



Neue Glykopeptide, Lipopeptide und Peptide

Neue Glyco-/Lipopeptidantibiotika

Code	Name	Gruppe	Wirksam gegen	Abstract
A54145		Lipopeptid	<i>Staphylococcus aureus</i>	F1-262 ; F1-372
MX-2401		Lipopeptid	Multiresistente Staphylokokken <i>S. epidermidis</i>	F1-363 ; F1-364 ; F2-385
--	Dalbavancin	Lipoglycopeptid	Gram(+) Staphylokokken Streptokokken	C1-151 ; C1-180 ; M-743
--	Oritavancin	Lipoglycopeptid	Gram(+) Staphylokokken Streptokokken	A-1893 ; A-3563 ; A-971 ; A-994 ; B-1009 ; B-1011 ; C1-142 ; C1-143 ; C1-144 ; C1-145 ; C1-146 ; C1-186 ; C1-187 ; C1-198 ; C1-199 ; C1-3711 ; C1-3717 ; C1-3720 ; C1-3835 ; C1-4182 ; L-1514 ; L-1515
--	Telavancin	Lipoglycopeptid	Gram(+) Staphylokokken Streptokokken	A-1878 ; A-1879 ; A-973 ; A-976 ; A-978 ; A-980 ; B-1002 ; B-1004 ; B-1012 ; B-3573 ; C1-147 ; C1-148 ; C1-149 ; C1-150 ; C1-181 ; C1-191 ; C1-3718 ; F1-362 ; K-528 ; K-529 ; K-530

Neue Peptidantibiotika

Code	Name/Substanz	Gruppe	Wirksam gegen	Abstract
--	Arenicin-3	Peptid	Gram(-) multiresistente Bakterien	F1-3986
--	Parasin I	Peptid	Gram(-) Bakterien	F1-3991
--	Omiganan	Peptid	Gram(-) Bakterien	C1-3845
BL-2060	Oligopeptid	Peptid	<i>Ps. aeruginosa</i>	F1-3987
PMX30063	Defense Protein		<i>Staphylococcus</i> spp.	F1-3993
POL7080	Polykationisches Peptid	Peptid	<i>Pseudomonas</i> spp.	F1-3995 ; F1-3996
RTA3	--	Peptid	Streptokokken	F1-3998 ; F1-3199

A-971

Intracellular Activity of Oritavancin against MSSA, MRSA and VISA Strains in a Model of Human Macrophages

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Background: *S. aureus* survives and thrives inside eukaryotic cells, which contributes to recurrence or persistence of infections. Oritavancin, a new lipoglycopeptide with multiple modes of action, is highly bactericidal against the extracellular forms of multiresistant *S. aureus*, including strains poorly susceptible to vancomycin, and has proven active against intracellular forms of a reference strain of MSSA (AAC 2003; 47:2283-92). Our aim was to assess the activity of oritavancin towards the intracellular forms of *S. aureus* using strains with relevant resistance phenotypes.

Methods: MICs and MBCs were determined by microdilution in cation-adjusted MH broth + 0.002 % Tween 80. Intracellular activity was measured after phagocytosis by human THP-1 macrophages (changes in CFU from initial inoculum over 24 h over a wide range of extracellular concentrations (0.006 to 60 µg/mL) with analysis of dose-response parameters [E_{max} and static concentration]; AAC 2006; 50:841-851).

Results:

Strain	Phenotype	MIC (mg/L)	MBC (mg/L)	Intracellular activity (24h)		
				E_{max} ^a ($\Delta\log$ cfu)	static conc. ^b (µg/mL)	(x MIC)
ATCC25923	MSSA	0.06	0.25	-4.1	5.0	83
NRS192	CA-MRSA	0.125	0.25	-3.9	2.4	80
N4112910	HA-MRSA	0.03	0.06	-1.9	5.4	43
NRS18	VISA	0.5	1	-1.6	7.1	14
NRS126	VISA	0.25	0.5	-1.1	1.2	5

^a extrapolated for oritavancin at infinite concentration (based on Hill equation) at 24h compared to post-phagocytosis value (t=0)

^b no apparent change from the original, post-phagocytosis inoculum

Conclusions: Oritavancin is active intracellularly against all strains, but shows a markedly lesser maximal efficacy (E_{max}) against VISAs (as already reported for another lipoglycopeptide [telavancin; JAC 2006;58:1177-84]). Oritavancin also shows a static effect at lower multiples of MIC against VISAs as compared to MRSA and MSSA. This suggests that the intracellular milieu modulates oritavancin activity in a differential fashion depending on the resistance phenotype.

A-973

Postantibiotic Effects of Telavancin Against 15 Gram-Positive Organisms

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Background: Telavancin is an investigational lipoglycopeptide with cidal activity against Gram-positives. We measured postantibiotic effect (PAE), sub-MIC effect (SME), and postantibiotic sub-MIC effect (PA-SME) of telavancin against 15 Gram-positive bacteria.

Methodology: PAE, SME, and PA-SME effects of telavancin were tested against 11 methicillin-resistant *Staphylococcus aureus* (MRSA), including *S. aureus* ATCC 33591, 2 hetero vancomycin-intermediate (hVISA), 4 vancomycin-intermediate (VISA) and 1 vancomycin-resistant (VRSA) MRSA (these 7 strains were isolated at Hershey Med. Ctr.), 1 *Streptococcus pyogenes*, 1 *S. agalactiae*, 1 vancomycin-susceptible *Enterococcus faecalis*, and 1 vancomycin-susceptible *E. faecium*. Telavancin MICs were by CLSI macrodilution. Freshly prepared cation-adjusted Mueller-Hinton broth (+ 5% lysed horse blood for streptococci) was used throughout the study. PAEs were induced by exposure to telavancin at 10 x MIC for 1 h. PA-SMEs were tested by diluting exposed cultures (1:1000) then adding telavancin at sub-MIC concentrations of 0.2 x, 0.3 x, 0.4 x MIC. SMEs measured direct effect of sub-MIC concentrations on unexposed cultures.

Results: Telavancin MICs varied between 0.06 and 0.5 µg/ml for all strains. The PAEs ranged between 0.4-6.7 h. Exposure to subinhibitory concentrations delayed bacterial growth. The PA-SMEs were longer than the PAEs and SMEs. For *S. aureus* the PA-SMEs at subinhibitory concentrations of 0.2 x, 0.3 x, and 0.4 x MIC ranged between 2.9-8.6 h, 4.8->10.6 h, and 6.7->10.7 h, respectively. For *S. pyogenes*, *S. agalactiae*, *E. faecalis* and *E. faecium* the PA-SMEs at 0.2 x, 0.3 x and 0.4 x MIC ranged between 0.8-10.7 h, 4.7->10.7 h, and >10->11 h, respectively.

Conclusions: Telavancin PAEs ranged between 0.4-6.7 h for all strains tested, and subinhibitory concentrations of telavancin produced long PA-SMEs against all of the strains. PA-SMEs were longer than PAE and PAE + SME at the subinhibitory concentrations tested. Subinhibitory concentrations of telavancin substantially extended duration of the PAE.

A-976

Pharmacokinetics of Telavancin in Patients with Complicated Skin and Skin Structure Infections (cSSSI)

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Background: Telavancin (TLV) is an investigational, rapidly bactericidal lipoglycopeptide with activity against clinically important Gram-positive bacteria. We report herein the pharmacokinetic (PK) disposition of TLV in patients with cSSSI.

Methods: A total of 337 patients with cSSSI, enrolled in 2 methodologically identical, randomized, active controlled Phase 3 studies, participated in the PK evaluations. Up to 4 blood samples were collected from each individual on dosing Day 3, 4, or 5. TLV concentrations in plasma were determined by liquid chromatography with tandem mass spectrometric detection. PK parameters were determined by non-compartmental analysis.

Results: In the combined study PK population, 54% of the patients were male and 17% had diabetes. The mean (\pm SD) age, body weight and estimated creatinine clearance (CL_{cr}) were 44 ± 15 years, 84.3 ± 27.6 kg and 104 ± 34 mL/min, respectively. The mean (\pm SD) TLV systemic exposure (AUC_{0-24}), maximum plasma concentrations (C_{max}) and trough plasma concentrations (C_{trough}) were 905 ± 269 μ g·hr/ml, 92.8 ± 51.7 μ g/mL and 8.8 ± 7.0 μ g/ml, respectively. These PK parameters were comparable to those observed in healthy subjects. The AUC/MIC target of 219 was achieved in all patients for organisms with minimum inhibitory concentration (MIC) of ≤ 2 μ g/mL. Body weight and CL_{cr} were identified as covariates for TLV AUC_{0-24} and C_{trough} . Gender and diabetes did not affect the TLV PK parameters. The effect of age was confounded by its relationship with CL_{cr} .

Conclusions: Our results highlight the predictable nature of TLV PK in patients with cSSSI. Creatinine clearance was identified as a strong covariate for TLV systemic exposure. These results support the weight-based, once-daily dosing strategy for TLV with dose adjustments guided by degree of renal dysfunction.

A-978

Activity of Telavancin (TLV) Against *Staphylococcus aureus* (SA) of Varying Vancomycin Susceptibilities in an In Vitro Pharmacokinetic/Pharmacodynamic (PK/PD) Model with Simulated Endocardial Vegetations (SEV)

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Background: TLV is an investigational lipoglycopeptide with broad activity against Gram-positive pathogens. We assessed the activity of TLV and vancomycin (VAN) alone and combined with gentamicin (GEN) or rifampin (RIF) versus 4 strains of SA in an in vitro PK/PD model with SEV.

Methods: The clinical isolates used were: methicillin-susceptible SA (MSSA) 1199, methicillin-resistant SA (MRSA) 494, heterogeneously glycopeptide intermediate SA (hGISA) R1629, and glycopeptide intermediate SA (GISA) NJ 992. Simulated doses of TLV 10 mg/kg q 24 h, VAN 1 g q 12 h, GEN 5 mg/kg q 24 h, and RIF 300 mg q 8 h were utilized in the PK/PD model with SEV at a starting inoculum of $\sim 10^9$ CFU/g over 96 h in duplicate. Bactericidal activity was defined as 99.9% kill from initial inoculum ($T_{99.9}$) and was determined by linear regression. Differences in CFU/g at 96 h were evaluated by ANOVA with Tukey's post-hoc test.

Results: TLV/VAN MIC values ($\mu\text{g/ml}$) were 0.125/0.5 for MRSA 494, 0.25/1 for MSSA 1199, 0.125/2 for hGISA R1629, and 0.5/8 for GISA NJ 992. In the PK/PD model, TLV demonstrated significantly greater kill than VAN ($p < 0.01$) against all strains except MRSA 494 ($p = 0.07$). TLV absolute reductions in CFU/g at 96 h were 2.8 ± 0.5 for MRSA 494, 2.8 ± 0.3 for MSSA 1199, 4.2 ± 0.2 for hGISA R1629, and 4.1 ± 0.3 for GISA NJ 992. Adding RIF to TLV improved kill over TLV alone resulting in an absolute reduction in CFU/g of 3.7 ± 1.2 ($T_{99.9} = 73$ h) and 3.8 ± 0.3 ($T_{99.9} = 52$ h) against MRSA 494 and MSSA 1199 at 96 h respectively. TLV plus GEN was the best combination against both MRSA 494 and MSSA 1199 producing absolute reductions in CFU/g of 4.1 ± 0.4 ($T_{99.9} = 66$ h) and 4.3 ± 0.3 ($T_{99.9} = 56$ h) respectively at 96 h. TLV alone was bactericidal at 64 h against hGISA R1629 and at 62 h against GISA NJ 992.

Conclusions: TLV was superior to VAN in most scenarios, particularly against hGISA and GISA. The addition of both GEN and RIF to TLV improved kill over TLV alone and may be potential therapeutic options.

A-980

Pharmacodynamics of Telavancin: Relationship of AUC/MIC to Antibacterial Effect and Emergence of Resistance

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Background: Telavancin is a new parenteral lipoglycopeptide antibiotic with broad *in vitro* potency against Gram positive pathogens, including MRSA. The relationship between free drug telavancin AUC/MIC and emergence of resistance (EoR) or antibacterial effect (ABE) are poorly established. We used an *in vitro* pharmacokinetic model (IVPKM) to perform a dose ranging study to define the relationship between free drug AUC/MIC and ABE or EoR for 5 strains of MRSA.

Methods: A single compartment dilutional IVPKM was used to simulate free drug telavancin concentrations associated with human doses of 10mg/kg/day reducing to 0.03mg/kg. For the 10g/kg simulation, the C_{max} was 10mg/L, t_{1/2} 8h. 5 strains of MRSA telavancin MICs 0.19-0.38mg/L were used. ABE was measured by log reduction in viable count at 24h (d24) and EoR by growth on MICx2 media at 24h. D24 was related to AUC/MIC using an inhibitory Sigmoid E_{max} model for each strain and also the pooled data set from all 5 strains.

Results: The free drug AUC/MIC for 24h static, -1 log, -2 log drop were 43 ± 17 , 50 ± 17 , 67 ± 21 respectively, meaning the individual strains. There was significant strain to strain variability in the AUC/MIC for each ABE but curve fit was excellent with each individual strain ($R^2 > 0.9$). Using the pooled data, the free drug AUC/MIC for static, -1, -2 log drop were 26, 53 and 101 (R^2 0.72). EoR occurred with all strains at AUC/MIC <50, and more markedly at <25.

Conclusions: Free drug telavancin AUC/MICs of 50-100 are associated with 1-2 log drop in MRSA bacterial counts and a minimum risk of EoR.

A-994

Pharmacokinetic-Pharmacodynamic (PK-PD) Target Attainment (TA) as Decision Support for Oritavancin (ORI) Susceptibility Breakpoints for *Staphylococcus aureus* (SA)

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Background: Integration of PK-PD relationships from non-clinical models & PK data from clinical studies can provide decision support for susceptibility breakpoints. PK-PD TA analyses were conducted for ORI, a novel lipoglycopeptide in development for complicated skin and skin structure infections, to identify PK-PD cut-off MIC values to support SA susceptibility breakpoints.

Methods: Monte Carlo simulation was used to assess PK-PD TA by MIC using non-clinical PK-PD targets and a population PK model from Phase 2/3 studies. Free-drug (*f*) AUC₀₋₂₄:MIC targets for different log₁₀ CFU reduction endpoints at 48 and 72 h were based on those from a 3-day neutropenic murine thigh-infection model for SA, using an ORI murine protein binding (PB) estimate = 93.6%. PK parameter estimates for 10,000 patients following ORI 200 mg once daily were simulated & average *f*AUC₀₋₂₄ was calculated over 48 and 72 h (ORI PB = 87.5%). The probability of PK-PD TA for each target by MIC was evaluated.

Results: Irrespective of analysis endpoint (48 or 72 h) and target, probabilities of TA approached 1.0 for MIC ≤ 0.12 µg/mL, decreased at MIC = 0.25 µg/mL and were < 0.1 at MIC = 0.5 µg/mL.

Conclusions: These data support an ORI MIC PK-PD cut-off value of 0.12 µg/mL for SA. Such data will need to be considered in the context of MIC population statistics for SA (MIC_{50/90} of 0.06/0.12 µg/mL, respectively) and clinical outcome statistics when setting SA susceptibility breakpoints.

A-1878

Protein Binding of [¹⁴C]-Telavancin in Plasma and Human Skin Blister Fluid

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Background: Telavancin (TLV) is an investigational, bactericidal lipoglycopeptide antibiotic with potent activity against a broad range of Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA). The objective of this study was to evaluate the *in vitro* binding of [¹⁴C]-TLV to plasma harvested from different species as well as human skin blister fluid.

Methods: The binding of 1.00, 10.0 and 100 µg/mL of [¹⁴C]-TLV to mouse, rat, dog, bovine, rabbit and human plasma as well as human skin blister fluids were determined by means of equilibrium dialysis through a semipermeable membrane (molecular weight cut-off of 12000 -14000) over a period of 12 hours at 37°C. The dialysis buffer consisted of 5% dextran in Krebs Physiological Buffer, pH 7.4. **Results:** Over the concentration range studied, the binding of [¹⁴C]-TLV to plasma proteins was species-independent and ranged from 83.3% to 92.9%. The mean protein binding values (±SD) for 100 µg/mL of [¹⁴C]-TLV are presented in the table. Within species, observed mean protein binding ranges across concentrations studied were as follows: mouse 90.4-93.8%; rat, 91.2-93.5%; dog 86.4-90.9%; rabbit, 87.6-90.7%; bovine, 80.0-90.4%; and human, 85.5 to 90.7%. Binding was not affected by the concentration of [¹⁴C]-TLV. At a concentration of 100 µg/mL, the mean (±SD) protein binding of [¹⁴C]-TLV in pooled human skin blister fluid was 85.8%(±1.6), similar to that observed in the pooled human plasma from the same subjects. **Conclusions:** TLV exhibited species-independent and concentration-independent protein binding in plasma. The fraction of TLV bound to plasma proteins was on average 90%. TLV exhibited similar degrees of protein binding in human plasma and skin blister fluid.

Mean % Protein Binding in Plasma (±SD)

[¹⁴ C]-TLV	Mouse (n=3)	Rat (n=3)	Dog (n=3)	Bovine (n=3)	Rabbit (n=3)	Human (n=4)
100 µg/mL	92.7(±1.0)	92.9(±0.8)	89.3(±1.7)	88.9(±1.3)	90.3(±0.4)	89.7(±0.9)

A-1879

Pharmacokinetics, Excretion, and Mass Balance of Telavancin Following Intravenous Administration of [¹⁴C]-Telavancin to Healthy Volunteers

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Background: Telavancin (TLV) is an investigational, bactericidal lipoglycopeptide antibiotic with potent activity against a broad range of clinically relevant Gram-positive pathogens *in vitro*. The objectives of this study were to define the disposition and excretion kinetics of [¹⁴C]-TLV in man following intravenous (IV) administration, and to characterize the metabolites present in plasma and urine.

Methods: A single 1-h IV infusion of 10 mg/kg of [¹⁴C]-TLV (~ 0.68 µCi/kg) was administered to each of 6 subjects. Blood, urine, and feces were collected at regular intervals for up to 216 h post-dose. Whole blood and plasma concentrations of total radioactivity and plasma concentrations of TLV were determined, urine and fecal recovery of total radioactivity was determined, and metabolites in plasma and urine were identified and characterized using LC-MS/MS.

Results: Following a single IV dose of [¹⁴C]-TLV, approximately 76% (mean percentage) of the total radioactivity was recovered from urine. Excretion *via* feces was very low (<1%) throughout the entire collection period. Total recovery was ~77% (71-82%). Overall 82.3% (mean percentage) of the total radioactivity in the urine was recovered as parent. Three minor mono-hydroxylated metabolites were identified in the urine. TLV accounted for >95% of the total radioactivity in plasma for up to 12 h post-dose, and 83% and 13% of the total plasma radioactivity at 24 and 48 h post-dose, respectively. Total plasma clearance of TLV was 15.7 ± 2.5 mL/h/kg. The mean renal clearance of [¹⁴C]-TLV (based on cumulative excretion of TLV in urine over 0-48 h) was 9.00 ± 1.32 mL/h/kg. Elimination half-life of TLV was 7.08 ± 0.50 h. The 1-h infusion of 10 mg/kg [¹⁴C]-TLV was well tolerated. Dysgeusia (taste disturbance) was the most common adverse event.

Conclusions: Urinary excretion is the major route of elimination for [¹⁴C]-TLV in humans. The majority of the dose was excreted in the urine as unmetabolized TLV.

A-1893

A Phase I, Double-Blind, Randomized, Placebo- and Positive-Controlled, Single Dose, Parallel Design Trial to Assess the Potential Electrocardiographic Effects of Oritavancin in Healthy Adults

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Background: Oritavancin is a lipoglycopeptide with broad activity against gram positive bacteria. This study evaluated potential drug effects on QTc per the 2005 FDA E14 Guidance.

Methods: 240 male or female subjects received the clinical (200 mg IV over 1 hour) or suprathapeutic (800 mg IV over 2 hours) oritavancin dose in a parallel design with positive (400 mg oral moxifloxacin) and placebo controls. QTc_{lb} is the ECG QT interval, measured and averaged from 3 beats on 3 separate ECGs at each timepoint, and corrected for heart rate for all subjects on the basis of their baseline QT-heart rate relationships. The primary analysis was a comparison of mean QTc_{lb} intervals for 800 mg oritavancin and placebo evaluated in a linear mixed-effect model. The mean and upper 95% one-sided CI of the baseline-adjusted differences between QTc_{lb} intervals (ddQTc_{lb}) was used to make the primary statistical comparison. Oritavancin PK parameters were AUC and C_{max}. Safety variables were also evaluated.

Results: The upper CI bound of ddQTc_{lb} for 800 mg oritavancin was <6 msec at all timepoints, thus below the level of regulatory concern (10 msec). The lower bound for moxifloxacin exceeded 5 msec at 1 hour, validating adequacy of assay sensitivity. Values for C_{max} were seen immediately after the end of infusion. Due to the different infusion times, C_{max} increases with dose were less than dose-proportional. Increase in AUC was dose proportional, indicating linear PK within the range of 200 mg to 800 mg. The C_{max} and AUC %CV were ~20% and did not change from 200 mg to 800 mg. Oritavancin was well-tolerated.

Conclusions: There was no clinically significant repolarization effect of single doses of 200 and 800 mg oritavancin IV in normal subjects.

A-3563

Pharmacokinetics-Pharmacodynamics (PK-PD) of Oritavancin (ORI) Efficacy in Patients with Complicated Skin and Skin Structure Infections (cSSSI)

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Background: ORI is a novel lipoglycopeptide in development for the treatment of cSSSI. Using Phase 3 clinical trial data, PK-PD relationships for efficacy in patients with cSSSI were evaluated.

Methods: Evaluable patients receiving ORI 1.5 or 3 mg/kg/day for 3-7 days with plasma PK were considered. Using a population PK model, individual post-hoc PK parameter estimates were used to estimate AUC₀₋₂₄. PK-PD analyses were conducted in 5 patient groups (grps) based on Gram + pathogens isolated at baseline, starting with the most homogenous grp (grp 1, *S. aureus* & *S. pyogenes*). Multivariate logistic regression (LR) was used to identify factors predictive of outcome. Breakpoints (bkpts) for continuous independent variables and interactions between demographic &/or disease characteristics and AUC:MIC ratio were evaluated using tree-based modeling.

Results: 69 patients were classified into 5 non-exclusive grps (n=43 to 69) according to baseline Gram + pathogens; overall clinical success was 64%. No overall PK-PD relationships for efficacy were detected. However in diabetic patients (n=24), % clinical success was higher for those with AUC:MIC ratios ³ vs those < 2253 (across grps, 75-83 vs 9-17%, p £ 0.01). A final multivariate LR model with diabetes (p=0.002) and its interaction with AUC:MIC bkpt (p=0.016) showed AUC:MIC bkpt to be predictive of clinical outcome for diabetics.

Conclusions: In diabetic patients, AUC:MIC ratio ³ 2253 appears predictive of clinical success, suggesting that greater ORI exposure may be required in the presence of this comorbidity. However, given the limited sample size, this finding should be further validated. In non-diabetics, no AUC:MIC bkpt was apparent. It is likely that the lack of a PK-PD relationship for efficacy in the overall population was due to the paucity of non-diabetics with ineffective AUC:MIC ratios.

B-1002

Efficacy of Telavancin in a Murine Model of Acute Suppurative Osteomyelitis Induced by Methicillin-Resistant *Staphylococcus aureus*

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Background: Acute suppurative osteomyelitis represents an inflammatory reaction to bacterial infection characterized by abscess formation at the diaphyseal end of the bones. Infections with methicillin-resistant *S. aureus* (MRSA) frequently have severe chronic-progressive course and are particularly difficult to treat. Telavancin (TLV) is an investigational lipoglycopeptide with potent bactericidal activity against Gram-positive organisms, including MRSA. We examined the therapeutic efficacy of TLV, in comparison to vancomycin (VAN), in a murine model of acute suppurative osteomyelitis induced by MRSA.

Methods: Acute suppurative osteomyelitis in 4 week old, male ICR mice was induced by intravenous (IV) injection of 1.7×10^7 CFU of MRSA strain #22048 (clinical isolate from Japan). The animals were randomly assigned to receive at 8 h post-infection and twice a day on the following 3 days a total of 7 doses of TLV 40mg/kg IV, VAN 110mg/kg IV or normal saline IV (control). A fourth group received 3 doses of VAN followed by 4 doses of TLV. Doses were selected to equate clinical AUC exposures. Cohorts of animals (n=6) were sacrificed at 8 h, 48 h and 96 h post-infection and bone marrow samples were collected for bacterial titer determination. Minimum inhibitory concentration (MIC) was determined by the broth microdilution method (CLSI).

Results: see table

Conclusions: Systemic antibiotic therapy with TLV was associated with greater reduction in bone marrow bacterial titers when compared to VAN. These preclinical results suggest that that TLV may have utility in the treatment of hematogenous acute suppurative osteomyelitis induced by MRSA.

Time post-infection (doses received)	Bacterial Titers in Bone Marrow (Log CFU/g; Mean \pm SE)			
	Control (n=6)	TLV (n=6)	VAN (n=6)	VAN then TLV (n=6)
8 h (pre-treatment)	4.82 \pm 0.06	n/a	n/a	n/a
48 h (3 doses)	6.99 \pm 0.07	2.36 \pm 0.20	5.68 \pm 0.31 ^a	
96h (7 doses)	7.49 \pm 0.09	1.15 \pm 0.07 ^b	5.07 \pm 0.46	1.96 \pm 0.15 ^b
MIC (μ g/ml)	-	0.25	1	-

^a After 3 doses of VAN;

^b $P < 0.01$ vs VAN (Dunnett multiple comparison test);

B-1004

Comparison of Telavancin and Vancomycin for Prevention of In Vivo Staphylococcal Device-Associated Infections

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Background: Telavancin (TLV) is an investigational lipoglycopeptide that operates through a multifunctional mechanism and displays bactericidal activity against Gram-positive pathogens including staphylococci. This study compared vancomycin (VAN) and TLV for the prevention of staphylococcal colonization and infection of surgically implanted devices.

Methods: Female rabbits were randomly administered a single IV dose of 5% dextrose (D5W), VAN 20 mg/kg, or TLV (15 mg/kg, 30 mg/kg or 45 mg/kg) before the subcutaneous implantation of catheter segments (6 segments/rabbit; 9 rabbits/54 devices per treatment group). The catheters were inoculated with *S. aureus* P1 MSSA strain (10⁵ CFU) and the wounds sutured. One week post surgery the animals were sacrificed and bacterial cultures were obtained from the devices, surrounding tissues, and blood.

Results: All groups of systemic antibiotic prophylaxis were associated with significantly ($p < 0.0001$) lower rates of device colonization and device-related infection than the control group (Table). TLV (15 mg/kg), at a clinically subtherapeutic dose, was non-inferior to VAN in preventing device colonization and device-related infection. Clinically therapeutic doses of TLV (30 or 45 mg/kg), were superior ($p < 0.0001$) to VAN in preventing device colonization and device-related infection. All blood cultures were sterile.

Conclusions: The results from this animal study suggest that further study of the preoperative administration of TLV as an effective approach to prevent staphylococcal colonization and infection of surgical implants in humans is warranted.

Device Colonization and Infection Rates

Colonization col./total(%)	Infection inf./total(%)
48/48 (100%)	48/48 (100%)
28/54 (52%)	28/54 (52%)
21/54 (39%)	19/54 (35%)
6/54 (11%)	5/54 (9%)
6/54 (11%)	6/54 (11%)

B-1009

Efficacy of Oritavancin (ORI) in the Mouse Bacteremia Model

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Background: ORI is a lipoglycopeptide active against gram-positive bacteria including *Staphylococcus aureus* (SA). ORI is active in several animal models of infection including SA rabbit endocarditis. We investigated the efficacy of ORI in the mouse SA bacteremia model to assist in dose selection for human studies. **Methods:** Infection was established in CD-1 mice (n=10/group) by injecting intraperitoneally (i.p.) 10⁷ colony-forming units (CFU) of methicillin-sensitive SA ATCC 13709 (MIC=0.06 µg/mL) in 5% gastric hog mucin. 1) To assess efficacy resulting from a single dose of ORI, mice were treated intravenously (i.v.) with ORI at doses of 0.002 to 20 mg/kg at 1h post-infection (PI). Mice were observed for 72h, and the dose at which 50% of the animals were protected (PD₅₀) was calculated by using the Probit method. 2) In a dose-ranging study, ORI was administered i.v. at 1h PI. Mice received a multiple dose regimen to simulate human drug concentration profiles for the following daily doses (QD): 100, 400, 800 mg for 72h, or at a single 1200 mg dose. Blood and spleen were harvested over 72h PI to determine the bacterial titers.

Results: 1) Single doses of ORI showed efficacy in the bacteremia mouse model. 2 mg/kg of ORI provided 100% protection at 72h PI while all untreated mice succumbed within 24h. The PD₅₀ was 0.15 mg/kg (CI 0.06-0.40). 2) ORI showed efficacy using human equivalent (HEQ) dose regimens. At 72h PI, the 100 mg QD eq. dose was sufficient to rescue 100% of the animals, and reduced bacterial density by 1.7 +/- 1.2 Log CFU/ml of blood (p<0.03) and 1.3 +/- 1.0 Log CFU/spleen (p<0.02). ORI administered at a single 1200 mg HEQ dose consistently cleared SA from blood and decreased bacterial titer by 1.8 +/- 0.4 Log CFU/spleen (p<0.01). **Conclusion:** ORI has potent anti-SA activity in the bacteremia mouse model. All tested HEQ doses of ORI showed efficacy supporting further development for bacteremia.

B-1011

Efficacy of Oritavancin (ORI), a Lipoglycopeptide Antibiotic, in a Rat *Staphylococcus aureus* Endocarditis (IE) Model: Microbiological and Bioluminescent Assessments

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Background: ORI is a lipoglycopeptide with potent activity against gram-positive bacteria. The mechanisms of action of ORI include inhibition of cell wall synthesis, but differ from vancomycin (VAN) in that ORI also disrupts cell membranes and inhibits RNA synthesis. In this study, we tested efficacy of ORI, vs. VAN, oxacillin (OX) and daptomycin (DAP) in real-time by using a rat IE model caused by a bioluminescently engineered, biofilm-positive methicillin-susceptible *S. aureus* strain (Xen29).

Methods: IE was induced following transcarotid-transaortic valve indwelling catheterization. At 14 h after IV infection with 10⁵ cfu *S. aureus*, animals were randomized to receive either: i) no therapy (control); ii) ORI 80 mg/kg, iv, once daily; iii) VAN 120 mg/kg, subQ, bid; iv) DAP 10 mg/kg, subQ, once daily; or v) OX 200 mg/kg, im, tid; for 4 days. Cardiac bioluminescence signals (CBLS) were quantified daily, using a highly sensitive in vivo imaging system (IVIS®). At 24 h after the last antibiotic dose, animals were sacrificed, and cardiac vegetations were quantitatively cultured.

Results: All regimens significantly decreased *S. aureus* densities in vegetations vs. controls (9.51 ± 0.75 ; 4.52 ± 1.58 ; 6.14 ± 2.35 ; 4.32 ± 0.62 ; and 4.49 ± 1.41 log₁₀CFU/g. veg. \pm SD for controls, ORI, VAN, DAP and OX; respectively, $P < 0.00001$ for ORI, DAP and OX vs. controls; and $P < 0.005$ for VAN vs. controls). ORI treated animals had ~ 1.5 log lower *S. aureus* densities in vegetations vs. VAN treated animals, although these differences did not reach statistical significance. In addition, all regimens prevented emergence of CBLS vs. controls.

Conclusion: ORI had greater efficacy vs. VAN in this IE model, supporting further development of ORI for the therapy of severe *S. aureus* infections (e.g., IE).

B-1012

Efficacy of Telavancin Against hVISA Infection in the Neutropenic Murine Bacteremia Model

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Background: Infections caused by heterogeneous vancomycin-intermediate *S. aureus* (hVISA) are associated with high rates of vancomycin (VAN) treatment failure. Telavancin (TLV) is an investigational, bactericidal lipoglycopeptide with activity against Gram-positive pathogens including organisms with reduced susceptibility to VAN. We used a neutropenic murine bacteremia model to compare the efficacy of TLV and VAN against hVISA infection.

Methods: Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method (CLSI). Immunocompromised female non-Swiss albino mice (n = 110) were infected by intraperitoneal inoculation (0.7 mL) of hVISA Mu3 (10⁷ CFU/mL). Infected animals were assigned to one of three different treatment groups that were scheduled to receive, starting 4 h post-inoculation, VAN (110 mg/kg, q 12 h, SC for 8 days), TLV (40 mg/kg, q 12h, SC for 4 days) or no treatment (control group). Doses were selected to equate clinical AUC exposures. Blood and spleen bacterial titers were quantified from cohorts of animals (n= 5) that were euthanized at pre-treatment and at 24 h intervals post-treatment for 8 days.

Results: All animals were bacteremic at the pre-treatment period. Mortality was 100% within 24 h post-infection in untreated controls.

Overall 4 days of therapy with TLV was associated with greater reduction in spleen bacterial titers, rates of bacteremia and mortality than 8-days of therapy with VAN.

Conclusions: The data from this study demonstrate that, at doses that approximate human exposure, TLV was effective in clearing hVISA infection in neutropenic bacteremic mice.

	MIC(μg/mL)	Bact. Titers Mean ±SD (log CFU/g)	Animals with bacteremia % (n/N)	Mortality % (n/N)
Pre-treatment	-	7.3 ± 0.24	-	-
VAN	2	4.0 ± 0.63 ^a	50% (2/4) ^a	7.5% (3/40) ^c
TLV	0.5	2.3 ± 0.10 ^{a,b}	0% (0/5) ^a	0% (0/40) ^c

^aend of 8-day study period; ^bp<0.05 vs VAN; ^coverall mortality during the 8-day period

B-3573

Associations Between Patient Outcome and Genotype of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates From a Multinational Trial of Complicated Skin and Skin Structure Infections (cSSSI)

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Background: We evaluated potential associations between bacterial genotype and clinical outcome using MRSA isolates from Phase 3 trials assessing telavancin (TLV) for treatment of cSSSI (ATLAS).

Methods: *S. aureus* isolates from microbiologically evaluable (ME) patients (clinically evaluable outcome and a Gram + pathogen recovered at baseline) with cSSSI enrolled in 2 double-blind, randomized, Phase 3 studies (ATLAS trial) were genotyped by pulsed-field gel electrophoresis (PFGE) and PCR for SCC*mec*, *agr*, and 30 other virulence genes. Test-of-cure was 7-14 days after end of study treatment.

Results: A total of 769 pooled ME patients from 92 centers in 15 countries had a single baseline pathogen of *S. aureus*. Of these, 522 (68%) were MRSA. Compared with MRSA isolates from other regions, North American MRSA isolates (n=483) were more likely to contain *pvl*, *fnba*, *fnbb*, *clfb*, *map*, *ica*, *chp*, and SCC*mec4*, and less likely to contain *cna*, *sea*, *seg*, *sei*, or *agrI* (p<0.0005 for each). Clinical cure in MRSA was associated with presence of *pvl* (90% vs. 78%, p=0.015), *fnba* (92% vs. 76%; p=0.001); *sdrC* (97% vs 88%; p=0.006); *map* (89% vs. 72%; p=0.003), and SCC*mec4* (90% vs. 78%; p=0.014). USA 300 PFGE profile was present in 71% of typeable MRSA isolates.

Conclusions: In this contemporary, international study of MRSA cSSSI isolates, the presence of specific virulence genes varied geographically, and was associated with clinical outcome. The presence of *pvl* in MRSA isolates was associated with better outcome among patients with cSSSI. These findings suggest that *pvl* alone might not result in more severe infection in patients with MRSA cSSSI.

C1-142

Activity of Oritavancin Tested Against Contemporary Gram-Positive Pathogens Submitted to a Global Surveillance Program (2008)

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Background: Oritavancin (ORI), a novel lipoglycopeptide active against Gram-positive (GP) pathogens, is under US-FDA review for complicated skin and skin structure infections. We evaluated ORI against contemporary GP isolates recovered from an international resistance (R) surveillance program.

Methods: Consecutive, non-duplicate patient isolates (2890) were submitted from medical centers in North America and Europe during the first quarter of 2008 to the monitoring laboratory, and susceptibility (S) tested using CLSI methods (M7-A7 and M100-S18) against ORI and comparator agents. Isolates originated from bloodstream, skin and soft tissue and respiratory tract infections.

Results: ORI was highly active against all tested isolates including staphylococci (highest MIC value/MIC₉₀: 0.5/0.06 µg/mL), streptococci (0.12/0.06 µg/mL), and enterococci (1/0.06 µg/mL). ORI was equally active against *S. aureus* and coagulase-negative staphylococci, including against oxacillin-S and -R strains (all MIC_{50/90} values, 0.03/0.06 µg/mL). Among vancomycin (VANC)-R *E. faecalis* (5.7%) and *E. faecium* (66.3%), ORI was 16- and 8-fold less potent (MIC₉₀), respectively, than among VANC-S strains, but all VANC-R strains had ORI MICs ≤ 1 µg/mL. Compared with VANC, ORI MIC₉₀ potencies for all 3 organism groups were 8 to ≥512-fold lower.

Conclusions: Among tested agents, ORI displayed highest potency against recent staphylococcal, enterococcal and streptococcal isolates, and retained modest activity against VANC-R enterococcal strains (MIC values, ≤ 1 µg/mL). Bactericidal activity combined with enhanced potency are key attributes of ORI. As with other antimicrobials, R emergence necessitates continued surveillance.

Organism (no. tested)	MIC (µg/ml)		VAN	
	ORI 50%	ORI 90%	50%	90%
<i>S. aureus</i> (1728)	0.03	0.06	1	1
Coagulase-negative staphylococci (187)	0.03	0.06	2	2
<i>E. faecalis</i> (280)	0.015	0.06	1	2
<i>E. faecium</i> (187)	0.03	0.06	>16	>16
<i>S. pneumoniae</i> (286)	≤ 0.004	≤ 0.004	≤ 1	≤ 1
β-haemolytic streptococci (157)	0.03	0.06	0.5	0.5
Viridans group streptococci (45)	≤ 0.004	0.03	0.5	0.5

C1-143

Comparative Activity of Oritavancin Against Methicillin-Resistant *Staphylococcus aureus* (MRSA) Bloodstream Isolates from Geneva University Hospital

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Background: Oritavancin (ORI) is a semi-synthetic lipoglycopeptide with activity against drug-resistant *S. aureus*. We assessed the activity of ORI and comparators against multiply drug-resistant MRSA bloodstream isolates from Geneva University Hospital (HUG).

Methods: Bloodstream MRSA isolates (n=56) displaying a range of vancomycin [VAN] MICs (0.25-4 µg/mL) were collected from 1995 to 2003 from the Clinical Microbiological Laboratory of HUG. >90% of MRSA isolates were also resistant to ciprofloxacin, gentamicin, and erythromycin. Susceptibility testing was performed by broth microdilution (BMD; CLSI M7-A7, M100-S18) against ORI and comparators (VAN; teicoplanin [TEI]; daptomycin [DAP]; linezolid [LIN]). **Results:** MRSA phenotype was confirmed for all isolates (OXA MIC ³ 4 µg/mL). By BMD testing, three of the isolates showed a VAN-intermediate phenotype (VISA; VAN MIC, 4 - 8 µg/mL). The remaining 53 isolates showed VAN-susceptible (VSSA) phenotype (VAN MIC \leq 2 µg/mL).

Conclusions: From MIC₉₀s, ORI is four times more active than comparators VAN, TEI, LIN and DAP against VSSA. ORI MIC range for VISA was identical to that of LIN, and at least two times more active than those of VAN, TEI and DAP. Considering MIC range against VISA, ORI was more potent than VAN, TEI and DAP.

Agent	MIC range (MIC ₉₀), µg/mL	
	VSSA MRSA (n=53)	VISA ^a MRSA (n=3)
ORI	0.03 - 0.5 (0.25)	0.5 - 1
OXA	8 -> 64 (>64)	>64
VAN	0.25 - 2 (1)	4
TEI	0.06 - 4 (1)	2 - 4
DAP	0.25 - 2 (1)	1 - 4
LIN	0.5 - 2 (1)	0.5 - 1

^a Only MIC ranges are shown for VISAs as n<10

C1-144

In Vitro Activity of Oritavancin Against Gram-Positive Organisms in Europe: A Comparative Study of Clinical Trial and Surveillance Results

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Background: Oritavancin (ORI), a lipoglycopeptide under development for the treatment of infections caused by gram-positive organisms, has completed phase 3 trials for complicated skin and skin structure infections (cSSSI). Because *in vitro* activity of ORI from clinical trials (CT) as it relates to clinical outcome will be considered by regulators when determining susceptibility breakpoints, it is important to determine whether *in vitro* data from CTs correlate to the current activity profile of ORI. For this reason, the ORI activity profile against target pathogens collected from Europe (EU) in a recent surveillance (SV) study was compared to the ORI profile against EU isolates from ORI CTs.

Methods: Diverse EU clinical isolates of *S. aureus* (SA), *S. agalactiae* (GBS), *S. pyogenes* (GAS) and enterococci (EN; *E. faecalis* and *E. faecium*) from SV ('05-'08) and 2 cSSSI CTs ('99-'02) were centrally tested by broth microdilution against ORI (CLSI M7-A7, M100-S18).

Results: See Table.

Conclusion: The modal MICs and MIC₉₀s for CT isolates were comparable to SV isolates, within one doubling dilution for SA, GBS, and VAN S EN (per exclusion criteria, VAN NS EN were not evaluated in CT), and within two doubling dilutions for GAS. The current activity profile of ORI in EU surveillance correlates well with prior EU clinical trials.

Organism	Study	Category	Total N	MIC (µg/ml)			
				Range	Mode	MIC ₅₀	MIC ₉₀
<i>S. aureus</i>	CT	All	213	0.015-0.25	0.06	0.06	0.12
	SV	All	687	0.008-1	0.06	0.06	0.25
<i>S. agalactiae</i>	CT	All	27	0.015-0.12	0.03	0.03	0.12
	SV	All	26	0.03-0.25	0.06	0.06	0.25
<i>S. pyogenes</i>	CT	All	13	0.008-0.12	0.015	0.03	0.06
	SV	All	185	<=0.0005-1	0.06	0.06	0.25
Enterococci	CT	VAN S	54	0.015-0.12	0.06	0.06	0.06
		All	354	<=0.0005-1	0.03	0.03	0.12
	SV	VAN S	302	<=0.0005-0.5	0.03	0.03	0.06
		VAN NS	52	0.008-1	0.5	0.12	0.5

VAN: vancomycin, S: susceptible, NS: non-susceptible

C1-145

Oritavancin Comparative In Vitro Activity Against Hospital-Acquired Enterococci from Multicenter Surveillance - The ORION Study

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Background: Oritavancin (ORI) is a semi-synthetic bactericidal lipoglycopeptide with multiple modes of action, possessing superior pharmacodynamic properties compared to vancomycin. The ORI Susceptibility Profile Initiative (ORION) study is designed to determine ORI activity against a variety of gram-positive pathogens. This analysis focuses on the in vitro activity of ORI and comparators against common clinical enterococci and resistant phenotypes.

Methods: 248 clinical isolates of *E. faecalis* and *E. faecium* were collected from 17 labs in the US in late 2007/early 2008. MICs were determined using broth microdilution following CLSI guidelines and interpretive criteria (M7-A7 and M100-S18).

Results: ORI MIC₉₀ (µg/mL) and %S compared to vancomycin (VAN), daptomycin (DAP) and linezolid (LIN) are summarized below:

Conclusions: ORI had the lowest MICs against all enterococci compared to VAN, DAP and LIN without regard to resistant phenotypes. ORI MIC₉₀ values were 8- to 16-fold lower than either DAP or LIN and >512-fold lower than VAN against *E. faecalis* and *E. faecium*. ORI demonstrates potent in vitro activity against these common gram-positive pathogens.

	ORI		VAN		DAP		LIN	
	MIC ₉₀	%S	MIC ₉₀	%S	MIC ₉₀	%S	MIC ₉₀	%S
<i>E. faecalis</i> (141)	0.25	nd	4	91.5	2	100	2	98.6
<i>E. faecalis</i> , VRE (10)	0.5	nd	>128	0	1	100	2	100
<i>E. faecium</i> (107)	0.25	nd	>128	14	4	99.1	4	84.1
<i>E. faecium</i> , VRE (92)	0.25	nd	>128	0	4	98.9	4	82.6
<i>E. faecalis/E. faecium</i> (248)	0.25	nd	>128	58.1	4	99.6	2	92.3
All VREs (102)	0.25	nd	>128	0	4	99	4	84.3
Linezolid Non-Sus (19)	0.25	nd	>128	15.8	4	100	4	0

C1-146

Activity of Oritavancin Against *S. aureus*, *S. epidermidis*, *Enterococcus* spp. and *S. pneumoniae*, Isolated from Canadian Hospitals: Results of CANWARD 2007

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Background: Oritavancin (ORI) is a new lipoglycopeptide antimicrobial agent. We assessed the activity of oritavancin and comparators against Gram-positive pathogens causing infections in Canadian hospitals.

Methods: 12 sentinel hospitals across Canada submitted pathogens from patients attending all ward types. Susceptibility testing was performed, using CLSI broth microdilution.

Results: MIC₅₀ and MIC₉₀ values for ORI, vancomycin (VAN) and linezolid (LZD) are displayed below.

Conclusions: Oritavancin is more active than vancomycin and linezolid versus MRSA, MRSE, VISA, VRSA and *Enterococcus* spp. including VRE

SPN-*S. pneumoniae*, *Enterococcus* spp.- *E. faecium* and *E. faecalis*, VRE-vancomycin-resistant enterococci, HA-healthcare-associated, CA-community-associated, VISA-vancomycin-intermediate *S. aureus*, VRSA-vancomycin-resistant *S. aureus*.

*Median MIC

**Isolates obtained through the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program: supported under NIAID, NIH Contract No. N01-AI-95359.

Organism (# isolates)	ORI MIC ₅₀ /MIC ₉₀	VAN MIC ₅₀ /MIC ₉₀	LZD MIC ₅₀ /MIC ₉₀
SPN-All (171)	≤ 0.002/0.004	≤ 0.25/£ 0.25	0.5/1
- PenS (142)	≤ 0.002/0.004	≤ 0.25/£ 0.25	0.5/1
- PenI (23)	≤ 0.002/0.004	≤ 0.25/£ 0.25	0.5/1
- PenR (6)	≤ 0.002/0.004	≤ 0.25/0.5	0.5/1
MSSA (371)	0.25/0.5	1/1	2/4
CA-MRSA (23)	0.25/0.5	1/1	2/2
HA-MRSA (91)	0.25/0.5	1/1	2/4
MSSE (43)	0.25/0.5	1/2	0.5/1
MRSE (9)	0.5*	2*	1*
<i>E. faecalis</i> (81)	0.12/0.25	1/2	2/2
<i>E. faecium</i> (37)	0.03/0.12	0.5/>8	2/2
<i>Enterococcus</i> spp. (25)	0.12/0.5	1/2	2/2
VRE (2)	0.03*	>8*	2*
**VISA (12)	1/1	4/4	1/2
**VRSA (7)	0.5*	>8*	2*

C1-147

Bactericidal Activity of Telavancin, Vancomycin, and Metronidazole Against *Clostridium difficile*

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Background: Telavancin (TLV) is a rapidly bactericidal lipoglycopeptide active against a range of gram-positive bacteria. As *C. difficile* associated disease (CDAD) continues to increase in frequency and severity, new treatments are being investigated. This study compared the activity of TLV with those of vancomycin (VAN) and metronidazole (MET) against five *C. difficile* strains including REA types BI, G, K, and ATCC 700057 in cultures containing predominantly vegetative cells or spores.

Methods: Time-kill studies were conducted against 4 recent clinical isolates obtained from patients with CDAD. Agents were tested at concentrations of 2X, 4X, and 8X their respective MICs, which were previously determined by the agar dilution method. Starting inocula representing high and low vegetative cell to spore ratio were tested. Inoculum was a suspension from Brucella agar equal to the turbidity of a 0.5 McFarland standard diluted to give a starting concentration of approximately 10^6 cfu/ml in tubes containing Brucella broth with vitamin K₁ and hemin and the appropriate drug concentration. After inoculation, the tubes were placed at 37°C with constant agitation and were sampled for quantitative subcultures at various time points. Plates were incubated for 24-48h before colony counts were determined. All procedures were performed under anaerobic conditions.

Results: TLV MICs ranged between 0.125 and 0.25 µg/ml and were one to three-fold dilutions lower than vancomycin MICs. TLV was bacteriostatic at each MIC multiple, reducing the inoculum by 1 - 2.5 log₁₀ after 24h. Similar results were obtained for VAN (count reduction of 1.5 - 3 log₁₀ at 24h). Metronidazole demonstrated a concentration-dependent rapidly bactericidal activity with a 2-3 log₁₀ count reduction by 4 h. No differences were observed for the different inoculum forms or the REA types.

Conclusion: TLV demonstrated bacteriostatic activity similar to VAN against *C. difficile* while MET was bactericidal.

C1-148

Antimicrobial Activity of Telavancin Tested Against Contemporary Gram-Positive Pathogens: Results from an International Surveillance Program (2007)

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Background: Telavancin (TLV), an investigational lipoglycopeptide has been studied in Phase III clinical trials of skin and skin structure infections against Gram-positive (GP) pathogens, and is under regulatory review in the US, EU and Canada. We evaluated TLV potency against staphylococci, enterococci (ESP) and streptococci collected in 2007 as part of a global surveillance protocol.

Methods: Non-duplicate clinical GP isolates (10,700 total; see Table) were submitted from medical centers in North America (45.2%), Europe (25.0%), the Asia-Pacific region (22.4%) and Latin America (7.4%) participating in TLV surveillance. Identifications were confirmed by the central monitor and susceptibility (S) tested using CLSI methods.

Results: TLV was highly potent against year 2007 isolates originating from four continents, inhibiting all *S. aureus* (SA; 45.1% oxacillin-resistant [OX-R]) and coagulase-negative staphylococci (CoNS; 78.0% OX-R) at £0.5 mg/mL; all vancomycin (VAN)-S ESP at £1 mg/mL; and all *S. pneumoniae* (SPN), viridians group streptococci (VGS) and b-haemolytic streptococci at £0.25 mg/mL. While TLV MIC values were elevated among VAN-R ESP (16.7% overall), 26.8% and 76.6% of VAN-R strains had TLV MIC values of £1 mg/mL and £2 mg/mL, respectively. OX-R among SA and penicillin non-susceptibility among SPN and VGS had no adverse effect on TLV activity.

Conclusions: TLV was the most potent (MIC₉₀) agent tested against GP isolates originating from a 2007 TLV global surveillance study. Pending regulatory agency approval and clinical introduction, continued monitoring for potential resistance emergence to TLV and other Gram-positive-targeted agents will be necessary.

Organism (no. tested)	MIC (µg/mL)		Cum. % inhibited at MIC (µg/mL)				
	50%	90%	£0.12	0.25	0.5	1	2
<i>S. aureus</i> (5,895)	0.12	0.25	84	>99	100	-	-
CoNS* (1,030)	0.12	0.25	85	>99	100	-	-
<i>E. faecalis</i> (1,229)	0.25	0.5	21	83	98	98	98
<i>E. faecium</i> (680)	0.12	2	58	59	61	69	92
<i>S. pneumoniae</i> (180)	0.03	0.03	100	-	-	-	-
β-haemolytic strep (125)	0.03	0.06	>99	100	-	-	-
Viridans group strep (28)	0.03	0.06	100	-	-	-	-
Corynebacterium spp. (21)	0.03	0.03	100	-	-	-	-

*CoNS = Coagulase-negative staphylococcus

C1-149

Activity of Telavancin Against Gram-Positive Isolates from Phase 3 Studies of Hospital-Acquired Pneumonia (ATTAIN)

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Background: Telavancin (TLV) is an investigational, bactericidal lipoglycopeptide with a broad and potent Gram-positive spectrum of activity. The efficacy and safety of TLV have been studied in patients with hospital acquired pneumonia (HAP) in two methodologically identical, Phase 3 studies (ATTAIN 1 and ATTAIN 2). Here, we report the susceptibility testing results for TLV and comparators against the Gram-positive isolates collected in these studies.

Methods: A total of 738 baseline aerobic Gram-positive isolates were obtained from 1503 HAP patients enrolled worldwide. All isolates were identified and susceptibility tests were performed at a central laboratory. MIC values were determined by the CLSI broth microdilution method.

Results: The most frequently isolated Gram-positive pathogens were *S. aureus* (n=650 [88 %]; of which 63 % were MRSA) and *S. pneumoniae* (n=54 [7 %]; of which 35% were penicillin-resistant). Activity of TLV against target HAP pathogens is shown in the table. Based on MIC₉₀ comparisons, TLV was 2-fold more potent than vancomycin and teicoplanin against *S. aureus*. TLV was equally active against penicillin-susceptible and -resistant *S. pneumoniae* (MIC₉₀ = 0.03 µg/mL). Daptomycin MIC values were similar to TLV against staphylococci, but were higher for *S. pneumoniae* (MIC₉₀ = 0.25 µg/mL). Linezolid MIC₉₀ values were 2 µg/mL against all target organisms.

Conclusions: TLV was among the most active agents tested against common Gram-positive pathogens in HAP. These data highlight the potential therapeutic use of TLV in the treatment of HAP due to Gram-positive bacteria.

Organism	No. of isolates	TLV MIC (µg/mL)		
		MIC range	MIC ₅₀	MIC ₉₀
<i>S. aureus</i>	650	0.06-1	0.25	0.5
MSSA	241	0.12-1	0.25	0.5
MRSA	409	0.06-1	0.5	0.5
<i>S. pneumoniae</i>	54	0.008-0.06	0.015	0.03

C1-150

Activity of Telavancin Against Gram-Positive Cocci Isolated from Canadian Hospitals: CANWARD 2007

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Background: Telavancin is a novel lipoglycopeptide with rapid bactericidal activity against a broad spectrum of gram-positive pathogens. The purpose of this study was to assess the activity of telavancin against gram-positive cocci obtained from Canadian hospitals as part of the CANWARD 2007 study.

Methods: From January to December 2007, 12 sentinel hospitals across Canada submitted isolates from patients attending hospital clinics, emergency rooms, medical/surgical wards, and intensive care units. Each centre was asked to submit pathogens (consecutive, one per patient per infection site) from blood, respiratory specimens, urine, and wound/IV sites. 7881 isolates were collected in total, including 3473 gram-positive cocci. Susceptibilities to telavancin and comparators were determined by CLSI broth microdilution.

Results: MIC₅₀ and MIC₉₀ values for telavancin and vancomycin are shown below.

Conclusions: Telavancin is more active in vitro than vancomycin against *S. pneumoniae*, MSSA, MRSA, and *Enterococcus* spp.

Organism (n)	Telavancin MIC ₅₀ /MIC ₉₀	Vancomycin MIC ₅₀ /MIC ₉₀
<i>S. pneumoniae</i> - All (656)	≤ 0.06 / £0.06	≤ 0.25 / £0.25
- PenS (519)	≤ 0.06 / £0.06	≤ 0.25 / £0.25
- PenI (103)	≤ 0.06 / £0.06	≤ 0.25 / £0.25
- PenR (34)	≤ 0.06 / £0.06	≤ 0.25 / 0.5
MSSA (1088)	0.25 / 0.5	1 / 1
CA-MRSA (74)	0.25 / 0.25	1 / 1
HA-MRSA (290)	0.25 / 0.25	1 / 1
<i>E. faecalis</i> (154)	0.5 / 1	1 / 2
<i>E. faecium</i> (58)	0.12 / 0.5	0.5 / >8

MSSA, methicillin-susceptible *S. aureus*;
MRSA, methicillin-resistant *S. aureus*; CA,
community-associated; HA, hospital-
associated.

C1-151

Activity of Dalbavancin Against *S. aureus*, *S. epidermidis*, *S. pneumoniae*, and *Enterococcus* spp. Isolated from Canadian Hospitals: CANWARD 2007

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Background: Dalbavancin (DAL) is a new teicoplanin-related lipoglycopeptide with activity against gram-positive organisms. We compared the activity of DAL and vancomycin (VAN) against pathogens causing infections in Canadian hospitals.

Methods: 12 sentinel hospitals across Canada submitted pathogens from patients attending all ward types. Susceptibility testing was performed, using CLSI broth microdilution methods.

Results: The activity (MIC₅₀ and MIC₉₀ µg/ml) of DAL and VAN against select pathogens is described below.

Conclusions: Dalbavancin is more active than vancomycin versus MRSA, MRSE, VISA and *Enterococcus* spp.

SPN-*S. pneumoniae*, *Enterococcus* spp- *E. faecium* and *E. faecalis*, VRE-vancomycin-resistant enterococci, HA-healthcare-associated, CA-community-associated, VISA-vancomycin-intermediate *S. aureus*, VRSA-vancomycin-resistant *S. aureus*.

*Isolates obtained through the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program: supported under NIAID, NIH Contract No. N01-AI-95359.

**median MI ≤

Organism (# isolates)	DAL MIC ₅₀ /MIC ₉₀	VAN MIC ₅₀ /MIC ₉₀
SPN-All (661)	≤ 0.03/ ≤ 0.03	≤ 0.25/ ≤ 0.25
- PenS (516)	≤ 0.03/ ≤ 0.03	≤ 0.25 ≤ 0.25
- PenI (103)	≤ 0.03/ ≤ 0.03	≤ 0.25/£0.25
- PenR (34)	≤ 0.03/ ≤ 0.03	≤ 0.25/0.5
MSSA (1088)	0.06/0.06	1/1
CA-MRSA (71)	0.06/0.06	1/1
HA-MRSA (285)	0.06/0.06	1/1
MSSE (110)	≤ 0.03/0.06	1/2
MRSE (20)	≤ 0.03/0.06	1/2
<i>E. faecalis</i> (154)	0.06/0.06	1/2
<i>E. faecium</i> (58)	0.12/0.25	0.5/>8
<i>Enterococcus</i> spp. (232)	0.06/0.12	1/2
VRE (8)	8**	16**
*VISA (12)	0.25/2	4/4
*VRSA (7)	16**	16**

C1-180

In Vitro Activity of Dalbavancin Tested Against a Worldwide Collection of 81,677 Gram-Positive Bacterial Isolates

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Background: Dalbavancin (DAL) is a lipoglycopeptide with a novel once-weekly dosing schedule that has been developed for the treatment of skin and skin structure infections (SSSI) caused by Gram-positive pathogens. This study was designed to generate a large volume of susceptibility (S) data for DAL and comparator agents from worldwide geographic locations.

Methods: 210 medical centers in 33 countries (4 continents) contributed *S. aureus* (SA; 46,773 isolates), coagulase-negative staphylococci (CoNS; 12,308), including oxacillin-resistant (OXA-R; MRSA) strains. Viridans group (VGS; 2,148) and b-haemolytic (bHS; 5,316) streptococci, *E. faecalis* (EF; 10,375) and *E. faecium* (EFM; 4,754), including vancomycin (VANC)-S and -R isolates, were also tested. Isolates, during 2002-2007, were S tested with CLSI broth microdilution methods using validated panels (TREK Diagnostics) and appropriate supplements for processing fastidious species.

Results: Nearly all (99.9%) of tested *Staphylococcus* spp. were inhibited by ≤ 0.25 $\mu\text{g/ml}$ of DAL. All (100.0%) bHS and VGS were inhibited by ≤ 0.25 mg/ml. The VANC-S enterococci were readily inhibited by DAL (>99% at ≤ 0.12 mg/ml; MIC_{50} , ≤ 0.03 - 0.06 mg/ml) compared to the VANC-R strains (MIC_{50} , >4 mg/ml).

Conclusions: DAL demonstrated potent activity (MIC_{90} , ≤ 0.12 mg/ml) against this large preclinical collection of SA, CoNS, VGS and BHS. VANC-R will likely be categorized as DAL-R among the majority of EF and EFM isolates (VAN A patterns). DAL represents an important, once weekly alternative therapy for SSSI caused by common Gram-positive pathogens which are increasingly becoming more R to other antimicrobial classes, especially the emerging community-acquired MRSA.

Organism group (no.)	Cumulative % inhibited at MIC (mg/ml)							
	≤ 0.03	0.06	0.12	0.25	0.5	1	2	
OXA-S SA (26,721)	30.8	93.9	99.8	100.0	-	-	-	
OXA-R SA (20,052)	28.4	91.5	99.6	>99.9	100.0	-	-	
OXA-S CoNS (2,836)	62.9	91.9	98.9	99.8	99.9	100.0	-	
OXA-R CoNS (9,472)	51.4	82.6	95.4	99.2	99.8	>99.9	100.0	
BHS (5,316)	94.9	99.0	99.8	100.0	-	-	-	
VGS (2,148)	93.5	99.3	100.0	-	-	-	-	
EF (10,026)	50.1	93.6	97.4	97.6	97.6	97.6	97.8	
EFM (2,578)	14.8	38.9	55.8	59.2	61.4	64.1	67.5	

C1-181

Capability of Telavancin to Select Resistant Mutants of Vancomycin-Susceptible and Non-Susceptible MRSA and Vancomycin-Susceptible Enterococci

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Background: Telavancin is an investigational lipoglycopeptide active against Gram-positives. We used multistep selection to compare capability of telavancin, daptomycin, vancomycin, teicoplanin, linezolid to select resistant mutants of 10 MRSA and two enterococci.

Methods: Recent Hershey isolates comprised 10 MRSA strains (4 vanco susceptible MRSA, 2 h VISA, 4 VISA), 1 *E. faecalis*, 1 *E. faecium* (both vanco-susceptible). MICs were by CLSI macrodilution. Serial passages were done daily in MHB (Ca²⁺ for dapto) in subinhibitory drug concentrations, taking for each subsequent passage an inoculum from the tube 1-2 dilutions below MIC that matched turbidity of a growth control. Daily passages were continued until a >4-fold increase in MIC was found (min. 14, max. 50 passages). Resistant clones were subcultured 10 x in drug-free medium to test stability of selected resistance. Identity between parents and resistant clones was confirmed by PFGE or multiple-locus VNTR fingerprinting.

Results: Parental MICs (µg/ml) were: tela, 0.25-1; dapto, 0.5-16; vanco, 1-8; teico, 0.5-16; linez, 2-4. Tela yielded raised MICs after 43 days in 1 of 10 MRSA strains with MIC rising from 0.25 µg/ml (parent) to 2 µg/ml. MICs for this clone did not go higher when passages were continued for the maximum 50 days. Dapto had resistant clones after 14-35 days in 6/12 strains with MICs rising from 1-4 µg/ml (parents) @ 4-16 µg/ml (R clones). Teico had resistant clones after 14-21 days in 2/12 strains with MICs rising from 1-2 µg/ml (parents) @ 4-16 µg/ml (R clones). Linez had resistant clones after 22-48 days in 2/10 strains with MICs rising from 4 µg/ml (parents) @ 32 (R clones). Vanco yielded no resistant clones in all 12 strains tested but MICs rose from 1-8 µg/ml to 1-16 µg/ml after 50 days. No cross resistance was found with any clone/antimicrobial combination. Resistant enterococci developed with dapto only.

Conclusions: Telavancin yielded a clone with an elevated MIC (2 µg/ml) in 1 of 12 strains tested.

C1-186

Oritavancin Activity Against 200 Vancomycin-Susceptible and Non-Susceptible MRSA by MIC Testing

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Background: Methicillin-resistant *S. aureus* (MRSA) strains are therapeutic problems all over the world, and life-threatening infections caused by community-acquired MRSA strains are increasingly found. Also, MRSA strains which are not susceptible to glycopeptides have appeared in many locations. Oritavancin is an experimental lipoglycopeptide with a very long half-life. This study describes the anti-MRSA activity of oritavancin compared to that of vancomycin, teicoplanin, linezolid, daptomycin, tigecycline, quinupristin/dalfopristin, minocycline, rifampin, trimethoprim-sulfamethoxazole and fusidic acid.

Methods: Two hundred recent MRSA isolates were tested: of these 127 were community-acquired and isolated from sites throughout the US, and 43 were hospital-acquired. Strains also comprised 2 hetero-vancomycin intermediate (hVISA), 23 vancomycin-intermediate (VISA) and 5 vancomycin-resistant (VRSA). Both hVISAs, 2 VISAs and 1 VRSA were isolated in Hershey. MICs were determined by CLSI broth microdilution.

Results: MICs ($\mu\text{g/ml}$) were as follows. (**Tabelle siehe nächste Seite**)

Oritavancin MICs against VISA strains were usually 2 dilutions higher than against vancomycin-susceptible strains; two VISA strains had oritavancin MICs of 2 $\mu\text{g/ml}$. All strains were susceptible to linezolid ($\leq 4 \mu\text{g/ml}$), 99% to quinupristin/dalfopristin ($\leq 1 \mu\text{g/ml}$) and 94% to tigecycline (EUCAST $\leq 0.5 \mu\text{g/ml}$). Sixteen of the 23 VISA strains were daptomycin non-susceptible (MIC $>1 \mu\text{g/ml}$).

Conclusions: Oritavancin was potent against the group of staphylococci tested, with MIC₉₀ for VISA isolates two dilutions higher compared to vancomycin-susceptible strains.

C1-186 (Forts.)

Drug	Community-acquired (127)		Hospital-acquired (43)		hVISA (2)	VISA (23)	VRSA (5)	
	Range	MIC90	Range	MIC90	Range	Range	MIC90	Range
Oritavancin	0.03-0.5	0.25	0.06-1	0.25	0.12-0.25	0.12-2	1	0.12-1
Vancomycin	0.5-2.0	1	0.5-2	1	1	2-8	8	8->256
Teicoplanin	0.25-1	0.5	0.25-4	1	0.5-1	1-16	8	2-8
Linezolid	1-4	2	1-4	4	2	1-4	4	2
Daptomycin	0.25-1	0.5	0.25-1	1	0.5-1	0.5->8	4	0.25-1
Tigecycline	0.06-1	0.5	0.12-1	0.5	0.25	0.12->1	1	0.12-0.5
Quinu/dalfo	0.25-1	0.5	0.25-2	1	0.5-1	0.25-2	1	0.5-1
Minocycline	0.06-0.12	0.12	≤ 0.03-0.5	0.12	0.06-0.12	≤ 0.03-16	8	0.06-1
Rifampin	0.008-0.016	0.016	≤0.004-0.016	0.016	0.008	≤ 0.004->4	>4	≤ 0.004->4
Trimeth/sulf	0.06-0.5	0.25	0.06->4	0.5	0.5-1	0.06->4	>4	0.12->4
Fusidic acid	≤ 0.06-0.25	0.25	≤ 0.06-0.5	0.25	≤ 0.06-0.25	≤ 0.06->0.5	0.5	≤ 0.06-0.25

C1-187

In Vitro Activity of Oritavancin against CA-MRSA, VISA and Daptomycin-non-susceptible *Staphylococcus aureus* (DNSSA)

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Background: This study evaluated the *in vitro* activity of oritavancin, a semi-synthetic lipoglycopeptide with multiple mechanisms of action against isolates of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA), vancomycin-intermediate *Staphylococcus aureus* (VISA) and DNSSA.

Methods: For 92 CA-MRSA, 23 VISA and 6 DNSSA isolates microdilution tests using Mueller-Hinton broth were used to determine minimal inhibitory concentrations (MIC) of oritavancin and 7 additional antimicrobial agents for MRSA. Minimal bactericidal concentrations (MBC) were also determined. Pulse-field gel electrophoresis was performed using SmaI restriction endonuclease on the CA-MRSA isolates. Staphylococcal cassette chromosome (SCC) *mec* typing was determined by multiplex-polymerase chain reaction (PCR). Panton-Valentine leukocidin (*PVL*) genes were identified by PCR.

Results: CAMRSA: 43% *PVL*(+), 57% SCCType IV, 41% SCCType II, **VISA:** 100% *PVL*(-), 91% SCCType II, **DNSSA:** 4 isolates SCCType II and *PVL*(-), 1 isolate SCCType III, *PVL*(-), 1 isolate SCCType IVa and *PVL* (+)

Conclusions: Oritavancin was active against all CA-MRSA and DNSSA isolates and demonstrated greater activity than most comparators against VISA.

	CAMRSA (n=92)	VISA (n=23)	DNSSA (n=6)
	MIC 90 (MBC 90/MIC 90)	MIC 90 (MBC 90/ MIC 90)	MIC Range (MBC Range)
Oritavancin	0.12 (2)	2(1)	0.06-0.5 (0.12-1)
Vancomycin	1 (1)	8 (1)	2 (2)
Daptomycin	1 (1)	4 (1)	4 (4-8)
Teicoplanin	0.5 (2)	8 (2)	1-2 (1-2)
Linezolid	2 (>4)	2 (>4)	1-2 (2->8)
TMP/SMX	0.5/9.5 (2)	>4/76(1)	0.12/2.4->4/76 (0.12/2.4 >4/76)
Quinupristin/ Dalfopristin	0.5 (>16)	1 (>8)	0.12-0.5 (0.12->8)
Tigecycline	0.25 (>8)	0.5 (>4)	0.06-0.25 (0.12->2)

C1-191

In Vitro Activity of Telavancin and Other Antimicrobial Agents Against Vancomycin Susceptible and Non-Susceptible MRSA

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Background: Telavancin (TLV) is a rapidly bactericidal lipoglycopeptide active, via a multifunctional mechanism, against a range of Gram-positive bacteria. This study compared the activity of TLV with those of daptomycin (DAP), vancomycin (VAN), linezolid (LZD), quinupristin/dalfopristin (Q-D), teicoplanin, oxacillin, clindamycin, ciprofloxacin, telithromycin, erythromycin, gentamicin and cotrimoxazole against a collection of methicillin-resistant *S. aureus* (MRSA) isolates expressing VAN-susceptible (VSSA), VAN-intermediate (VISA), heterogenous-VISA (hVISA) and VAN-resistant (VRSA) phenotypes.

Methods: In total, 67 MRSA isolates were tested. The CLSI microdilution method was used for minimum inhibitory concentration (MIC) determination.

Results: The activities of TLV, VAN, DAP, LZD and Q-D are displayed in the table; 100% of VISA were susceptible to telavancin at £1 µg/ml, whereas 12/26 (46%) of these isolates were nonsusceptible to DAP at the same concentration. VRSA strains were more susceptible to DAP than to TLV. All strains were susceptible to LZD and Q-D and resistance was found to all other drugs tested.

^aConfirmed by population analysis profiling; ^bMIC range only;

Conclusion: TLV demonstrated potent activity against all VSSA isolates as well as against VISA and hVISA resistance phenotypes, which are increasingly recognized as important causes of VAN treatment failure. TLV MICs were elevated against VRSA strains. By comparison, DAP was active against VRSA but less active against VISA strains.

	MIC Range (MIC90) in µg/ml			
	VSSA, n=33	hVISA ^a , n=2 ^b	VISA, n=26	VRSA, n=6 ^b
TLV	0.25-1 (0.25)	0.25-0.5	0.25-1 (1)	2-4
VAN	0.5-1 (1)	2	2-8 (8)	>32
DAP	0.25-1 (0.5)	0.5-1	0.5->1 (>1)	0.25-1
LZD	2-4 (4)	2-4	≤ 0.5-4 (4)	2-4
Q-D	0.25-1 (1)	0.5	≤ 0.12-2 (1)	0.5-1

C1-198

Pretreatment of Model Surfaces of Indwelling Devices with Oritavancin Prevents *In Vitro* Biofilm Formation by *Staphylococcus epidermidis*

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Background: Oritavancin (ORI), a synthetic derivative of the glycopeptide antibiotic chloroeremomycin (CEM), is in clinical development for treatment of serious gram-positive infections. Because ORI binds avidly to labware surfaces and is active against *in vitro* biofilms, we studied whether it could prevent biofilm formation (BF) by *S. epidermidis*, a prominent cause of indwelling medical device infections.

Methods: 96-well polystyrene microtiter plates (PMPs) were incubated overnight (O/N) at room temperature (RT) with solutions of ORI, CEM, vancomycin (VAN), daptomycin (DAP), or nafcillin (NAF) in water. In a catheter biofilm model, silicone tubing (ST) pieces (VWRbrand select silicone, 1.0 mm inner diameter, 2.2 mm outer diameter; 1 cm lengths cut longitudinally in half) were likewise incubated with ORI or CEM. All surfaces were washed 3 times (with water) prior to and following (with saline) establishment of biofilms with *S. epidermidis* ATCC 35894 (10^5 CFU/mL) O/N at 37°C in tryptic soy broth supplemented with 1% glucose. Residual biofilm burden in PMPs was assessed by adding fresh broth to the wells and incubating for a further 24 hours at 37°C. Biofilm cell density (BCD) on ST was quantified by sonication to liberate adherent cells and serial dilution plating.

Results: Pretreatment of PMPs with 2 µg/mL ORI completely suppressed *S. epidermidis* ATCC 35984 BF whereas VAN, CEM, DAP or NAF at concentrations up to and including 2048 µg/mL were ineffective. Prevention of BF on ST by ORI was concentration-dependent: BCD on ST pretreated with 5 mg/mL ORI was below the level of detection (200 CFU/mL). In contrast, control ST or ST incubated O/N in 20 mg/mL CEM had BCD of $(5.3 \pm 2.2) \times 10^5$ CFU/mL and $(3.8 \pm 2.5) \times 10^5$ CFU/mL, respectively. Stability studies showed that incubation of ORI-coated ST in water for 7 days at RT did not affect ORI capacity to prevent BF.

Conclusion: These results highlight the potential of ORI as a stable surface-coating agent that can prevent BF.

C1-199

Comparative Study of Membrane Permabilization Induced by Oritavancin (ORI) vs. Vancomycin (VAN) in Liposomes and Role of an Acidic Phospholipid

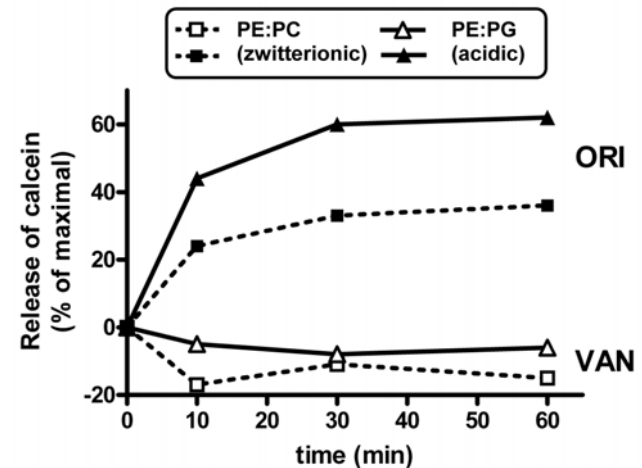
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Background: Novel lipoglycopeptides like ORI, exert a marked and rapid bactericidal effect against Gram (+) bacteria. Beyond inhibiting peptidoglycan biosynthesis (as VAN), permeabilization of the bacterial membrane by ORI contributes to its antibacterial properties, owing to the presence of a 4'-chlorobiphenylmethyl group absent from VAN. Since (i) ORI contains an additional aminogroup compared to VAN, and (ii) bacterial membranes are rich in phosphatidylglycerol (PG), we have also examined the role of this acidic phospholipid.

Method: Liposomes (5 μ M phospholipids; zwitterionic: phosphatidylethanolamine (PE) + phosphatidylcholine (PC) [8:4, mol:mol]; acidic: phosphatidylethanolamine (PE) + PG [8:4, mol:mol] containing calcein at self-quenched concentration were exposed at pH 7.4 and 37°C to ORI or VAN (0.6 μ M [0.87-1.07 mg/L]) and monitored for change in fluorescence signal (increase = unquenching of calcein upon release; 100 % = detergent-treated liposomes).

Results: In contrast to VAN, ORI caused a marked release of calcein from liposomes, which was further increased when PG was present in the membrane.

Conclusion: Both hydrophobic and electrostatic interactions may play a role in the membrane permeabilization induced by ORI.



C1-3711

Efficacy of Oritavancin Against In Vitro Biofilms Derived from Clinical Isolates of *Staphylococcus epidermidis*

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Background: *S. epidermidis* (SE) forms biofilms that are notoriously tolerant to antimicrobial agents and that complicate infections of chronic wounds and indwelling medical devices. In this study, the efficacy of oritavancin (ORI), vancomycin (VAN), linezolid (LIN) and rifabutin (RFB) was determined against *in vitro* biofilms derived from SE clinical isolates (CI).

Methods: *In vitro* biofilms for 10 CI of SE (5 catheter and 5 wound isolates) and reference strains ATCC 12228 (low biofilm-slime production [BSP]), ATCC 35983 (moderate BSP) and ATCC 35984 (high BSP) were established in MBEC™ P&G Assay plates (Innovotech; Edmonton). Planktonic minimal inhibitory concentrations (MIC) and minimal biofilm eradication concentrations (MBEC; the concentration of drug needed to sterilize the biofilm) were determined following the manufacturer's protocol. BSP was determined by growing SE isolates overnight in borosilicate glass tubes in tryptic soy broth containing 1% glucose at 37°C and comparing the biofilm (stained with 0.1% crystal violet) formed on the tubes to the ATCC reference strains. MBECs of ORI + RFB were determined against 3 high BSP isolates using a checkerboard technique.

Results: ORI sterilized the SE biofilms at MBECs ranging from 2 to 16 µg/mL with the exception of two high BSP isolates (ATCC 35984 and one CI) that had MBECs between 16-64 µg/mL: for all isolates ORI activity was only slightly affected by growth in a biofilm as MBECs were only 2 to 4-fold higher than their planktonic MIC determined in MBEC™ plates. RFB also sterilized the SE biofilms, with MBECs ranging from 0.06 to 2 µg/mL. In contrast, VAN and LIN were ineffective at sterilizing the biofilms as MBECs were >128 µg/mL. Against 3 high BSP isolates (ATCC 35984 and 2 CI), the combination of ORI (MBEC range of 2-4 µg/mL) + RFB (MBEC range of 0.03-0.5 µg/mL) was highly effective.

Conclusion: ORI alone sterilized *in vitro* SE biofilms and exhibits enhanced activity in combination with RFB against biofilms with high BSP.

C1-3717

In Vitro Time Kill Studies of Oritavancin Against Vancomycin-Intermediate *Staphylococcus aureus* (VISA)

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Background: Oritavancin (ORI), a semi-synthetic lipoglycopeptide, exerts bactericidal activity against gram-positive bacteria including vancomycin (VAN)-nonsusceptible *S. aureus* and enterococci. To understand the kinetics of ORI activity *in vitro*, we performed time-kill (TK) assays against recent clinical isolates of VISA and heterogenous VISA (hVISA) and reference strains under conditions that minimize ORI loss to vessel surfaces.

Methods: VISA (n=7) and hVISA (n=6) were tested in TK assays based on CLSI M26-A guidelines. ORI assays included 0.002% polysorbate-80 throughout. ORI and comparators VAN, teicoplanin (TEI), linezolid (LIN) and daptomycin (DAP) were tested at static concentrations approximating their free peak (fC_{max}) and free trough in plasma when administered at standard doses (intended label doses for complicated skin and skin structure infections [cSSSI]). Cell counts were determined by serial dilution plating.

Results: ORI showed concentration-dependent activity against all strains tested. At its fC_{max} (predicted from a 200 mg dose in humans), ORI was bactericidal ([≥] 3 log kill at 24 h relative to starting inoculum) against all 6 hVISA strains and against 6 of 7 VISA strains tested, with hVISA strains more rapidly killed than VISA strains at fC_{max} concentrations of ORI. Further, at fC_{max} predicted from an 800 mg dose, ORI was bactericidal against all 13 strains tested. While no correlation was observed between ORI MIC and rate of kill for VISA strains (r²=0.14), MIC correlated with the time to 3 log kill (r²=0.88) for hVISA isolates.

Conclusions: ORI displayed concentration-dependent killing of VISA and hVISA *in vitro* and was more rapidly bactericidal than were VAN, TEI, LIN or DAP at pharmacologically-relevant concentrations. These data support the conclusion that ORI exerts concentration-dependent cell eradication activity against recent, VAN-intermediate isolates of *S. aureus*.

C1-3718

Time-Kill Activity of Telavancin (TLV) Alone and in Combination Against hVISA, VISA, and VRSA

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Background: TLV is an investigational anti-Gram + lipoglycopeptide; time-kills tested interaction of TLV with other drugs against MRSA.

Methods: Ten strains were 2 hVISA, 7 VISA, 1 VRSA. MICs were by CLSI broth microdilution. TLV was tested by time-kill alone and combined with rifampin (RIF), gentamicin (GEN), cefepime (FEP), ceftriaxone (CRO), oxacillin (OXA), meropenem (MEM), ciprofloxacin (CIP). Tubes were incubated in a shaking water-bath with viability at 3, 6, 12, 24 h. Synergy was a $\geq 2 \log_{10}$ CFU/mL difference in reduction between comb. and the most active agent compared to 0 h. In comb., at least 1 drug was present at a conc. minimally affecting growth when tested alone. Drugs with MICs $>128 \mu\text{g/mL}$ were tested at 32 and 64 $\mu\text{g/mL}$.

Results: MICs ($\mu\text{g/ml}$) for drugs alone were: TLV, 0.5-1 for VISA and hVISA and 4 against VRSA; RIF, 0.002- >512 ; GEN, 1- >512 ; FEP, 4-512; CRO, 16- >512 ; OXA, 4-512; MEM, 0.25-64; CIP, 0.5-512. TLV + RIF were synergistic at 12 h for 2 strains at MICs ($\mu\text{g/ml}$) of 0.5-2 + 0.001 and for 7 strains after 24 h at 0.12-0.5 + 0.001-32. TLV + GEN were synergistic at 3 h for 3 strains at 0.12-0.5 + 0.5-1; 5 strains showed synergy at 6 h (0.12-0.25 + 0.25-1), 6 strains at 12 h (0.12-0.5 + 0.25-1), and 8 strains at 24 h (0.12-0.5 + 0.25-32). TLV + FEP were synergistic for 1 strain at 3 h and 6 h (0.25 + 2; 0.25 + 1), 4 strains at 12 h (0.12-1 + 1-64), 9 strains at 24 h (0.25-1 + 2-64). TLV + CRO showed synergy for 1 strain at 6 h (1 + 32), 4 strains at 12 h (0.12-1 + 4-64), 9 strains at 24 h (0.12-1 + 4-64). TLV + OXA were synergistic for 1 strain at 6 h and 12 h (both 1 + 32), 10 strains at 24 (0.25-1 + 1-64). TLV and MEM were synergistic at 6 h for 1 strain (1 + 8), 4 strains at 12 h (0.12-1 + 0.06-16), 9 strains at 24 h (0.12-1 + 0.06-16). TLV + CIP were synergistic at 6 h for 1 strain (1 + 32), at 12 h for 3 strains (0.25-2 + 32), for 7 strains at 24 h (0.25-1 + 0.25-64). All other comb. were additive. No antagonism was seen.

Conclusions: TLV was active ($\leq 1 \mu\text{g/ml}$) against all hVISA and VISA, and synergized at subMIC conc. with a wide range of drugs including all tested β -lactams, GEN, RIF, CIP against all strains, including 1 VRSA.

C1-3720

Oritavancin (ORI) Combinations with Linezolid (LIN) and Nafcillin (NAF) are Synergistic Against Vancomycin (VAN)-non-susceptible *Staphylococcus aureus* (SA)

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Background: ORI is a lipoglycopeptide with bactericidal activity against VAN-intermediate SA (VISA) and VAN-resistant SA (VRSA). We assessed synergy *in vitro* between combinations of ORI + LIN against VISA isolates and ORI + NAF against the 8 available VRSA isolates from the Network on Antimicrobial Resistance in *S. aureus* (NARSA).

Methods: Synergy between ORI + LIN against NARSA VISA isolates NRS26, NRS56, NRS65 and NRS402 and against ATCC 700699 (Mu50; prototypic VISA) was evaluated by time-kill methodology at concentrations below their broth microdilution minimal inhibitory concentrations (MIC) as determined following CLSI guidelines: 32-log CFU decrease between the combination and its most active constituent alone at 24 hours was considered synergistic (CLSI, document M26-A). Synergy between ORI + NAF against VRSA isolates VRS1 to VRS8 (note that all VRSA isolates are methicillin resistant) was assessed by the checkerboard technique: fractional inhibitory concentration index (FIC_i) was calculated using the equation $FIC_i = (A/MIC_A) + (B/MIC_B)$ where A & B are the MIC of each agent obtained in combination; FIC_i of ≤ 0.5 were considered synergistic whereas FIC_i > 0.5 were indifferent.

Results: see table

Conclusion: ORI + LIN exhibited synergy against all 5 VISA isolates tested. Furthermore, ORI + NAF were synergistic against 5 of the 8 VRSA isolates. Novel combinations such as those described here may enhance ORI activity against VISA and VRSA isolates.

Isolate	Phenotype	ORI + LIN (log CFU reduction)
ATCC 700699	VISA	S (2.4)
NRS26	VISA	S (4.1)
NRS56	VISA	S (2.7)
NRS65	VISA	S (3.0)
NRS402	VISA	S (2.3)
Isolate	Phenotype	ORI + NAF (FIC _i)
VRS1	VRSA	I (FIC _i = 0.51)
VRS2	VRSA	S (FIC _i = 0.50)
VRS3	VRSA	S (FIC _i = 0.50)
VRS4	VRSA	S (FIC _i = 0.50)
VRS5	VRSA	S (FIC _i = 0.31)
VRS6	VRSA	S (FIC _i = 0.31)
VRS7	VRSA	I (FIC _i = 0.63)
VRS8	VRSA	I (FIC _i = 0.75)

C1-3835

Impact of Human Serum Albumin (HSA) on Oritavancin (ORI) In Vitro Activity Against Enterococci

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Background: ORI is a lipoglycopeptide with activity against gram-positive pathogens including vancomycin (VAN)-resistant enterococci (VRE). The impact of HSA on ORI activity against enterococci was assessed *in vitro* using MIC and time-kill (TK) methods.

Methods: MICs for 6 strains of enterococci including 1 VAN-susceptible *E. faecalis* (VSE; ATCC 29212) and 5 clinical isolates of VRE (*E. faecalis*, n=3; *E. faecium*, n=2) were determined by CLSI broth microdilution (CLSI M7-A7; M100-S18) ± 4% HSA. In TK assays (CLSI M26-A) with 4% HSA, ORI and comparators (VAN, teicoplanin, linezolid & daptomycin) were tested at their predicted total peak and trough concentrations in plasma from intended label doses for complicated skin and skin structure infections. Viable cell counts were determined by serial dilution plating.

Results: The impact of HSA on ORI activity was more pronounced for isolates of *E. faecalis* (MIC up to 8 fold higher with HSA) than *E. faecium* (MIC maximally 2 fold higher with HSA). In TK assays in the presence of HSA, ORI at its total peak concentration eradicated VSE and VRE more rapidly than comparators: only ORI had bactericidal activity against VSE (3 log kill in 24 h). ORI was the only agent active against all VRE tested including *E. faecalis* and *E. faecium* VanA (ATCC 51559, ID#1058946 & ID#1119060: -1.4 ± 0.04 , -1.2 ± 0.03 & -1.5 ± 0.05 Δ log CFU/mL at 24h, respectively) and isolates of *E. faecalis* and *E. faecium* VanB (ATCC 51299 & ID#1119175: -1.6 ± 0.1 & -1.4 ± 0.4 Δ log CFU/mL at 24h, respectively). ORI also retained activity against VSE (-0.48 ± 0.02 Δ log CFU/mL at 24h) and VanB *E. faecalis* ATCC 51299 (-1.2 ± 0.06 Δ log CFU/mL at 24h) when tested at its total trough concentration with HSA.

Conclusions: ORI MICs were up to 8 fold higher in the presence of HSA for enterococci, consistent with its propensity to bind serum protein. In TK assays with HSA, ORI retained activity against all tested strains and killed VSE and VRE more rapidly than did comparators.

C1-3845

Activity of Omiganan, a Novel Peptide, Tested Against Contemporary Gram-Negative Pathogens: Results from an International Surveillance Program (2008)

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Background: Omiganan (OMI), a cationic peptide being developed for topical use in prevention of catheter-related infections, has a broad spectrum of activity to Gram-negative (GN) and -positive bacteria and fungi. We present 2008 results from a global surveillance program on OMI activity against prevalent GN pathogens.

Methods: Consecutive, non-duplicate patient isolates (GN; 4153) were submitted from medical centers in the USA (75.8%) and Europe (24.2%) for identification and susceptibility (S) testing to OMI and comparator agents by CLSI MIC methods. Isolates originated from bloodstream, respiratory tract, and skin and skin structure infections.

Results: Among prevalent GNs, all *E. coli* (EC), *Klebsiella* spp. (KSP), *P. aeruginosa* (PSA) *Acinetobacter* spp. (ASP) and >99% of *Enterobacter* spp. (EBS) were inhibited by £1024 µg/ml of OMI, results well below the 1% topical gel concentration (10,000 µg/ml). Only *Serratia* spp, *P. mirabilis* and indole-positive Proteae consistently displayed MIC values to OMI of >1024 µg/ml (93.0%). EC strains had the lowest MIC₅₀ and MIC₉₀ results (both 32 µg/ml) followed by ASP and EBS. MIC₅₀ and MIC₉₀ potencies for EC and KSP isolates displaying ESBL phenotypes were no higher than for wild type (WT) strains. AmpC-producing EBS showed lower MIC₅₀ and MIC₉₀ results (32 and 64 µg/ml, respectively) than WT strains. All PSA were inhibited by £512 µg/ml with no difference for carbapenem-R strains.

Conclusion: All major GN pathogen groups associated with skin and skin structure, and catheter-related infections, including strains expressing prevailing R mechanisms, were inhibited by OMI well below the clinically used topical gel concentration (10,000 µg/ml).

Organism (no.)	Omiganan MIC values (µg/ml)		
	MIC ₅₀	MIC ₉₀	Range
<i>E. coli</i> (390)	32	32	8-256
<i>Klebsiella</i> spp. (202)	64	512	1-1024
<i>Enterobacter</i> spp. (114)	32	512	16->1024
<i>Pseudomonas aeruginosa</i> (199)	256	512	8-512
<i>Acinetobacter</i> spp. (23)	32	128	8-256

F1-3986

Arenicin-3 : A Novel Antimicrobial Peptide Showing Potent *In Vitro* Activity Against Gram-negative Multi-resistant Clinical Isolates

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Background: Arenicin-3 is an antimicrobial peptide isolated using Transposon-Assisted-Signal -Trapping from the lugworm *Arenicola marina* living on sediments in the tidal water.

Structural analysis showed that Arenicin-3 belonged to the beta-hairpin peptides. This class of AMPs are known to exhibit cidal activities towards a diverse number of microorganisms. Interestingly, susceptibility data on clinical isolates of *Klebsiella pneumoniae*, *Salmonella enterica*, *Pseudomonas aeruginosa* and *Escherichia coli* showed very potent activities of Arenicin.

Methods: Minimal inhibitory concentrations were performed according to the general guidelines for susceptibility measurements using micro-broth dilution provided by CLSI/ NCCLS (M7-A5)

All isolates were tested by a standard time-kill methodology as described by CLSI document M26-A: *Methods for Determining Bactericidal Activity of Antimicrobial Agents; approved guideline*, with the exception of taking earlier time points than normal due to the rapid bactericidal nature of Arenicin-3.

Results: Gram-negative bacteria including multi-resistant clinical relevant isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella* Typhimurium, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* were susceptibility tested to Arenicin-3. The results for isolates of both *Enterobacteriaceae* (n=148) and non fermentors (n=53) populations, MIC₉₀ was < 1 mg/ml. The antimicrobial activity is markedly bactericidal (MBC~1-4xMIC), causing 3-log (99.9%) reduction in the viable bacteria population within 1-2 hours of Arenicin-3 exposure.

Conclusions: Arenicin-3 has shown potential antimicrobial activity, even against multi resistant clinical isolates (ESBL positive, fluoroquinolone resistant, aminoglycoside resistant)

C1-4182

Oritavancin Disrupts Membrane Integrity to Effect Cell Killing of Vancomycin (VAN)-Nonsusceptible *Staphylococcus aureus* (SA) and VAN-Resistant *Enterococcus faecium* (VRE)

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Background: Unlike VAN, the lipoglycopeptide oritavancin (ORI) perturbs membrane integrity (potential $[\Delta\psi]$ and permeability) of VAN-susceptible SA and enterococci and exerts rapid bactericidal activity. We assessed whether ORI activity against VAN-nonsusceptible SA and VRE also includes a $\Delta\psi$ and membrane permeability component.

Methods: $\Delta\psi$ and membrane permeability of VAN-intermediate SA (VISA, ATCC 700699 & NRS402), heterogeneous VISA (hVISA ATCC 700698), VAN-resistant SA (VRSA VRS5 & VRS1) and VanA VRE (*E. faecium* ATCC 51559) were investigated by measuring DiSC₃(5) and SYTO-9/propidium iodide fluorescence of exponential-phase cells (10^6 CFU/mL) with a TECAN Ultra reader. ORI and VAN challenges approximated their free peak (fC_{max}; 4 and 16 $\mu\text{g/mL}$, respectively) and free trough concentrations (0.5 and 4 $\mu\text{g/mL}$, respectively) in plasma from intended label dose for complicated skin and skin structure infections. Bacterial viability was assessed by serial dilution plating.

Results: ORI effects on $\Delta\psi$, permeability and viability were temporally correlated and concentration-dependent: challenge with 4 $\mu\text{g/mL}$ of ORI depolarized VRSA and hVISA membranes and decreased cell counts by 0.9 ± 0.1 and 1.1 ± 0.0 log respectively within 15 min while 16 $\mu\text{g/mL}$ ORI depolarized VISA cell membranes over 15 minutes leading to a 1.0 ± 0.02 log killing. Similarly, 4 $\mu\text{g/mL}$ ORI reduced VanA VRE cell viability by 1.2 ± 0.3 log in 10 min. In contrast, VAN at 16 $\mu\text{g/mL}$ did not affect $\Delta\psi$, membrane permeability or cell viability over 2 h for any tested strain.

Conclusions: ORI profoundly affected $\Delta\psi$ and membrane permeability of VAN-nonsusceptible SA and enterococci. These effects were temporally correlated with a significant loss of cell viability and they required the chlorobiphenyl methyl group of ORI. This recently proposed mechanism of ORI action helps to explain its rapid, potent bactericidal activity against VAN-nonsusceptible SA and VRE *E. faecium*.

F1-362

In Vitro Activity of Telavancin (TEL) & Vancomycin (VAN) against Biofilm-Producing *Staphylococcus aureus* (SA), *S. epidermidis* (SE) & *Enterococcus faecalis* (EF)

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Background: Infections caused by biofilm (BF) producing strains of SA, SE & EF are difficult to eradicate. TEL, a new lipoglycopeptide with activity against these bacteria possess a secondary mechanism of action (MOA) that causes depolarization & loss of the functional integrity of the membrane. It was our intent to evaluate TEL against BF producing SA, SE & EF.

Methods: We evaluated TEL & VAN activity against known (ATCC controls) BF-producing SA (35556), SE (35984) & EF (29212). Non-BF forming isolates were used as controls and CLSI MIC testing was performed. Using the Calgary Biofilm Device (CBD) each agent was evaluated in a pre-formed 24hBF. Additionally, efficacy in preventing BF formation was quantified using a colorimetric microtiter plate assay (OD570).

Results: TEL & VAN respective MIC were as follows: SA, 0.0625 & 1mg/l; SE, 0.125 & 2mg/l; & EF 0.25 & 2mg/l. MIC's of TEL were 1 to 4 dilutions higher in established BF (CBD) when compared to organisms grown planktonically (0.25 & 0.0625mg/L for SA; 0.5 & 0.125mg/L for SE & 0.5 & 0.25mg/l for EF, respectively). VAN MICs in planktonic vs. BF formed bacteria were 1 & 4mg/L for SA; 2 & 4mg/L for SE & 2 & 8 for EF respectively. MICs for the non-BF forming organisms did not increase in the BF assay. TEL inhibited BF development against SA, SE & EF using concentrations at or below the isolates respective MIC (>0.03mg/L). VAN inhibited BF development at concentrations at or above each isolate's respective MIC. The non-BF forming controls did not produce BF (OD570 of 0.01 ±0.02).

Conclusions: MICs for TEL were 8 to 16x lower than VAN. Unlike VAN, TEL concentrations below the isolates respective MIC inhibited the development of BF mass. In established BFs, significantly lower concentrations of TEL are required to demonstrate activity. These findings maybe explained by TEL secondary MOA. TEL demonstrates promise in BF forming SA, SE & EF. Further investigations are warranted.

F1-363

In Vitro Activity of MX-2401 a Novel Lipopeptide Against Multi-Drug Resistant (MDR) *Staphylococcus aureus* (SA)

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Background: MX-2401, a novel lipopeptide, has demonstrated potent in vitro activity against Gram positive pathogens and in vivo activity against SA in a mouse thigh infection model. The purpose of this study was to examine the in vitro activity of MX-2401 against MDR SA including vancomycin resistant (VRSA) and vancomycin intermediate resistant (VISA) strains.

Methods: MICs/MBCs were determined against 62 susceptible and resistant SA as described by CLSI utilizing the approved microbroth format. Colony counts confirmed inoculum and appropriate ATCC control organisms were utilized.

Results: The MIC/MBC range, MIC₅₀ and MIC₉₀ (ug/ml) are shown below:

Conclusions: MX-2401 was potent and demonstrated bactericidal activity against SA including both CA and HA-MRSA and vancomycin intermediate and resistant isolates. The in vitro antibacterial effects of MX-2401 supports the potential of this agent in the treatment of MDR SA infections.

<i>S. aureus</i> Phenotype (N)	MIC Range	MBC Range	MIC ₅₀ /MBC ₅₀	MIC ₉₀ /MBC ₉₀
MS (10)	2	2 - 8	2 / 2	2 / 8
CA-MR (17)	2 - 4	2 - 8	2 / 2	2 / 8
HA-MR (17)	1 - 4	2 - 32	2 / 4	4 / 8
*VISA (11)	2 - 8	4 - 16	8 / 8	8 / 16
*VRSA (7)	1 - 2	2 - 8	-	-

*Isolates obtained through the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program: supported under NIAID, NIH Contract No. N01-AI-95359.

MS-methicillin-susceptible, MR-methicillin-resistant, CA-community associated, HA-healthcare associated.

F1-364

MX-2401 Bactericidal Activity and Membrane Depolarization in *Staphylococcus epidermidis*

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Background: MX-2401, is a calcium-dependant antimicrobial lipopeptide in development for treating serious Gram positive infections. MX-2401, an analogue of amphotericin, is bactericidal in vitro and in vivo against a broad spectrum of Gram positive microorganisms, including MRSA. To understand the mechanism of action of MX-2401, we studied the effect of MX-2401 on bacterial membrane depolarization. Daptomycin, a calcium-dependant lipopeptide antibiotic whose bactericidal activity has been correlated to its depolarization effect, was used as a control.

Methods: *Staphylococcus epidermidis* (ATCC 12228) in exponential phase were incubated 0 to 90 minutes in presence of sub- to supra-MIC concentrations of MX-2401 or daptomycin. After treatment, 3,3'-dipropylthiadicarbocyanine iodide, a membrane-potential-sensitive dye, was added to the cells to monitor the changes in *S. epidermidis* membrane potential.

Results: MX-2401 did not affect membrane polarity in *S. epidermidis* when incubated with cells for 0-30 minutes. This was confirmed in *Staphylococcus aureus* (ATCC 29213) in which dye partition was not affected by MX-2401. MX-2401 reduced the uptake of the membrane-potential-sensitive dye only when the bacteria were exposed to MX-2401 for 60 minutes or more. The dye uptake by MX-2401 was dose-dependent, requiring high doses of MX-2401 and occurred before cell viability was significantly affected. In contrast, inhibition of the uptake of the membrane-potential-sensitive dye by daptomycin was rapid (within minutes of incubation), occurred at lower doses, and correlated with its bactericidal effect.

Conclusions: Our data suggest that membrane depolarization by MX-2401, in contrast to depolarization by daptomycin, occurs before significant bactericidal effect and is time dependent. The mechanism of action of MX-2401 is being further investigated.

F1-372

Generation of Hybrid Lipopeptide Antibiotics with Improved Properties by Genetic Engineering

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Background: Daptomycin is a cyclic lipopeptide antibiotic approved for treatment of skin and skin structure infections, and bacteremia/endocarditis caused by *Staphylococcus aureus*, including methicillin resistant strains. However, daptomycin is inferior to standard treatment for community acquired pneumonia, probably because its activity is inhibited by lung surfactant. In this study, novel hybrid molecules of the structurally related lipopeptide A54145 were biosynthesized with a goal of identifying compounds with significantly reduced *in vitro* surfactant inhibition and acute toxicity in mice relative to A54145E.

Methods: Novel hybrid A54145 nonribosomal peptide synthetase biosynthetic genes were constructed by λ -Red mediated exchanges of single or multiple modules, and coupled with deletion of the methyltransferase *lptI* gene involved in the formation of the 3-methyl-glutamic acid residue in A54145E. These hybrid biosynthetic pathways were expressed in *Streptomyces fradiae* to produce novel lipopeptides by fermentation. The products were isolated and used in assays for *in vitro* antibacterial activity in the presence of increasing concentrations of bovine surfactant, and for acute toxicity in mice.

Results: Combinations of 8 module exchanges with the *LptI* inactivation resulted in the production of 16 novel lipopeptides, some of which were as active as A54145E against *S. aureus* (MIC 1-2 $\mu\text{g}/\text{mL}$). Modifications at the amino acid residues 2, 3 and 13 were identified as important for lipopeptide properties. While A54145E activity was inhibited 32 fold by 5% bovine surfactant, activity of two novel compounds, CB-182575 and CB-182561, was inhibited only 2 fold. In addition, CB-182561 showed reduced acute toxicity in mice.

Conclusions: This study demonstrated the use of combinatorial biosynthesis to produce novel lipopeptide antibiotics related to daptomycin and A54145, including ones with improved properties.

F1-385

Development of a Novel Method for Determining MX-2401 Drug Potency

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Background: MX-2401 is a new antimicrobial lipopeptide which targets Gram positive bacteria including multi-drug resistant isolates. The effects of various conditions on an MX-2401 diffusion microbial assay were investigated.

Methods: The conditions tested in the assay were pH (6.6 or 8.3), inoculum (10^5 or 10^6 CFU/mL) in the overlay, calcium concentration in the overlay (25 to 100 $\mu\text{g/mL}$) and diluent. *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 14154, and *Staphylococcus epidermidis* ATCC 12228 were inoculated at either 10^5 or 10^6 CFU/mL in the agar overlay. Solid phase agar diffusion assay with penicylinders was used for all tests. Antibiotic USP-81 assay medium 1 was used for *M. luteus* only and antibiotic USP-81 assay medium 11 was used for the 3 species tested. The MX-2401 concentration range was from 12.5 - 100 $\mu\text{g/mL}$.

Results: Collected data included: zone sizes, dynamic range (range of concentrations giving a readable zone), delta values (zone size difference between 2 concentrations), R^2 and %CV. Only the zone sizes, dynamic range, and delta values varied between the 3 species tested while all other parameters were similar. The organism with the largest dynamic range and largest zone sizes at the same concentration of MX-2401 was *S. epidermidis* followed by *S. aureus* and *M. luteus*. Against *S. epidermidis*, the pH effects on dynamic range, delta values, and zone sizes were minimized by addition of 75 $\mu\text{g/mL}$ calcium. Increasing the inoculum size of *M. luteus* from 10^5 to 10^6 CFU/mL produced smaller zone sizes and dynamic range but similar delta values. Increasing calcium concentrations in the overlay increased dynamic range, delta values, and zone sizes. Some diluents induced changes in dynamic range, delta values, and zone sizes. Water, saline, USP-81 buffer No. 3, ammonium carbonate, calcium chloride, 0.002% tween 80 and CAMHB showed equivalent delta values.

Conclusions: A cylinder dilution method optimized for these conditions can be used to determine the potency of manufactured batches of MX-2401 or for in vitro analysis of MX-2401 containing samples.

F1-3199

The Activity of RTA-3, a Novel Antimicrobial Peptide, Against *Staphylococcus aureus* (SA) of Various Resistance Phenotypes

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Background: SA infections cause serious disease in community and hospitals worldwide. The establishment and emergence of resistance phenotypes to meticillin (M) and glycopeptides makes demands upon treatment options and health budgets. RTA-3 is a 16 amino acid antimicrobial peptide derived from a peptide produced by *Streptococcus mitis*. It has been developed to optimise activity against multiply resistant Gram negatives including *Pseudomonas aeruginosa*, *Acinetbacter* spp., and *Stenotrophomonas maltophilia*. The current study assesses the activity of RTA-3 against SA with different susceptibilities to M or vancomycin (V) using modified *in-vitro* testing.

Methods: 48 strains were tested: 12 M sensitive (MSSA), 12 M resistant/V sensitive (MRSA), 12 hetero-V intermediate resistant (hVISA), 12 V intermediate (VISA) from an international collection. Susceptibility was determined by microbroth and agar dilution using Mueller-Hinton media, solidified with agarose.

Results: The MICs were comparable between microbroth and agar dilution. The table shows susceptibilities (mg/L) plus MIC₅₀ and MIC₉₀s for the strains tested:

Conclusions: MICs were generally between 8 - 64mg/L, with VISA and MSSA showing the most susceptibility. These results are encouraging for the further modification of the peptide to optimise activity against *S. aureus*. The finding of activity against Gram positive organisms supports the hypothesis that RTA-3 has an extra site of action in addition to the Gram negative outer membrane.

	MIC ₅₀	MIC ₉₀	Range
MSSA	16	>64	8->64
MRSA	64	64	16-64
hVISA	32	64	16-64
VISA	64	>64	8->64

F1-3987

BL-2060 A Broad Spectrum Antimicrobial Peptidomimetic for the Treatment of Severe Lung Infections

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Background: BL-2060, a novel peptide-mimetic oligomer that imitates natural host defense antimicrobial peptides, was found to exert rapid broad-spectrum bactericidal activity (Nat Biotechnol, 2007, 25(6):657-9). In murine peritonitis-sepsis model, BL-2060 demonstrated potent antimicrobial activity against multidrug resistant bacterial strains with high safety index. Here, we show that BL-2060, administered by inhalation, is effective in treating *Pseudomonas aeruginosa* induced pneumonia.

Methods: Acute infection in lungs was established by intratracheal administration of 1×10^5 CFU of *P. aeruginosa* PAO1. Four hours after infection, mice were placed in nose-only restraint tubes and connected to exposure chambers using Positive Flow-By nose cones (In-Tox Products, LLC; Albuquerque, NM). The nebulizer was filled with a pre-mixed stock solution of either sterile water, Tobramycin or BL-2060, operated at constant pressure. Treatments by inhalation were given for up to 6 hours. Exposure duration was determined by the target deposited dose of the test article. At day 3 post-infection, animals were euthanized, lungs excised, homogenized, and bacterial counts were determined by dilution plate count method.

Results: BL-2060, administered by inhalation, substantially reduced lung bacterial population by up to 2 logs as compared to inoculated vehicle controls. Study results also show that prolonged exposure to high dose levels of BL-2060 (up to 100µg in the respiratory tract) was not associated with toxicity.

Conclusions: This study demonstrated the ability of BL-2060 to reduce bacterial load of *P. aeruginosa* after inhalation exposure in murine pneumonia infection, indicating that BL-2060 has potential for use as antimicrobial agent in treatment of severe lung infections caused by gram negative pathogens.

F1-3991

Structure-activity Relations Of Parasin I, A Histone H2A-derived Antimicrobial Peptide

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Background: Parasin I is a H2A derived 19-residue antimicrobial peptide (KGRGKQGGKVRKAKTRSS) isolated from the skin mucus of wounded catfish, *Parasilurus asotus*, by the action of cathepsin D. Parasin I formed an amphipathic alpha-helical structure (residues 9-17) flanked by two random coil regions (residues 1-8 and 18-19) in helix-promoting environments.

Methods: The structures of parasin I and its truncation analogs were determined by NMR and circular dichroism(CD) analysis. The antimicrobial activity of each peptide was determined using the broth microdilution assay. Finally, peptide localization in *E. coli* and the permeabilization of the *E. coli* outer membrane were investigated by confocal microscopy and the NPN uptake assay, respectively.

Results: Deletion of the lysine residue at the N-terminal [Pa(2-19)] resulted in loss of antimicrobial activity, but did not affect the alpha-helical content of the peptide. The antimicrobial activity was recovered when the Lys residue was substituted with another basic residue, Arg ([R(1)]Pa), but not with polar, neutral, or acidic residues. Progressive deletions from the C-terminal [Pa(1-17), Pa(1-15)] slightly increased the antimicrobial activity (1-4µg/ml) without affecting the alpha-helical content of the peptide. But, further deletion [Pa(1-14)] resulted in nearly complete loss of antimicrobial activity and alpha-helical structure. Confocal analysis and membrane permeabilization assays showed that parasin I and its analogs with comparable antimicrobial activities localized to the cell membrane and subsequently permeabilized the outer and cytoplasmic membranes, but Pa(1-14) lost membrane-permeabilizing activity, whereas Pa(2-19) showed poor membrane-binding and -permeabilizing activities.

Conclusions: The presence of a basic residue at the N-terminal end is essential for the antimicrobial activity and the membrane-binding activity of parasin I and its analogs. Furthermore, alpha-helical structure is necessary for membrane-permeabilizing activity.

F1-3993

***In Vitro* Antimicrobial Activities of a Novel Host Defense Protein Mimetic, PMX30063, Against Multiple Isolates of Staphylococci with Defined Susceptibility Phenotypes**

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Background: We have developed small molecule non-peptidic mimetics that capture the structural and biological properties of host defense proteins. Their small size (MW < 1,000) and fully synthetic origins provide significant advantages over peptides. *In vitro* antimicrobial activities and mammalian cell cytotoxicities for a lead arylamide mimetic, PMX30063, are described.

Methods: Susceptibility assays were carried out *in vitro* by broth microdilution according to CLSI recommendations. Cytotoxicity was evaluated in a bioreduction assay using transformed human liver (HepG2) and embryonic mouse (NIH/3T3) cell lines. Hemolysis of human erythrocytes was evaluated by measuring release of hemoglobin.

Results: A susceptibility screen was performed against 150 isolates of *S. aureus* and coagulase-negative Staphylococci with defined antibacterial susceptibilities to other antimicrobials. MIC₉₀ values (ug/ml) for PMX30063 were: 1 for *S. aureus* (n = 80), 0.5 for *S. epidermidis* (n = 60) and 1 for *S. haemolyticus* (n = 10). MIC ranges (ug/ml) were nearly identical between methicillin-susceptible strains (0.25 - 1, n = 30) and methicillin-resistant (0.5 - 2, n = 50), daptomycin non-susceptible (0.25 - 1, n = 9), linezolid non-susceptible (0.5 - 2, n = 5), vancomycin intermediate (0.25 - 1, n = 6) and vancomycin resistant (0.5 - 1, n = 5) strains of *S. aureus*. Serial passage of methicillin-susceptible (ATCC 29213) and resistant (ATCC 33591) strains of *S. aureus* at 0.5x MIC concentrations for 17 passages (triplicate cultures) did not result in any change in MIC values. EC₅₀ ranges were 298 - 727 µg/ml with NIH/3T3 cells (n = 4), 588 - 1031 µg/ml with HepG2 cells (n = 4), and 60 - 316 ug/ml with isolated human erythrocytes.

Conclusion: PMX30063 demonstrates potent antimicrobial activity against Staphylococcus spp., a low incidence for development of resistance *in vitro*, and high selectivity for bacteria versus mammalian cell types.

F1-3995

Mechanism of Action of POL7080, a Novel Anti-*Pseudomonas* Compound

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Background: The literature on polycationic antimicrobial peptides often implicates membrane lysis, mediated by non-specific binding to negatively-charged membrane surfaces, as a primary mode of action of these molecules. Here we describe a non-lytic mode of action for POL7080 and a series of novel compounds with specific activity against *Pseudomonas aeruginosa* (PA).

Methods: PA membrane lysis in presence of test compounds was assessed over 3 h using Sytox® green. Resistance mutants were selected on agar containing 5 µg/ml POL7080. Chromosomal libraries were made in pUCP19 and individual genes were cloned into pVLT31 for over-expression.

Results: The selectivity of POL7080 against PA suggests the target is restricted in expression or sequence homology. Enantiomers of POL7080 are inactive (MIC >32 µg/ml) compared to the parent molecule (MIC <0.5 µg/ml), indicating the stereospecificity of target-compound interaction. Neither enantiomeric form is significantly lytic toward PA or human erythrocytes. POL7080-resistant clones of PA01 (PA^{RES}) were generated spontaneously at a frequency of <10⁻¹⁰, and chromosomal libraries from PA^{RES} were transduced into PA01. Clones were selected for resistant phenotype on agar containing compound and carbenicillin. Sequencing revealed an in-frame insertion in the same open reading frame (ORF) from 12 clones. Overexpression of the mutant but not wild type ORF in PA01 confers resistance to POL7080. PA^{RES} strains are not cross-resistant with other antibiotics, including cationic peptides or colistin.

Conclusions: The selectivity and high potency of POL7080 as well as a non-lytic mode of action, differentiates its activity from typical antimicrobial peptides. POL7080 interacts with a PA specific protein target. Over-expression and mutagenesis experiments point toward a potential target involved in outer membrane biogenesis.

F1-3996

POL7080, a Novel Anti-*Pseudomonas* Compound: Determination of the Predictive PK/PD Parameter for Efficacy in Murine Thigh and Lung Infection

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Background: POL7080 is a new compound with potent activity restricted to *Pseudomonas spp.* including multidrug-resistant (MDR) *P. aeruginosa* (PA). The aim of this study was to demonstrate activity of POL7080 in neutropenic mice concomitantly infected in the thigh and lung, and to identify PK/PD indices correlating with efficacy.

Methods: PK analysis was performed after subcutaneous administration of POL7080 at doses ranging from 0.625 to 20 mg/kg. The efficacy in terms of CFU-reduction was studied in neutropenic mice (n = 5) infected via intranasal and intramuscular routes with 5×10^3 CFU of PA DSM 12055 followed by subcutaneous administration of POL7080 (0.625 to 20 mg/kg). After 24 h, the bacterial load in thigh muscle and lungs were determined. Dose fractioning studies were performed in the same model (n = 3) with six dose groups and dosing frequencies of q3, 6, 12, 24 h. Minimal inhibitory concentrations (MICs) were determined by microdilution assay.

Results: A 1-compartment model best describes the pharmacokinetics of POL7080 with good dose linearity for C_{max} and AUC ($r^2=0.99$ and 0.97 respectively). Single administrations resulted in dose-dependent killing in the thigh and lungs indicating good tissue penetration, with similar activity in both tissues. Greater than 5-logs killing was achieved at a dose of 10 mg/kg. In dose fractioning studies, AUC_{24}/MIC emerges as the best predictive index for efficacy ($R^2=0.82$). An AUC_{24}/MIC ratio of 76 has a net static effect. Although time above MIC was not predictive, a minimum uninterrupted time of 5 hours over the MBC appears necessary for complete efficacy of POL7080.

Conclusions: POL7080 is efficacious at low concentrations in lung and thigh muscle infection of neutropenic mice. Efficacy is driven by AUC_{24}/MIC and to a lesser extent by time above MIC. The unique selectivity of POL7080 makes it a potentially valuable agent against resistant PA infections.

F1-3998

Membrane-Selective Interactions in the Antimicrobial Mechanism of RTA3, a Commensal-Derived Peptide

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Background: A novel cysteine-containing peptide RTA3, derived from the commensal organism *Streptococcus mitis*, has high activity against multi-drug resistant Gram negative pathogens, and very low mammalian cell toxicity. Here we resolve the role of the Cys-15 residue, and characterise membrane-selective effects of RTA3 relating to synergy and bacterial killing, and the transcriptional response of *E. coli* to sub-lethal concentrations of RTA3.

Methods: Selective inner (IM)- and outer-membrane (OM) permeabilisation was measured by increased substrate accessibility of cytoplasmic β -galactosidase and periplasmic β -lactamase. Synergies were assessed using chequerboard titrations, expressed as Fractional Inhibitory Concentrations (FIC). Transcriptional profiling at 1/5th the MIC of RTA3 was determined by microarray using the *E. coli* MG 1655 genome.

Results: RTA3 peptides (MIC ~ 2 μ M) showed synergy with rifampicin (FIC 0.25-0.38), erythromycin (0.16-0.25) and polymixin E (0.38), but not with aminoglycosides, β -lactams or quinolones. Synergy correlates with permeabilisation of the OM, since the very-weakly-antimicrobial derivative RTA3-C15S (MIC 138 μ M), exhibited synergy and OM permeabilisation but no IM permeabilisation. Recovery of anti-Gram negative activity in RTA3-C15L (MIC = 8 μ M) indicates that the C15 residue functions largely as a bulky hydrophobic residue and is unlikely to have a role in homo- or hetero-disulphide formation. Array analysis indicates that both active and inactive RTA3 variants that perturb OM induce upregulation of envelope stress genes, but only strongly antimicrobial variants induce upregulation of periplasmic serine endopeptidase.

Conclusions: While RTA3 synergy with cytoplasmic acting antibiotics is mediated via OM permeabilisation, only strongly antimicrobial peptides permeabilise the IM, and this may be the dominant site of action of RTA3 peptides.

K-529

Telavancin for Hospital-Acquired Pneumonia Caused by *S. aureus*: Efficacy Analysis According to the *In Vitro* Susceptibility to Vancomycin

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Background: Telavancin (TLV) is an investigational lipoglycopeptide that is rapidly bactericidal against Gram-positive pathogens including methicillin-susceptible and -resistant *S. aureus* (MSSA, MRSA). The clinical cure rates of patients treated for monomicrobial *S. aureus* hospital-acquired pneumonia (HAP) with TLV or vancomycin (VAN) in the ATTAIn study were analyzed by the minimum inhibitory concentration (MIC) of VAN against the respective baseline isolates.

Methods: ATTAIn 1 and 2 were methodologically identical, randomized, double-blind, multinational, phase 3 clinical studies. Patients aged \geq 18 years with HAP caused by suspected or confirmed Gram-positive pathogens were randomized to receive TLV 10 mg/kg IV q 24 h or VAN 1 g IV q 12 h for 7-21 days. Test-of-cure visit was scheduled 7-14 days after end of study treatment. Microbiologically evaluable (ME) patients were those who met pre-specified criteria for evaluability and from whom a Gram-positive pathogen was recovered at baseline. MICs were determined by broth microdilution (CLSI).

Results: TLV clinical cure rate was numerically higher than VAN for all subgroups and statistically significantly higher for patients infected with *S. aureus* with a VAN MIC \geq 1 μ g/mL (Table).

Conclusions: TLV appears more effective than vancomycin for the treatment of HAP due to *S. aureus* with VAN MIC \geq 1 μ g/mL.

Pooled clinical cure rates (ME patients) at TOC, % (n/N)

VAN MIC mg/mL	Pooled clinical cure rates (ME patients) at TOC, % (n/N)			
	TLV	VAN	TLV	VAN
Treatment group				
<i>S. aureus</i>	89% (33/37)	79% (22/28)	87% (74/85) ^c	74% (78/105)
MRSA	92% (11/12)	86% (12/14)	86% (50/58)	75% (66/88)
MSSA	88% (22/25)	71% (10/14)	89% (24/27)	71% (12/17)

^aAll MICs are 0.5 mg/mL, except for 1 TLV patient with MIC \leq 0.25 mg/mL.

^bAll MICs are 1 mg/mL, except for 2 TLV patients with MIC = 2 mg/mL.

^c p<0.05 vs VAN

K-530

Telavancin for Treatment of Hospital-Acquired Pneumonia (HAP) Caused by MRSA and MSSA: The ATTAIN Studies

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⁷Theravance, Inc., South San Francisco, CA.

Background: Vancomycin (VAN) is the standard-of-care for the treatment of methicillin-resistant *S. aureus* (MRSA) pneumonia but is felt to be less effective for infections with methicillin-susceptible (MSSA) strains. Telavancin (TLV) is an investigational, bactericidal lipoglycopeptide with potent activity against Gram-positive pathogens *in vitro*. We compared the efficacy of TLV and VAN for treatment of HAP caused by Gram-positive bacteria.

Methods: ATTAIN 1 and 2 were randomized, methodologically identical, double-blind, phase 3, clinical studies. Patients (³18 years of age) with HAP suspected or documented to be due to Gram-positive pathogens were randomized to receive TLV 10 mg/kg IV q 24 h or VAN 1 g IV q 12 h for 7-21 days. Respiratory and blood specimens were to be obtained at baseline. Test-of-cure (TOC) visit was conducted 7-14 days after end of study treatment. The microbiologically evaluable (ME) population consisted of clinically evaluable patients (met pre-specified criteria for evaluability) with Gram-positive pathogen isolated at baseline.

Results: see table

Conclusions: TLV achieved numerically higher clinical cure rates in patients with HAP caused by MRSA and MSSA. These results suggest that the methicillin resistance status of *S. aureus* may be less important when treating with TLV compared with VAN.

L-1514

Oritavancin in the Treatment of Complicated Skin and Skin Structure Infections (cSSSI): Combined Results of Two Phase 3 Multinational Trials

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Background: Oritavancin (ORI), a novel semisynthetic lipoglycopeptide with activity against a wide range of gram-positive bacteria, including vancomycin-resistant staphylococci and enterococci, is presently under regulatory review for treatment of cSSSI.

Methods: Two randomized, double-blind, multicenter, phase 3 trials were designed to test whether 3 to 7 days of oritavancin was noninferior to 10 to 14 days of vancomycin/cephalexin in the treatment of cSSSI. Patients received IV ORI every 24 hours for 3 to 7 days followed by placebo or IV vancomycin followed by oral cephalexin every 12 hours. Both treatment groups were given for a total of 10 to 14 days as determined by the investigator. In the first study, ORI included two dose groups (1.5 and 3.0 mg/kg/day); ORI dose for the second study was 200 mg/day (300 mg for patients >110 kg). Test-of-cure (TOC) occurred between Days 21 to 35.

Results: 1763 patients received study medication (1173 ORI; 590 VAN). Stratified by disease category, 511 patients were enrolled with wound infection, 734 with major abscess, and 518 with cellulitis. Of the ORI recipients, 956 received a total daily dose of 180 to 330mg ($\pm 10\%$ of proposed dose range) [ORI-DR group]. In clinically evaluable (CE) patients, mean duration of total active dosing was 5.2 days in the ORI-DR group compared to 11.3 days in the VAN group. Clinical cure rates in the CE population at TOC were 77.7% (597/768) in ORI-DR compared to 75.8% (347/458) for VAN (95% CI: -2.9, 6.9). For CE patients with a documented pathogen at baseline (ME population), the microbiological success rate at TOC for ORI-DR was 74.0% (378/511) compared to 72.5% (232/320) for VAN (95% CI: -4.7, 7.7). Adverse events (AEs) were generally mild to moderate in nature with nausea, headache and pruritus being the most common drug-related AEs in ORI patients and pruritus and vomiting in VAN patients.

Conclusions: Oritavancin demonstrated efficacy with short course therapy (3-7 days) in phase 3 trials in the treatment of cSSSI.

L-1515

Incidence of Histamine-like Infusion Reactions (HLIRs) in 2 Phase 3 Studies Comparing Oritavancin (ORI) and Vancomycin (VAN) in the Treatment of Complicated Skin and Skin Structure Infections (cSSSI)

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Background: Histamine-like infusion reactions are a well-recognized adverse effect of glycopeptide administration.

Methods: We sought to compare the frequency and characterize the nature of HLIRs in 1173 ORI and 590 VAN intent-to-treat patients (pts) in 2 randomized, double-blind, phase 3 studies comparing ORI with VAN in the treatment of presumed or proven gram-positive bacterial cSSSI. We defined a possible HLIR as the onset of ³¹ of the following events during or within 6 hours (h) of a patient's completion of an active ORI or VAN infusion, and resolution within 24 h of infusion completion: flushing, erythema, erythematous maculopapular rash on the face, neck, shoulders, chest, back, or arms; urticaria; pruritus; musculoskeletal pain or spasm; angioedema; hypotension; wheezing. A physician, blinded to treatment assignment, reviewed verbatim and preferred adverse-event (AE) terms in the integrated studies safety database and identified terms that might represent HLIR signs/symptoms. Subsequent unblinded physician review identified 104 pts with events that met our definition of possible HLIR. Of the 104 pts with possible HLIRs, 3 were excluded from analyses; 2 due to non-specific AEs, 1 due to an investigator-attributed AE of specific etiology. Fisher's exact test was applied to the data.

Results: A significantly ($p < 0.001$) lower percentage of ORI vs VAN pts had ³¹ possible HLIR (3.1% [36 of 1173 ORI]; 11.0% [65 of 590 VAN]). No significant differences were observed in the percentages of ORI and VAN pts who received concomitant medications due to an HLIR, or who discontinued therapy, study, or both due to an HLIR.

Conclusions: When compared with vancomycin in the treatment of pts with cSSSI, the semisynthetic lipoglycopeptide antibiotic oritavancin was associated with a lower incidence of HLIRs.

K-528

Telavancin for Hospital-Acquired Pneumonia Caused by *S. aureus*: Efficacy Analysis According to the *In Vitro* Susceptibility to Vancomycin

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Background: Telavancin (TLV) is an investigational lipoglycopeptide that is rapidly bactericidal against Gram-positive pathogens including methicillin-susceptible and -resistant *S. aureus* (MSSA, MRSA). The clinical cure rates of patients treated for monomicrobial *S. aureus* hospital-acquired pneumonia (HAP) with TLV or vancomycin (VAN) in the ATTAIN study were analyzed by the minimum inhibitory concentration (MIC) of VAN against the respective baseline isolates.

Methods: ATTAIN 1 and 2 were methodologically identical, randomized, double-blind, multinational, phase 3 clinical studies. Patients aged ³ 18 years with HAP caused by suspected or confirmed Gram-positive pathogens were randomized to receive TLV 10 mg/kg IV q 24 h or VAN 1 g IV q 12 h for 7-21 days.

Test-of-cure visit was scheduled 7-14 days after end of study treatment. Microbiologically evaluable (ME) patients were those who met pre-specified criteria for evaluability and from whom a Gram-positive pathogen was recovered at baseline. MICs were determined by broth microdilution (CLSI).

Results: TLV clinical cure rate was numerically higher than VAN for all subgroups and statistically significantly higher for patients infected with *S. aureus* with a VAN MIC ³ 1 µg/mL (Table).

Conclusions: TLV appears more effective than vancomycin for the treatment of HAP due to *S. aureus* with VAN MIC ³ 1 µg/mL.

	Pooled clinical cure rates (ME patients) at TOC, % (n/N)			
	VAN MIC µg/mL	≤0.5 ^a	31 ^b	
Treatment group	TLV	VAN	TLV	VAN
<i>S. aureus</i>	89% (33/37)	79% (22/28)	87% (74/85) ^c	74% (78/105)
MRSA	92% (11/12)	86% (12/14)	86% (50/58)	75% (66/88)
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^aAll MICs are 0.5 µg/mL, except for 1 TLV patient with MIC ≤ 0.25 µg/mL.

^bAll MICs are 1 µg/mL, except for 2 TLV patients with MIC = 2 µg/mL.

^c p<0.05 vs VAN

M-743

Dalbavancin May Interact with Mannose Binding Lectin to Perpetuate Fungal Infection

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Background: The Mannose Binding Lectin (MBL) complement pathway is activated when MBL binds to surface sugars of fungus and ends in phagocytosis. Selected glycopeptides like teicoplanin and dalbavancin contain similar surface sugars such as D-mannose, N-acetyl-D-glucosamine and N-acyl-D-glucosamine, which may be capable of binding to MBL. In a candidemia model (3 groups of 10 BALB/c mice injected intraperitoneally with *Candida albicans* ATCC 44858 (1.4×10^6 cfu), we previously demonstrated “accelerated infectivity” in mice administered teicoplanin (30 mg/kg IV, then 15 mg/kg IV). Additionally, we alluded to a potential dalbavancin-MBL and teicoplanin-MBL interaction using protein polyacrylamide gel electrophoresis.

Methods: We preinfected 9 BALB/c mice, 10 - 12 weeks old, with *C. albicans* ATCC 44858 (1.74×10^6 cfu) 2 hours prior to the administration of a single intraperitoneal dose of 80 mg/kg dalbavancin.

Results: Mice administered dalbavancin died within 12 hours of infection. Teicoplanin-dosed mice died within 56 hours in the previous candidemia model.

Conclusions: Clinically, dalbavancin and teicoplanin glycopeptides may compete with fungus for MBL. This potential interaction could impede phagocytosis and increase the incidence of fungal infection, which may be more aggressive with dalbavancin, in an immunocompromised patient population.