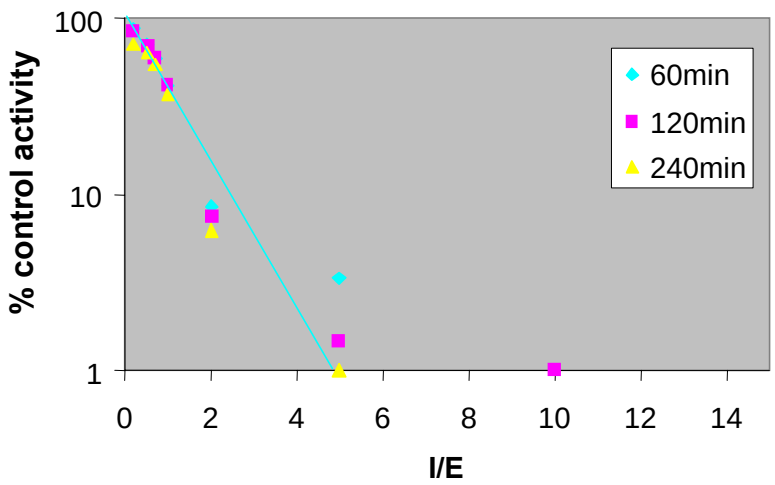
NXL104 - Class C P99 inhibition



P99	NXL104	Tazobactam	Sulbactam	Clavulanic acid
IC_{50} (nM)	80	5000	42000	10^6

NXL104 – Class C P99 kinetics

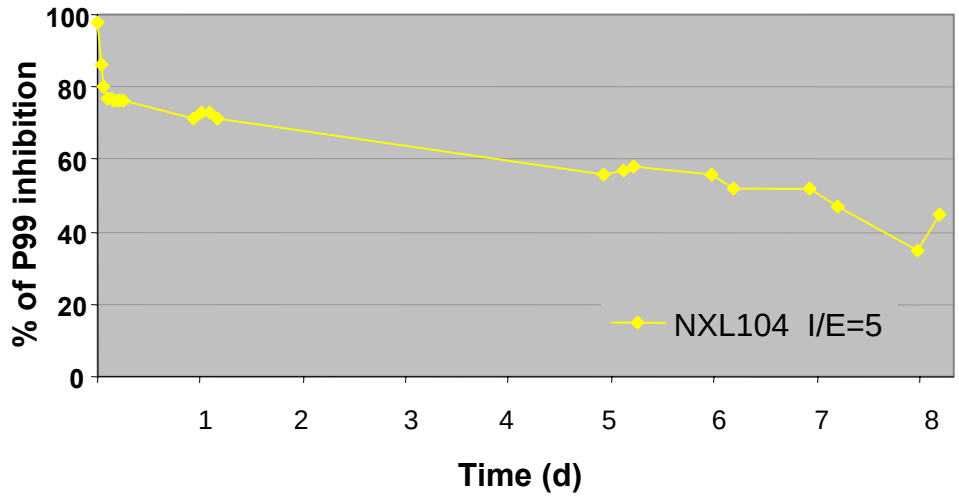
Turnover number



Turnover number for P99 at t=60 mn

NXL104	**Tn = 5**
Tazobactam	**Tn = 55**

Enzyme deacylation



Recovery of P99 β-lactamase activity:

~ 50% at Day 7

Summary of NXL104 activity against purified β-lactamases

- Broad spectrum activity - active on Class A and C β-lactamases
- Lower turnover number than β-lactam based inhibitors
- More stable covalent intermediate ⇨ longer half-life of the covalent intermediate

		NXL104	Tazobactam	Sulbactam	Clavulanic acid
IC_{50} (nM)	TEM-1	8	40	2300	130
	P99	80	5000	42000	100,000
Tn	TEM-1	2 / 318	nd	nd	214
	P99	5	55	nd	nd
Deacylation $t_{1/2}$	TEM-1	~ 7 d at Tn 1	nd	nd	~ 7 mn
	P99	~ 7 d	~ 290 mn	nd	nd

NXL104 – Inhibition of Class C β-lactamases

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Class C β-lactamases	IC_{50} (nM)	
	NXL104	TAZOBACTAM
E. cloacae P99 AmpC	190	2157
E. aerogenes 293 AmpC	552	7519
E. aerogenes 298 AmpC	254	5405
P. aeruginosa 34 AmpC	437	2132
P. aeruginosa CF17 AmpC	402	2840

β-lactamase activity in crude cell extracts

NXL104 - Selectivity for the target

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- **Cytotoxicity**
  - **No cytotoxicity up to 30 µM (max conc tested) against 5 human cell lines**
  - **No cytotoxicity up to 350 µM (max conc tested) against rat hepatocytes**
- **Receptor binding profile**
  - **No or low affinity at 100 µM in an *in vitro* binding assay on a panel of 50 receptors, ion channels and transporters**
- **Serine proteases**
  - **No significant inhibition against 12 clinically relevant human serine proteases (IC_{50} > 1 mM)**

NXL104

Inhibition of β-lactamases

***in vitro* antibacterial activity**

in vivo antibacterial activity

DMPK and safety

Phase I clinical trial

NXL104 *in vitro* antibacterial activity

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- **No significant antibacterial activity alone on most species**

  Only marginal activity on a few *E. coli* isolates (MIC ≥ 4 µg/mL)

- **PBP inhibition**

E. coli PBP	IC_{50} (µM)
PBP1a	>100
PBP1b	>100
PBP2	3.7
PBP3	>100
PBP4	>100
PBP5	>100
PBP6	>100

NXL104 - Class A producing strains

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Strain			Ceftazidime			Ceftriaxone			Cefpodoxime		
		+ inhibitor 4 µg/ml	0	NXL104	Clav	0	NXL104	Clav	0	NXL104	Clav
E. coli	250BE1	SHV4	32	0.25	0.25	2	0.03	0.03	32	0.5	0.5
E. coli	250IP7	SHV5	32	0.5	0.5	2	0.06	0.06	32	0.5	0.5
E. coli	250BE5	TEM3	32	0.5	0.5	4	0.06	0.06	>32	1	0.5
E. coli	250CF2	TEM5	32	0.25	0.12	2	0.03	0.03	32	0.12	0.25
E. coli	250SJ1	TEM7	32	0.03	0.03	0.12	<=0.015	<=0.015	8	0.03	0.12
E. coli	250KB3	CTXM3 like	>32	1	1	>32	1	1	>32	2	4
K. pneumoniae	283IP13	SHV4	>32	0.5	0.5	4	0.12	0.12	>32	0.5	0.5
K. pneumoniae	283CF5	SHV5	>32	1	1	0.5	0.25	0.5	4	1	1
K. pneumoniae	283IP98	TEM4	16	0.25	0.25	2	0.06	0.06	16	0.12	0.12
K. pneumoniae	283IP1	TEM21	16	0.12	0.12	4	<=0.015	<=0.015	>32	0.12	0.25
K. pneumoniae	283KB2	CTXM19	>32	2	4	32	0.25	0.25	>32	0.5	0.5

When combined with Ceftazidime, Ceftriaxone or Cefpodoxime, NXL104 activity against Class A producing strains is similar to Clavulanic Acid activity

NXL104 - Class C producing strains

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Strain			Ceftazidime			Ceftriaxone			Cefpodoxime		
		+ inhibitor 4 µg/ml	0	NXL104	Clav	0	NXL104	Clav	0	NXL104	Clav
E. coli	250KB8	ACC1	>32	4	>32	16	0.25	16	>32	2	>32
K. pneumoniae	283KB4	DHA2	>32	0.5	>32	4	0.06	8	>32	0.5	>32
C. freundii	261CO3	AmpC	16	0.5	>32	16	0.12	32	>32	0.5	>32
C. freundii	261GR6	AmpC	>32	1	>32	32	0.25	32	>32	0.5	>32
E. cloacae	293CO20	AmpC	>32	2	>32	>32	0.5	>32	>32	8	>32

When combined with Ceftazidime, Ceftriaxone, or Cefpodoxime, NXL104 restores antibacterial activity against Class C β-lactamase producing strains

NXL104 – Class A CTX-M producing strains

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58 *Enterobacteriaceae* isolates producers of CTX-M enzymes

Mechanism	Organism	Nb of isolates
CTX-M-1 group	*K. pneumoniae*	10
CTX-M-3	*E. coli*	2
CTX-M-15	*E. coli*	8
CTX-M-32	*E. coli*	1
CTX-M-2 group	*E. coli*	5
CTX-M-9 group	*E. coli*	9
CTX-M-9 group	*K. pneumoniae*	8
CTX-M-9 group	*E. cloacae*	10
CTX-M-14 (group 9)	*E. coli*	1
CTX-M-19 (group 9)	*E. coli*	1
CTX-M-40 (group 8)	*E. coli*	1
CTX-M-26	*E. coli*	1

NXL104 (4 mg/L) in combination with cefotaxime or ceftazidime

- **Cefotaxime + NXL104 (4 mg/L) : MICs reduced from >128 to 0.03 - 0.06 mg/L**
- **Ceftazidime + NXL104 (4 mg/L) against CTX-M-15, CTX-M-32 isolates: MICs reduced from >64 to 0.25-1 mg/L**
- **All CTX and CAZ / NXL104 combinations had MIC < 1 mg/L**

NXL104 - Class A KPC producing strains

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Organism	Specimen ID	Mechanism	Tazocillin	Imipenem	Ceftazidime	Ceftazidime + NXL104 4 mg/L
E. coli	DH5α	KPC-3	>128	8	128	0.25
K. pneumoniae	CL-5761	KPC-3	>128	>32	>128	<=0.015
K. pneumoniae	CL-5762A	KPC-3	>128	32	>128	<=0.015
K. pneumoniae	CL-5762B	KPC-3	>128	32	>128	<=0.015
K. pneumoniae	CL-5763	KPC-3	>128	>32	>128	<=0.015
Enterobacter	E624	KPC-4	>128	>32	>128	32

NXL104 – Strains with mixed mechanisms

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ID	*Organism*	Mechanisms	TZC	IPM	CAZ	CAZ + NXL104 4 mg/L	CTX	CTX + NXL104 4 mg/L
H04416107	*E. aerogenes*	AmpC Erta-R	128	1	>128	0.5	128	0.125
H043100370	*E. aerogenes*	AmpC Carbapenem R	>128	>32	>128	64	>128	1
H043100371	*Enterobacter spp*	AmpC Erta-R	16	0.5	32	1	64	0.5
H060340523	*E. aerogenes*	AmpC Erta-R	>128	8	>128	2	>128	2
H053520202	*E. cloacae*	AmpC Erta-R	>128	4	>128	2	>128	2
H045100290	*E. cloacae*	CTX-M 1 & 9 group Erta-R	64	1	128	0.5	>128	0.25
H050980341	*E. cloacae*	ESBL? Erta-R	128	1	>128	2	128	0.25
H051000441	*E. cloacae*	ESBL? Erta-R	>128	8	>128	2	>128	1
H042640247	*E. aerogenes*	ESBL? Erta-R	128	16	>128	1	>128	1
H053720086	*Klebsiella sp*	CTX-M 1 group Erta-R	>128	0.5	>128	1	>128	0.25
H054000396	*Klebsiella sp*	CTX-M 1 group Erta-R	>128	1	128	1	>128	0.25
H054120535	*Klebsiella sp*	CTX-M 1 group Erta-R	>128	2	>128	2	>128	0.25
H054120566	*K. pneumoniae*	CTX-M 1 group Erta-R	>128	1	>128	2	>128	0.5
H054200417	*Klebsiella sp*	CTX-M 1 group Erta-R	>128	0.5	>128	0.5	>128	0.125
H055120250	*Klebsiella sp*	CTX-M 1 group Erta-R	>128	4	>128	4	>128	1
H061260284	*Klebsiella sp*	CTX-M 1 group Erta-R	>128	0.25	>128	0.031	>128	0.031
H051880451	*E. cloacae*	CTX-M 9 group Erta-R	128	2	128	2	>128	1

17 isolates with CTX-M or AmpC enzymes together with impermeability

All* isolates are susceptible to cefotaxime+NXL104 and ceftazidime+NXL104 combinations (MICs < 4 mg/L)

*** : one anomaly with ceftazidime, to be confirmed**

NXL104 *in vitro* activity

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Summary of activity against characterized strains

- **NXL104 effectively inhibits:**
  - **Class A TEM enzymes, including ESBL and some IRTs, CMTs**
  - **Class A SHV enzymes, including ESBL**
  - **Class A CTX-M enzymes, including CAZ-hydrolyzing enzymes**
  - **Non classical class A enzymes (VEB, PER, GES)**
  - **Class A carbapenemases, including KPCs**
  - **Class C enzymes , both plasmid-borne and chromosomal enzymes**
  - **Some OXA enzymes (OXA-1, -31, -48 variants)**

- **NXL104 has no significant activity against metallo-enzymes and most Class D enzymes**

NXL104 *in vitro* antibacterial activity

Antibacterial activity of β-lactam / NXL104 combinations

- **Antibiotic activity restored against resistant Enterobacteriaceae Class A and C β-lactamase producers :**

  E. coli*, *Klebsiella spp.*, *Salmonella spp.*, *Enterobacter spp.*, *Citrobacter spp.*, *Proteus spp.*, *M. morganii.*, *M. catarrhalis

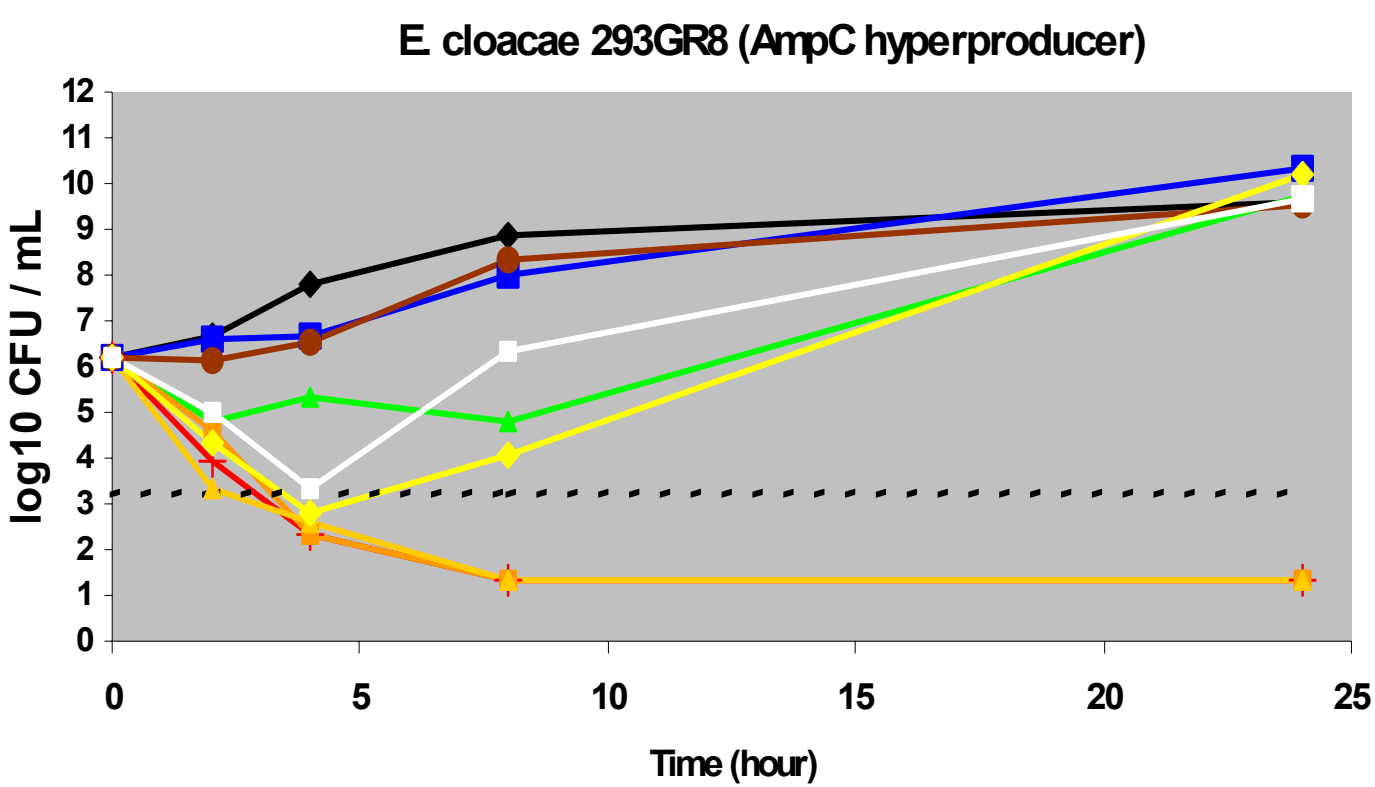
- **Association with NXL104 shown effective for a variety of β-lactams:**

  Piperacillin, Aztreonam, Ceftazidime, Ceftriaxone, Cefpodoxime, …

- **NXL104 restores Ceftazidime activity against CAZ-R *P. aeruginosa* for most Class A and C β-lactamase producers**

in vitro time-kill activity of CAZ / NXL104 combination

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



MICs at high inoculum

Tazo : 64

CAZ : >64

CAZ/CLA (4/1) : >64

CAZ/NXL104 (4/1) : 4

- Control
- CAZ 64 µg/ml
- Tazo 64 µg/ml (8/1)
- CAZ 64 µg/ml / CLA 4/1
- CAZ 16 µg/ml / NXL104 4/1
- CAZ 8 µg/ml / NXL104 4/1
- CAZ 4 µg/ml / NXL104 4/1
- CAZ 2 µg/ml / NXL104 4/1
- CAZ 1 µg/ml / NXL104 4/1
- 3 log efficacy

NXL104

Inhibition of β-lactamases

in vitro antibacterial activity

***in vivo* antibacterial activity**

DMPK and safety

Phase I clinical trial

NXL104 - Mouse septicaemia model

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Combination Ceftazidime (CAZ) / NXL104 at ratio 4/1 (s.c. dosing)

Strains	Reference	β-lactamase	Drug	MIC (mg/l)	PD_{50} (mg/kg)
E. cloacae	293GR8	AmpC	CAZ	64	>90
			CAZ/NXL104	1	<10
			Tazocillin	16	>90
			Coamoxiclav	>64	>90
K. pneumoniae	283IP10	SHV-4	CAZ	>64	>50
			CAZ/NXL104	1	5
			Tazocillin	8	>50
			Coamoxiclav	8	20
E. coli	250BE1	SHV-4	CAZ	>64	>50
			CAZ/NXL104	2	16
			Tazocillin	16	40
			Coamoxiclav	8	50
C. freundii	261GR6	AmpC	CAZ	64	>50
			CAZ/NXL104	2	<5
			Tazocillin	32	>50
			Coamoxiclav	>64	>50

Intraperitoneally induced infection ; therapy 0 and 4 hours after infection

NXL104 - Mouse pneumonia model (1)

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- **Immunosuppression (cyclophosphamide 150 mg/kg ; 3 days and 6 hours prior to infection)**
- **Intratracheal inoculation of ~ 10^8 – 10^9 cfu/mouse**
- **Development of bacteremic pneumonia and fatal disease within 2 – 4 days**
- **Lung bacterial burden 16-18h post infection is 10^{11} – 10^{13} cfu/gr lung**
- **s.c. treatment initiated 16-18h post-infection, tid for 2 days**
- **In vivo efficacy monitored using bacterial lung clearance at 24h and 48h post-infection**

- **2 *K. pneumoniae* Ceftazidime resistant strains used in this model**

NXL104 - Mouse pneumonia model (2)

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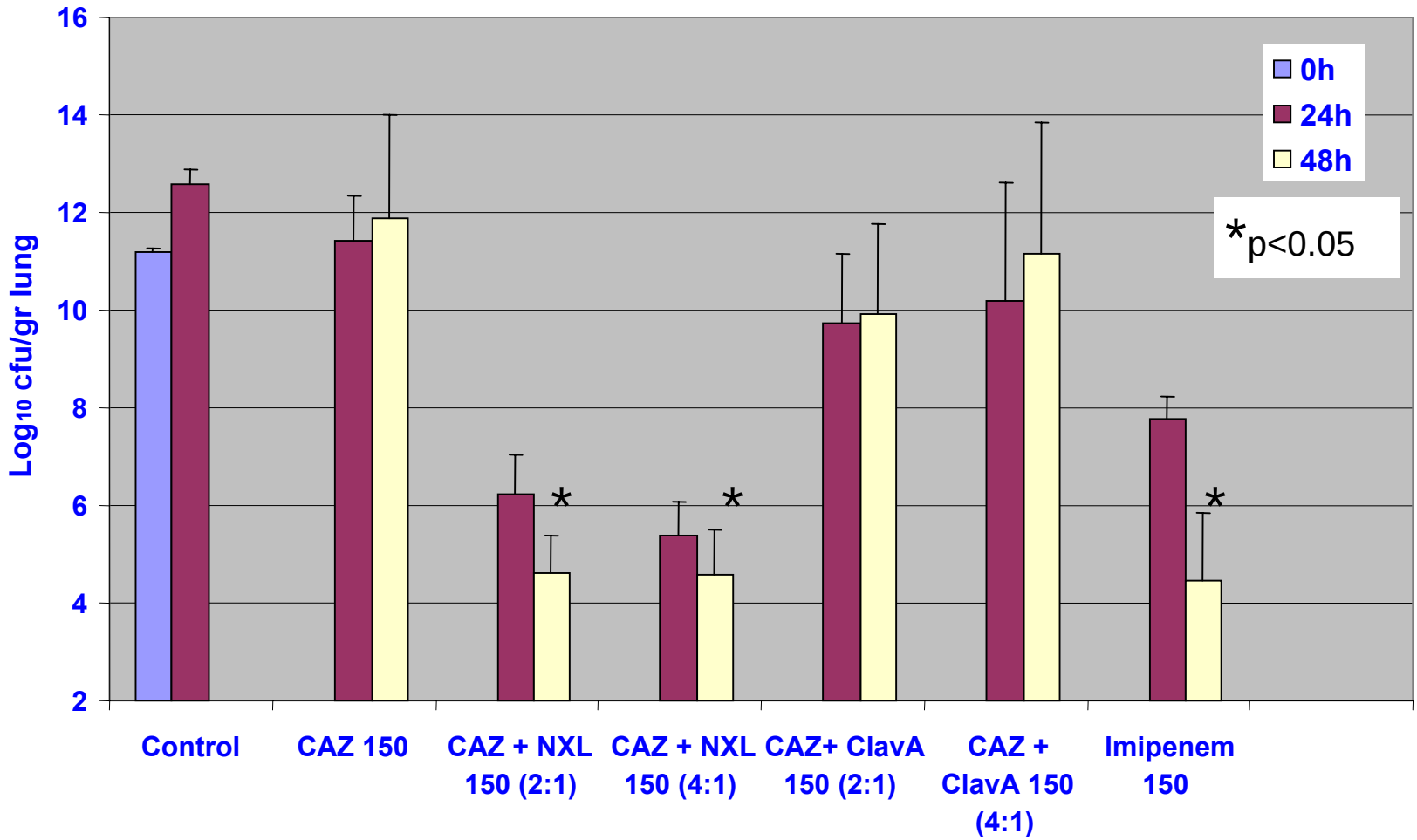
Lung counts of *K. pneumoniae* 283KB5 - AmpC + Class A SHV-11



MICs (µg/ml)
CAZ : 32
CAZ/104 : 1
CAZ/CLA : 8
IMI : 1

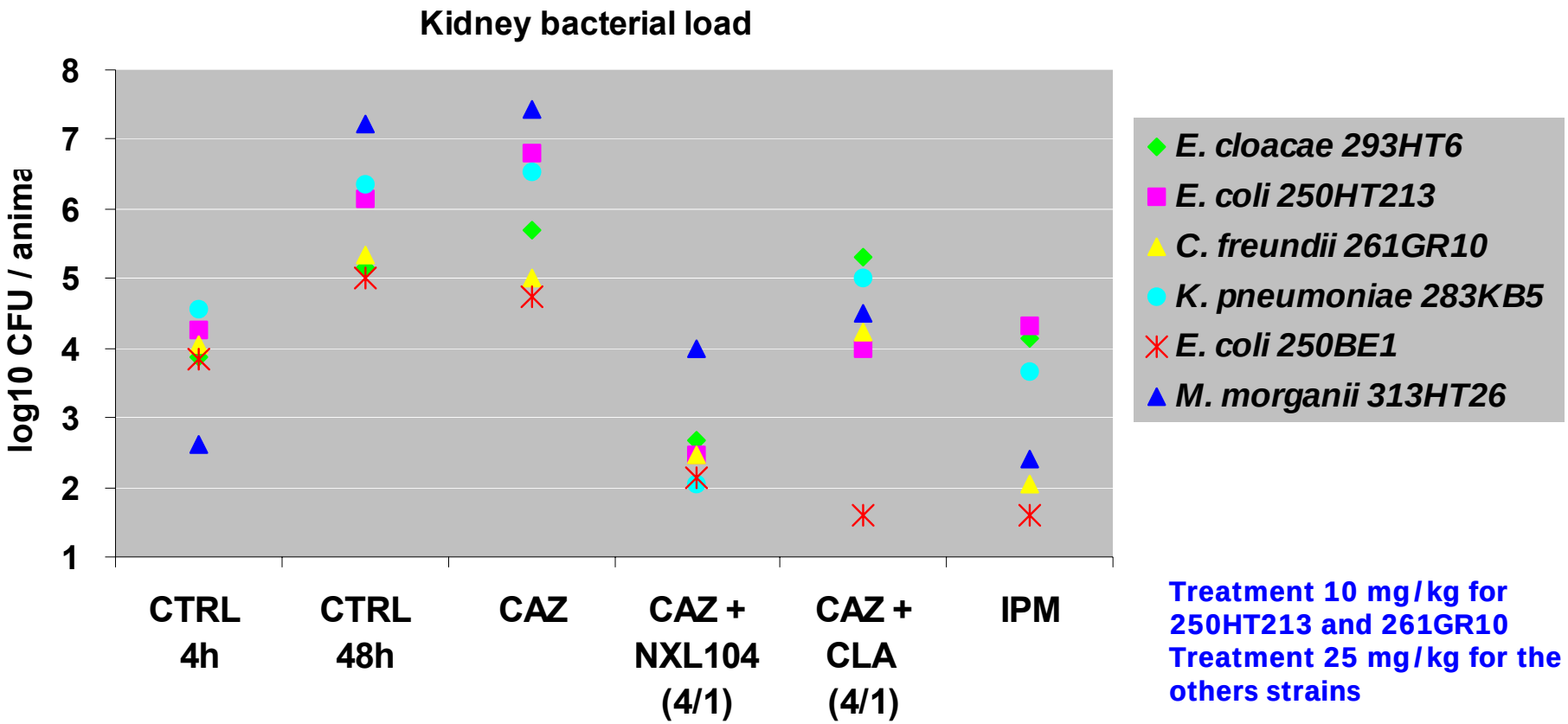
Slight immunosuppression before intratracheal infection
Therapy s.c. initiated 16-18 h post infection ; t.i.d for 2 days

NXL104 – Mouse pyelonephritis model (1)

			MIC (mg/L)			
Species	ID	Enzyme	CAZ	CAZ + NXL104	CAZ + CLA	IPM
E. cloacae	293HT96	AmpC	>128	4	>32	0.5
E. coli	250BE1	SHV-4	>128	2	1	0.5
C. freundii	261GR10	AmpC	>128	4	32	0.5
K. pneumoniae	283KB5	AmpC + SHV-11	64	2	32	0.5
M. morgannii	313HT26	AmpC	16	0.5	8	1
E. coli	250HT213	AmpC	16	1	16	1

- Immunosuppression (cyclophosphamide 150 mg/kg D-1 and day of infection)
- Direct kidney inoculation of ~ 10^3 – 10^4 CFU/mouse
- s.c. treatment at 4h, 8h, 24h and 32h post-infection
- Enumeration of bacterial load in kidney at 48h post-infection

NXL104 – Mouse pyelonephritis model (2)



Treatment 10 mg/kg for 250HT213 and 261GR10
Treatment 25 mg/kg for the others strains

Effective clearance of bacterial load in kidney with CAZ / NXL104 (4/1) : 2 – 4 log reduction

CAZ/NXL104 – Summary of *in vivo* activity

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***in vivo* efficacy of Ceftazidime/NXL104 combination has been demonstrated against CAZ-R enterobacterial strains, Class A and Class C β-lactamase producers:**

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

- **Significant survival improvement : ED_{50} 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**

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Inhibition of β-lactamases

in vitro antibacterial activity

in vivo antibacterial activity

DMPK and safety

Phase I clinical trial

NXL104 - ADME summary (1)

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DISTRIBUTION

Volume of distribution

Vdss (L/kg)	Rat :	0.6 – 0.7
	Dog :	0.3 – 0.7

in vivo plasma protein binding (^{14}C NXL104 iv administration)

Based on AUC :	38 % bound in dog plasma
	67 % bound in rat plasma

NXL104 - ADME summary (2)

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METABOLISM

in vitro metabolic stability in liver microsomes

Stable in every species (up to 200 µM) except rat (-12% at 90 min at 200 µM)

in vitro inhibition of human CYP isoforms

IC_{50} > 200 µM for isoforms CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4/5

in vitro induction of human CYP enzymes

no induction of isoforms 1A2, 2B6, 2C9, 3A4

slight induction potential of 2E1 at 5 mM - possibly irrelevant to the clinical situation

in vivo metabolic profiling (rat and dog)

>75% of the dose excreted as unchanged NXL104

→ **Good metabolic stability**

NXL104 - ADME summary (3)

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ELIMINATION

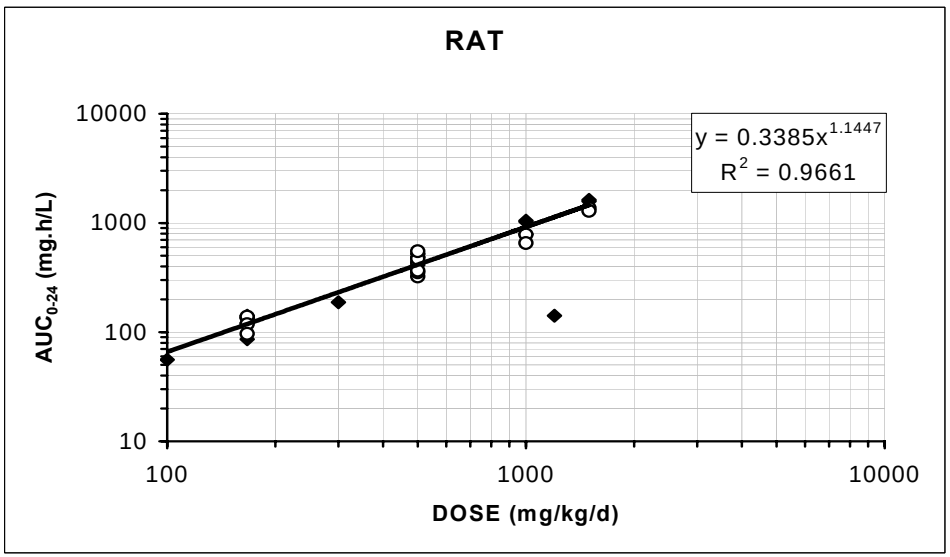
- **Rapid elimination (in 24h) ; mainly in urine**

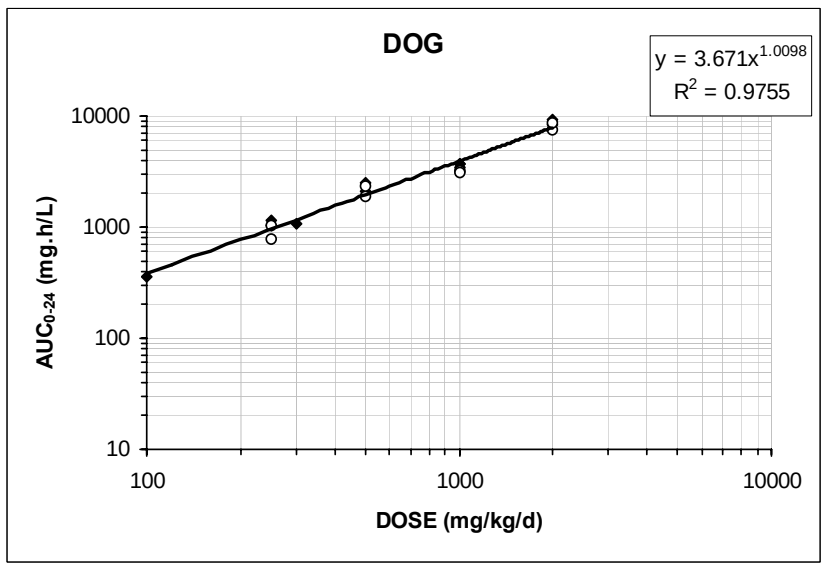
- **PK parameters**

	CL	$T_{1/2,z}$
Rat	0.79 - 0.84 L/h/kg	1.27 - 1.44 h
Dog	0.22 - 0.36 L/h/kg	3.38 - 3.88 h

NXL104 - TK summary

- **Rat**
  - **M > F**
  - **5% accumulation**
  - **AUC x 2.2 for dose x2**
- **Dog**
  - **M > F**
  - **13% accumulation**
  - **Linear PK**





NXL104 – Drug & Safety Evaluation

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SUMMARY : excellent safety profile

- Safety pharmacology package (100, 300, 1000 mg /kg) : no concern
- hERG channel assay: IC_{50} > 300 µM
- Genetic toxicology: no concern
- Good local tolerance (single dose in the rabbit)
- General toxicity (4-w regulatory tox studies)
  - Rat NOAEL for systemic toxicity: 500 mg/kg/d
    for local tolerance: 167 mg/kg/d
  - Dog NOAEL for systemic toxicity: 1000 mg/kg/d
    for local tolerance: 500 mg/kg/d
  - No target organ identified on histopathology examination

NXL104

Inhibition of β-lactamases

in vitro antibacterial activity

in vivo antibacterial activity

DMPK and safety

Phase I clinical trial

NXL104 – Phase I single dose study

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Safety, tolerance and pharmacokinetics in healthy volunteers

- Escalating single intravenous doses
- NXL104 alone and in combination with ceftazidime
- Randomized, double-blind, placebo-controlled study
- Nine cohorts of 10 subjects (8 active and 2 placebo)
- Seven cohorts received NXL104 alone at doses 50, 100, 250, 500, 1000, 1500 and 2000 mg
- After a washout period of 7 days, two cohorts received a combination of ceftazidime / NXL104 : CAZ 1000 mg + NXL104 250 mg
  CAZ 2000 mg + NXL104 500 mg

NXL104 – Phase I single dose study

The inphase of the study has been just completed : preliminary incomplete data available

- **No or few adverse events reported**
- **Hematology and blood chemistry parameters : no clinically significant abnormal values**
- **Good local tolerance**

Preliminary PK analysis for groups 50-250 mg :

- **PK is linear**
- **PK parameters are similar across doses**
- **No relevant influence of CAZ (1g) on NXL104 PK (250 mg)**

NXL 104

Conclusions

NXL104 programme

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 a cephalosporin / 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**
- **good safety and tolerability profile**

Clinical development of Ceftazidime + NXL104 to bring a solution to β-lactamase-mediated resistance in Gram-negatives in the hospital