

LORUS
therapeutics

Antimicrobial Program

2011

Emerging Infections with Multi-Drug Resistant Bacteria

- Incidence of severe infections with multi-drug resistant strains is increasing in hospitals and communities; multi-drug resistant infections have limited therapeutic options
- Only a few new antimicrobial agents have been recently approved for clinical use; the ability of bacterial pathogens for developing resistance, particularly Gram-positive species, makes the development of a diversified pipeline of antimicrobials a necessity
- Overall sales in the current antibiotics and new products market were nearly \$40 billion in 2008. It increased to \$41.5 billion in 2009. By 2014, it is projected to increase to \$65.5 billion, for a 5-year compound annual growth rate (CAGR) of 9.6%

Antimicrobial Program Opportunity

- Proprietary small molecules with potent antimicrobial activity against multi-drug resistant Gram-positive bacteria
- Active in vitro and in vivo with low toxicity observed in mouse models of infection
- Potential for treatment of other bacterial and fungal infections produced by important emerging pathogens such as *Mycobacterium tuberculosis*, *Streptococcus pneumoniae* and *Candida albicans*
- Unique mechanism of action – Opportunity for development of antimicrobial agents against strains that are resistant to approved drugs such as Vancomycin, Linezolid and Daptomycin
- Strong IP position

Antimicrobial Program - IP Portfolio

- PCT patent application: "2,4,5-Trisubstituted Imidazoles and Their Use as Anti-Microbial Agents"

➤ Applications filed worldwide from PCT as shown below:

COUNTRY	FILED	SERIAL #	STATUS	CLAIMS SUMMARY
Australia	8/19/2003	2003257329	ALLOWED	
Brazil	8/19/2003	PI0313763-5	PENDING	LOR-220 and related
Canada	8/19/2003	2,496,241	PENDING	molecules:
China	8/19/2003	3824355.5	ALLOWED	- Compositions
Europe	8/19/2003	3787546.5	PENDING	- Uses in the
Hong Kong	8/19/2003	5109435.9	PENDING	preparation of an anti-
Japan	8/19/2003	2004-528206	PENDING	microbial treatment
Mexico	8/19/2003	2005/002040	PENDING	- Methods for the
United States	8/19/2003	10/525,690	ALLOWED	treatment or prevention of a microbial infection

Antimicrobial Program - *Overview*

- Lorus has developed a novel class of proprietary antimicrobial compounds with potent antibacterial activity against multi-drug resistant Gram-positive strains
- Lead derivatives were discovered following in vitro screening of chemical libraries for molecules with activity against epidemic Methicillin-Resistant *S. aureus* (MRSA) strains
- Lead compound: LOR-220
 - First-in-class
 - Target: Novel eukaryotic-like Ser/Thr kinase (ESTK)
 - MOA: Block of translation of proteins involved in cell wall synthesis leading to cell death (ESTK signaling pathway)

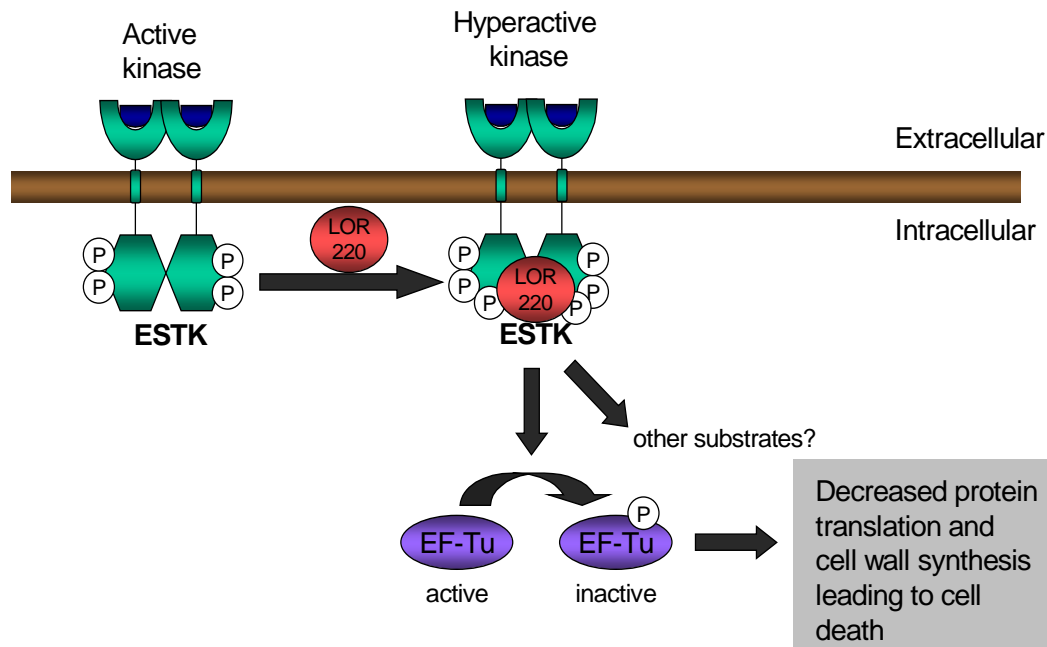
LOR-220: Features Summary

- Lead compound LOR-220 shows potent in vitro bactericidal activity against Methicillin, Vancomycin and Oxacillin resistant strains
- Antimicrobial susceptibility tests demonstrated higher antimicrobial activity of LOR-220 than the first line antimicrobial drugs Vancomycin and Linezolid against 330 clinical isolates
- LOR-220 treatment induced significant in vivo bactericidal effects in mouse models of infection
- LOR-220 increased the survival rate of mice infected with a lethal dose of Methicillin and Vancomycin resistant bacterial strains in a murine sepsis model - 100% survival effect at tolerated dose levels

LOR-220: Novel Target & MOA

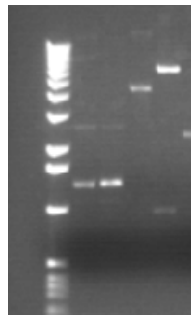
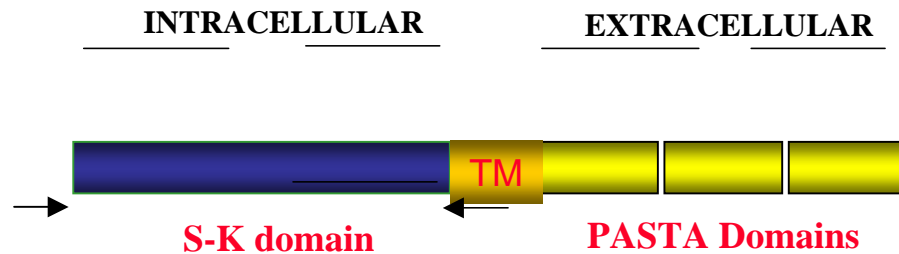
MOA model: LOR-220 induces eukaryotic-like Ser/Thr kinase (ESTK) autophosphorylation leading to inactivation of elongation factor EF-Tu

[Eukaryotic-like Serine/Threonine Kinase (ESTK) Signaling Pathway]

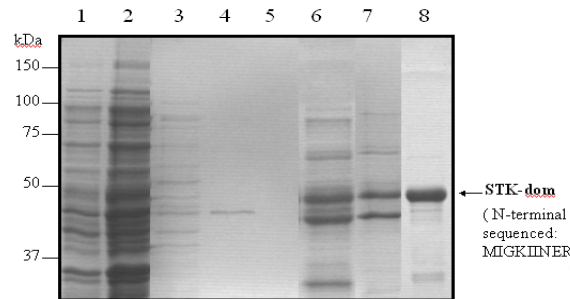


- Signaling pathway recently identified in Gram-positive bacteria and *M. tuberculosis*
- ESTK is associated to the bacterial cell membrane
- Comprise an extracellular domain, a transmembrane-spanning region (TMD) and a intracellular catalytic domain
- ESTK regulates the activity of the elongation factor Tu
- Critical functions:
 - Regulation of cell wall synthesis and developmental processes
 - Survival of intracellular pathogens inside macrophages (e.g. *M. tuberculosis*)

Cloning and Expression of the Recombinant ESTK from MRSA (N315)

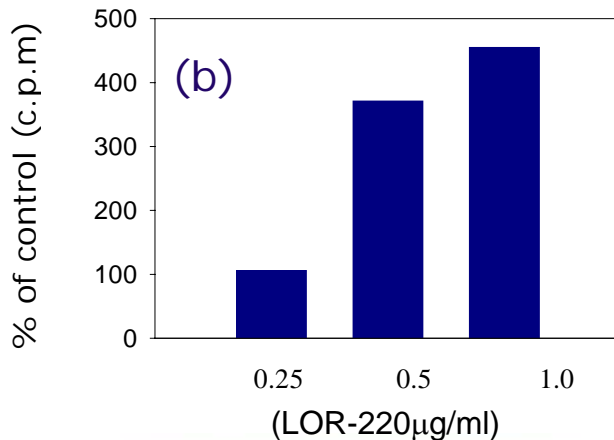
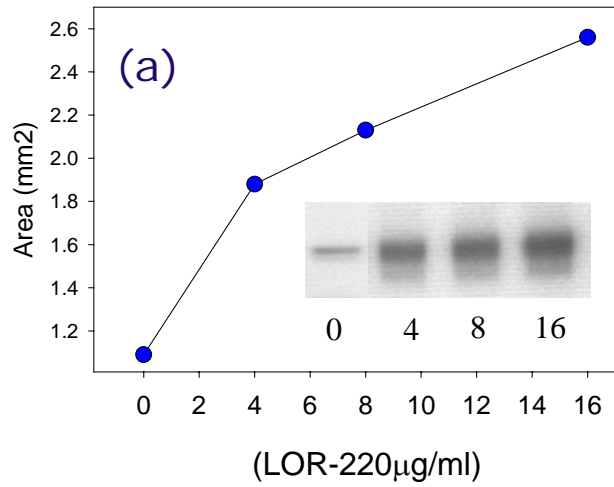


Purification of 6xHis-tagged STK-dom Protein by B-Per System



- The map sequence of the ESTK gene was determined from the full genome sequence from MRSA strain N315
- It comprises predicted extracellular (PASTA) domains believed to be involved in peptidoglycan-sensing signaling for cell wall synthesis initiation, a transmembrane domain and an intracellular (catalytic) domain.
- The catalytic domain of the ESTK gene was cloned and expressed as recombinant protein

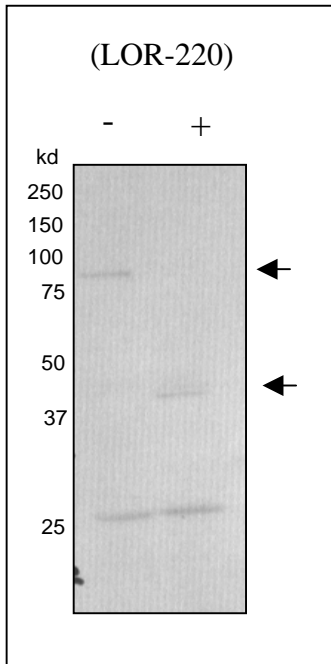
LOR-220-Series Compounds: Antimicrobial Mechanism of Action



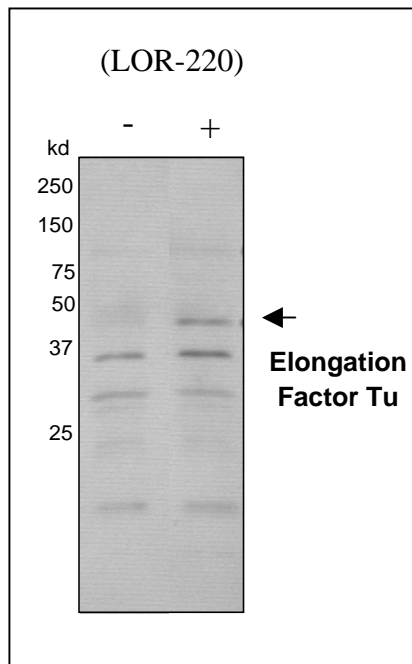
- LOR-220 induces auto-phosphorylation of the recombinant ESTK from MRSA (a) and a generic substrate (myelin basic protein) (b)
- Determined by γ -P32-ATP incorporation into the ESTK recombinant catalytic domain in the presence of increasing concentrations of LOR-220

LOR-220 treatment alters serine/threonine phosphorylation in MRSA including Elongation Factor Tu

“PKA substrate”
Anti-Phospho(ser/thr)



“PKC substrate”
Anti-Phospho(ser)

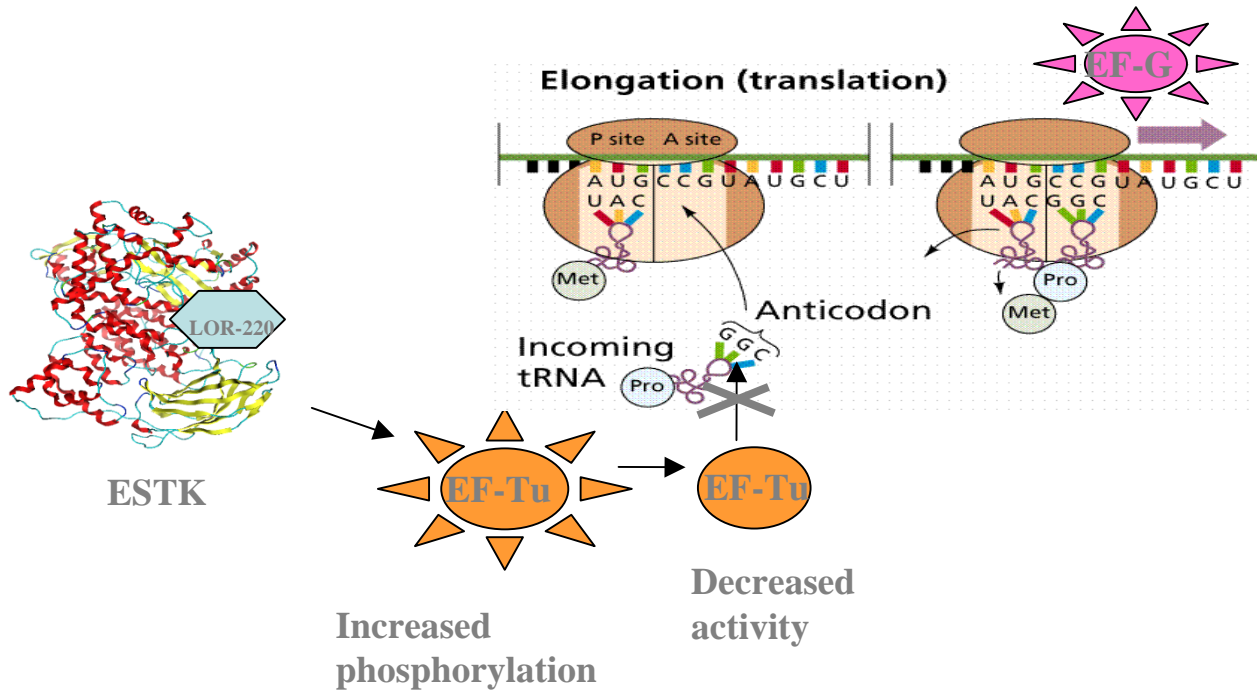


- Protein phosphorylation was determined by Western blot analysis of protein extracts from MRSA cells treated with LOR-220

- Changes in the phosphorylation levels of several proteins were detected, including increased phosphorylation of the elongation factor Tu (identity confirmed by LC/MS/MS analysis)

- Phosphorylation of EF-Tu is regulated by ESTK

Protein Translation: Cell Wall Synthesis



Antimicrobial Activity of Lorus derivatives

LOR-series	MIC (µg/ml)	LOR-series	MIC (µg/ml)
104	2-4	216	2.0
105	2-4	219	2.0
108	4.0	220	0.5-1
119	4.0	233	2.0
123	4.0	240	1.0
128	2.0	241	2.0
130	4.0	242	2.0
135	4.0	243	4.0
141	4.0	247	1.0
155	4.0	255	4.0
200	4.0	258	2.0
202	1.0	265	2.0
203	4.0	320	4.0
204	2-4	321	4.0
207	4.0	323	4.0
208	2-4	720	2.0
210	2-4	805	4.0
211	2.0	820	2.0
212	2.0	Linezolid	1-4
213	1.0	Oxacillin	0.5->128

- LORUS compounds are active *in vitro* against methicillin resistant (MRSA) and methicillin sensitive (MSSA) strains of *S. aureus* – Results shown are from 6 clinical isolates of MRSA and 2 ATCC strains of MSSA

- LOR-220 MIC value is similar to that of Linezolid

- LOR-220 is active against Oxacillin-resistant strains (MIC > 128)

(MIC= Minimum inhibitory concentration)

MIC values in Other Bacterial Species

Strain	ATTC	LOR-202	LOR-220	Vanc.
<i>B. subtilis</i>	14579	2	1	1
<i>B. subtilis</i>	6633	2	0.5	0.25
<i>E. faecalis</i>	29212	2	2	4
<i>E. faecalis</i>	51299	2	1	16
<i>E. faecium</i>	51559	2	2	>64
<i>M. luteus</i>	10240	1	0.25	0.25
<i>S. epidermidis</i>	13518	2	0.5	0.5
<i>S. epidermidis</i>	12228	2	0.25	2
<i>S. epidermidis</i>	35983	2	0.5	4
<i>S. saprophyticus</i>	15305	2	0.25	2
<i>S. pneumoniae</i>	49150	2	8	4
<i>S. dysgalactiae</i>	12388	2	16	0.5
<i>S. dysgalactiae</i>	12394	2	8	1
<i>S. pyogenes</i>	19615	2	8	1
<i>S. sanguinis</i>	10556	1	4	1
<i>S. agalactiae</i>	13813	2	8	1
<i>S. agalactiae</i>	12386	2	4	2
<i>E. coli</i>	12435	>64	>64	>64
<i>E. coli</i>	35333	>64	>64	>64
<i>P. aeruginosa</i>	19142	>64	>64	>64
<i>P. aeruginosa</i>	39324	>64	>64	>64
<i>S. typhimurium</i>	14028	>64	>64	>64
<i>S. typhimurium</i>	13311	>64	>64	>64

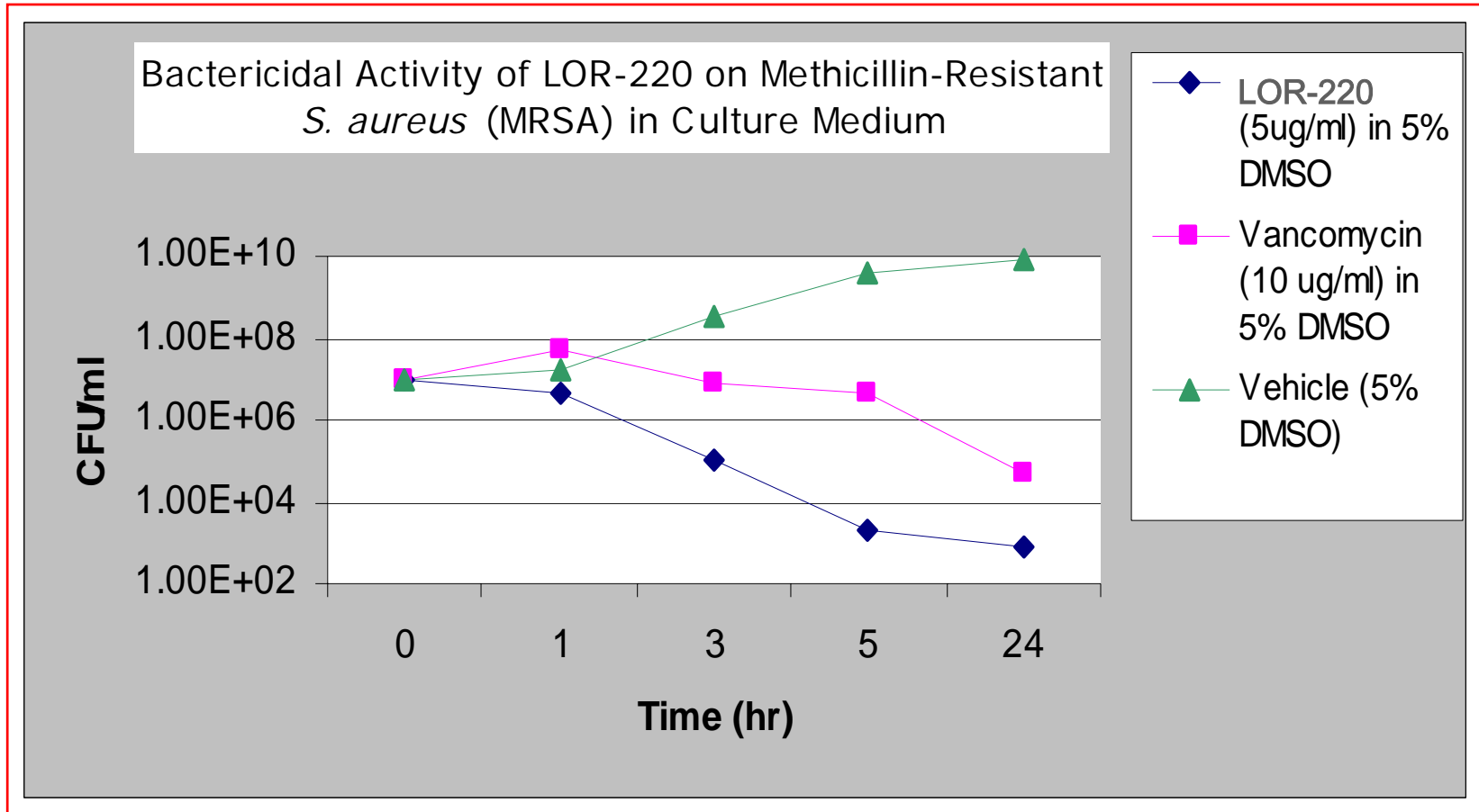
➤ LOR-220 is active against a range of Gram positive bacteria - Spectrum of activity is similar to first line antibiotic Vancomycin

➤ LOR-220 is active against Vancomycin resistant *E. faecium* (ATCC 51559) – Potential to treat Vancomycin resistant MRSA strains

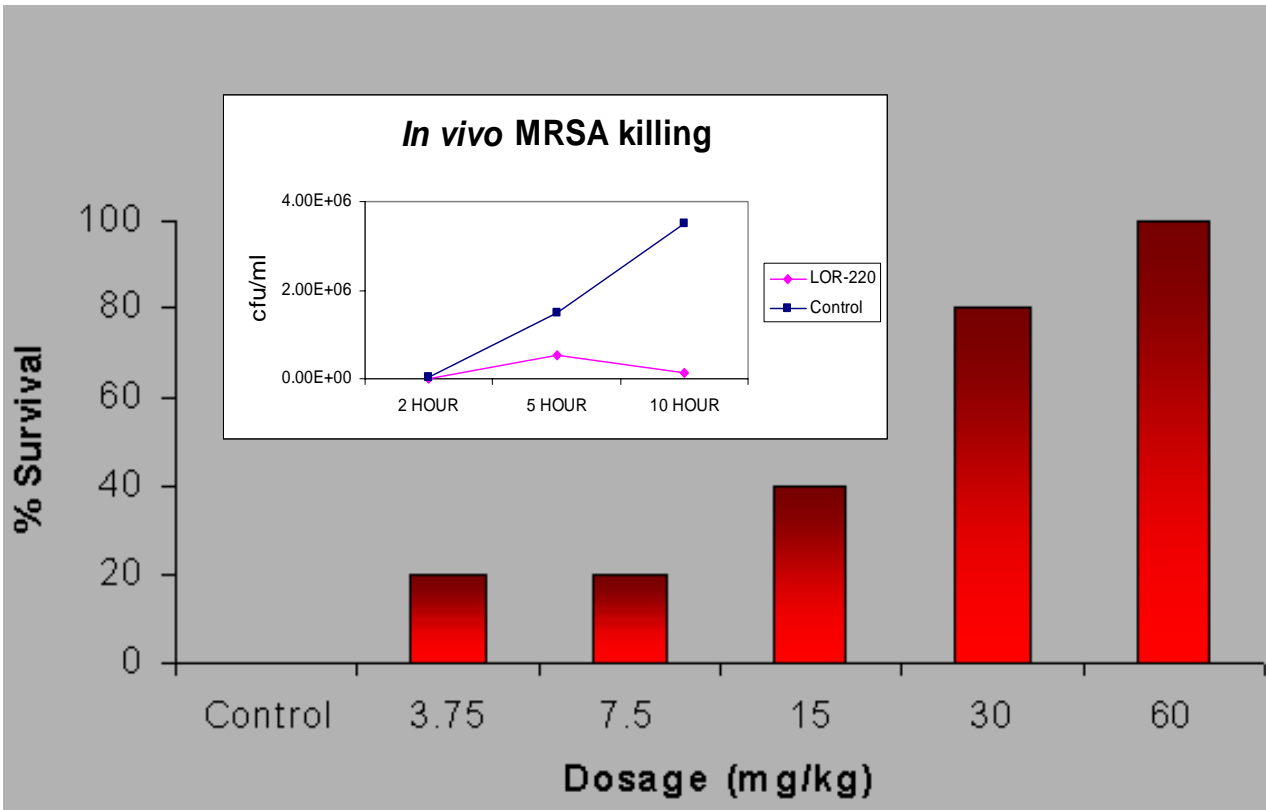
Antimicrobial Susceptibility of Clinical isolates

Organism	No. Strains	Antimicrobial	MIC50	MIC90	Range	Organism	No. Strains	Antimicrobial	MIC50	MIC90	Range
			— (µg/ml) —						— (µg/ml) —		
<i>S. aureus</i> (MRSA)	50	LOR-220 Vancomycin Linezolid	0.25 1.0 2.0	0.5 2.0 4.0	0.25-1.0 0.25-2.0 0.25-8.0	<i>E. faecium</i> (Vancomycin-Resistant)	25	LOR-220 Vancomycin Linezolid	2.0 128 2.0	32 >128 8.0	0.5-32 32->128 0.5-16.0
<i>S. aureus</i> (MSSA)	50	LOR-220 Vancomycin Linezolid	0.06 0.25 1.0	0.25 1.0 2.0	0.06-0.5 0.125-1.0 0.5-4.0	<i>S. pneumoniae</i> (PRSP)	20	LOR-220 Vancomycin Linezolid	0.125 0.5 1.0	0.5 1.0 2.0	0.06-1.0 0.125-2.0 0.25-4.0
<i>S. aureus</i> coag (-) (MRCNS)	30	LOR-220 Vancomycin Linezolid	0.25 0.5 1.0	0.5 1.0 4.0	0.06-2.0 0.125-2.0 0.25-8.0	<i>S. pneumoniae</i> (PSSP)	30	LOR-220 Vancomycin Linezolid	0.25 0.5 1.0	0.5 1.0 2.0	0.06-1.0 0.06-2.0 0.125-4.0
<i>S. epidermidis</i> (MRSE)	20	LOR-220 Vancomycin Linezolid	0.25 1.0 2.0	1.0 2.0 4.0	0.25-2.0 0.5-8.0 0.25-4.0	<i>S. pyogenes</i>	30	LOR-220 Vancomycin Linezolid	0.25 0.5 1.0	1.0 2.0 4.0	0.125-2.0 0.25-4.0 0.5-8.0
<i>S. epidermidis</i> (MSSE)	20	LOR-220 Vancomycin Linezolid	0.25 1.0 2.0	1.0 2.0 4.0	0.125-1.0 0.5-2.0 0.25-4.0	<i>H. influenzae</i>	10	LOR-220 Vancomycin Linezolid	1.0 2.0 4.0	2.0 4.0 8.0	0.5-4.0 0.5-8.0 2.0-16
Enterococcus spp (Vancomycin-Susceptible)	20	LOR-220 Vancomycin Linezolid	0.5 1.0 2.0	0.5 2.0 4.0	0.5-2.0 0.25-4.0 0.5-4.0	MRSA= Methicillin-Resistant <i>S. aureus</i> MSSA= Methicillin-Susceptible <i>S. aureus</i> MRCNS= Methicillin-Resistant Coagulase (-) <i>S. aureus</i> MRSE= Methicillin-Resistant <i>S. epidermidis</i> MSSE= Methicillin-Susceptible <i>S. aureus</i> PRSP= Penicillin-Resistant <i>S. pneumoniae</i> PSSP= Penicillin-Susceptible <i>S. pneumoniae</i>					
<i>E. Faecalis</i> (Vancomycin-Resistant)	25	LOR-220 Vancomycin Linezolid	0.5 64 1.0	1.0 >128 2.0	0.5-4.0 8->128 0.5-8.0						

LOR-220: *In Vitro* Bactericidal Activity



LOR-220: *In vivo* Antimicrobial Activity in a Murine Sepsis Model

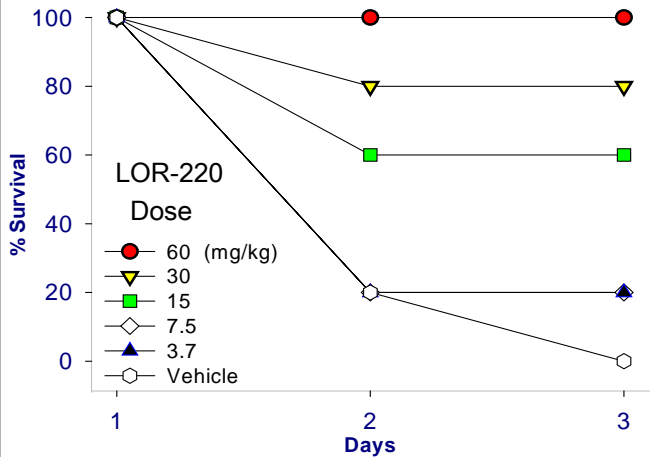


- LOR-220 showed a dose-response survival effect in a mouse sepsis model of MRSA strain 1B-387, with 100% survival following 2 ip injections of LOR-220 (60 mg/kg) at 1 and 3 hours after inoculation
- The number of viable bacteria (CFU) per ml of blood decreased dramatically, as shown in the inset (CFU measured at 2, 5 and 10 hours post inoculation)

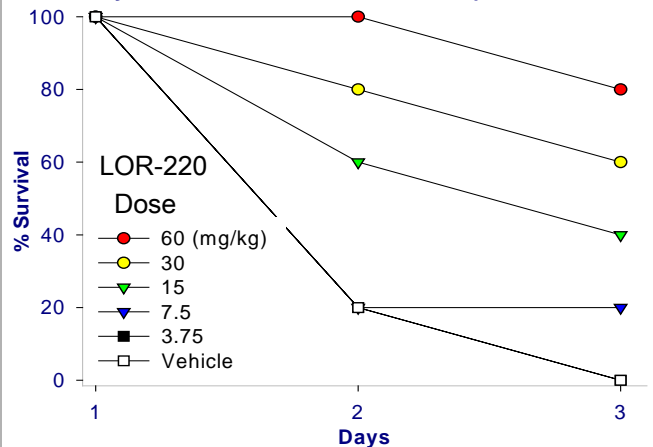
• In preliminary toxicity studies LOR-220 was tolerated at 60 mg/kg when given ip daily for 3 days; Additional studies are planned to determine the LD₅₀

LOR-220: *In vivo* Antimicrobial Activity in a Murine Sepsis Model

Vancomycin-Resistant *E. faecalis* (2.4×10^8 cfu/ ip)



Vancomycin-Resistant *E. faecium* (2.0×10^8 cfu/ip)



- LOR-220 was also tested in infections produced with other highly resistant Gram-positive bacterial strains
- Results obtained in a sepsis model of infection with 2 strains of Enterococci resistant to Vancomycin (MIC > 128 $\mu\text{g/ml}$)

Antimicrobial Program Development Status

- Additional experiments are planned to further characterize and validate LOR-220 mechanism of action
- Structural optimization/ formulation development studies planned to produce optimal therapeutic window in animal models of infections
- Active compounds will be further developed for submission to the U.S. IND within 1.5 - 2 years:
 1. Hit to Lead and Lead Optimization Studies include potency improvement, satisfactory PK, PD and ADME profiles, efficacy in various animal models, scalable synthesis and no in vivo tox and safety issues
 2. Therapeutic index determination and GLP-toxicology studies

Clinical Development Strategies

- Once a clinical lead candidate is identified, further efficacy studies will be conducted in various animal models including:
 1. VRSA
 2. Pneumonia
 3. Skin-soft tissues
 4. TB
 5. Immunosuppression
- These study results will support claims for clinical application in broader clinical indications