CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205435Orig1s000

MICROBIOLOGY / VIROLOGY REVIEW(S)

DIVISION OF ANTI-INFECTIVE PRODUCTS CLINICAL MICROBIOLOGY NDA REVIEW

NDA: 205435/205436 DATE REVIEW COMPLETED: 02/24/2014
Tedizolid Phosphate (Sivextro)

Date Company Submitted: 10/18/2016
Date received by CDER: 10/21/2013
Reviewer: Avery Goodwin, Ph.D

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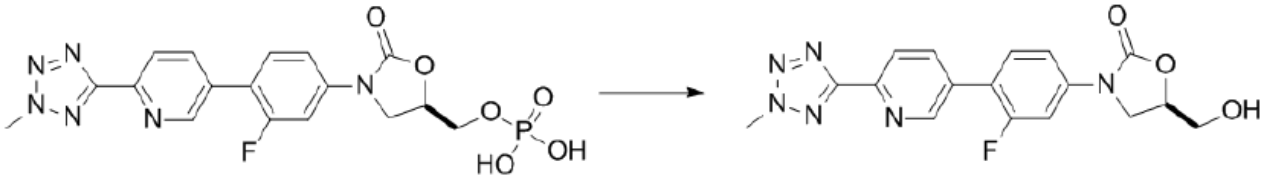
DRUG PRODUCT NAME:
Proprietary Name: Sivextro
Established Name: TR-701; Tedizolid phosphate
Structural Formula:



Tedizolid phosphate (TR-701 FA) Tedizolid (TR-700)

PROPOSED DOSAGE FORM AND STRENGTH:
Tedizolid phosphate: Injection for intravenous use and tablet.
Injection 200 mg sterile lyophilized powder; 200 mg tablet

ROUTE OF ADMINISTRATION AND DURATION OF TREATMENT:
For oral administration and IV administration: the recommended dosage of TRADENAME is 200 mg administered once daily for six (6) days either orally (with or without food) or as an intravenous (IV) infusion in patients (b) (4)

PROPOSED INDICATION:
Tedizolid phosphate (Sivextro) is indicated for the treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by susceptible isolates of the following gram- positive microorganisms: *Staphylococcus aureus* (including methicillin-resistant [MRSA] and methicillin-susceptible [MSSA] isolates, and cases with concurrent bacteremia), *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *Streptococcus*

NDA: 205435/205436 DATE REVIEW COMPLETED: 02/24/2014
Tedizolid Phosphate (Sivextro)

pyogenes, *Streptococcus agalactiae*, *Streptococcus anginosus* Group (including *Streptococcus anginosus, Streptococcus intermedius and Streptococcus constellatus), and Enterococcus faecalis.*

RELATED SUBMISSION REVIEWED:
IND 77,872; IND 106,307

TYPE OF SUBMISSION:
NDA submission

REVIEWER'S COMMENTS:

Based on the clinical microbiology data submitted by the Applicant, this NDA submission may be approved, provided that the Applicant makes the changes in the microbiology subsection of the proposed label recommended by the Agency. (b) (4)

FDA's version of the Tedizolid label:

12.4 Microbiology

Mechanism of Action

The antibacterial activity of tedizolid is mediated by binding to the 50S subunit of the bacterial ribosome resulting in inhibition of protein synthesis. (b) (4)

(b) (4)

Mechanism of Resistance

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Frequency of Resistance

Spontaneous mutations conferring reduced susceptibility to tedizolid occur *in vitro* at a frequency rate of approximately 10^{-10}. (b) (4)

Interaction with Other Antimicrobials

In vitro drug combination studies with tedizolid and aztreonam, ceftriaxone, ceftazidime, imipenem, rifampin, trimethoprim/sulfamethoxazole, minocycline, clindamycin, ciprofloxacin, daptomycin, vancomycin, gentamicin, amphotericin B, ketoconazole, and terbinafine demonstrate neither synergy nor antagonism.

Spectrum of Activity

Tedizolid has been shown to be active against most isolates of the following bacteria, both *in vitro* and in clinical infections, as described in (b) (4) *Indications and Usage (1)* (b) (4)

Aerobic and Facultative Gram-positive Microorganisms

- *Staphylococcus aureus* (including methicillin-resistant [MRSA] and methicillin-susceptible [MSSA] isolates)

(b) (4)

- *Streptococcus pyogenes*
- *Streptococcus agalactiae*
- *Streptococcus anginosus* Group (including *S. anginosus*, *S. intermedius*, and *S. constellatus*)
- *Enterococcus faecalis* (b) (4)

The following *in vitro* data are available, but their clinical significance has not been established. At least 90% of the following microorganisms exhibit an *in vitro* minimum inhibitory concentration (MIC) less than or equal to (b) (4) for tedizolid. However, the safety and effectiveness of TRADENAME in treating

clinical infections due to these microorganisms have not been established in adequate and well-controlled clinical trials.

Aerobic and Facultative Anaerobic Gram-positive Microorganisms

- *Staphylococcus epidermidis* ~~(including methicillin-susceptible and methicillin-resistant strains)~~
- *Staphylococcus haemolyticus*
- *Staphylococcus lugdunensis*

(b) (4)

- *Enterococcus faecium* (b) (4)

Susceptibility Test Methods

When available, the clinical microbiology laboratory should provide cumulative results of the *in vitro* susceptibility test results for antibacterial drugs used in local hospitals and practice areas to the physician as periodic reports that describe the susceptibility profile of nosocomial and community-acquired pathogens. These reports should aid the physician in selecting the most effective antibacterial drug product for treatment.

Dilution Techniques

Quantitative methods are used to determine antibacterial minimum inhibitory concentrations (MICs). These MIC values provide estimates of the susceptibility of bacteria to antibacterial compounds. The MIC values should be determined using a standardized procedure based on dilution methods (broth, agar, or microdilution)[1,3] or equivalent using standardized inoculum and concentrations of tedizolid. The MIC values should be interpreted according to the criteria provided in Table 3.

Table 1 Susceptibility Interpretive Criteria for TRADENAME

Pathogen	Minimum Inhibitory Concentrations (µg/mL)			Disk Diffusion Zone Diameter (mm)		
	S	I	R	S	I	R
Staphylococcus aureus (methicillin-resistant and methicillin-susceptible isolates)	≤0.5	1	≥2	≥19	16-18	≤15
(b) (4)						
Streptococcus pyogenes	≤0.5	-	-	≥18	-	-
Streptococcus agalactiae	≤0.5	-	-	≥18	-	-
Streptococcus anginosus Group*	≤0.25	-	-	≥17	-	-
Enterococcus faecalis	≤0.5	-	-	≥19	-	-

S=susceptible, I=intermediate, R=resistant

*Includes *S. anginosus*, *S. intermedius*, *S. constellatus*

Diffusion techniques

Quantitative methods that require measurement of zone diameters also provide reproducible estimates of the susceptibility of bacteria to antibacterial compounds. The standardized procedure[2,3] requires the use of standardized inoculum concentrations. This procedure uses paper disks impregnated with 20 mcg tedizolid to test the susceptibility of microorganisms to tedizolid. Reports from the laboratory providing results of the standard single-disk susceptibility test with a 20 mcg tedizolid disk should be interpreted according to the criteria in Table 3.

A report of "Susceptible" indicates that the (b) (4) is likely to inhibit growth of the pathogen if the antibacterial (b) (4) reaches the concentration (b) (4) A report of "Intermediate" indicates that the result should be considered equivocal, and if the microorganism is not fully susceptible to alternative (b) (4) drugs, the test should be repeated. This category implies possible clinical (b) (4) in body sites where the drug is physiologically concentrated. This category also provides a buffer zone that prevents small uncontrolled technical factors from causing major discrepancies in interpretation. A report of "Resistant" indicates that the (b) (4) is not likely to inhibit growth of the pathogen if the (b) (4) reaches the concentrations usually achievable at the infection site; other therapy should be selected.

Quality Control

Standardized susceptibility test procedures require the use of laboratory control microorganisms to monitor and ensure the accuracy and precision of supplies and reagents used in the assay, and the techniques of the individuals performing the test.[1, 2, 3] Standardized tedizolid powder should provide the following range of MIC values noted in Table 4. For the diffusion technique using the 20 mcg tedizolid disk, results within the ranges specified in Table 4 should be observed.

Table 2 Acceptable Quality Control Ranges for Susceptibility Testing

Quality Control Organism	Minimum Inhibitory Concentrations (µg/mL)	Disk Diffusion (zone diameter in mm)
Staphylococcus aureus ATCC 29213	0.25 - 1	Not Applicable
Staphylococcus aureus ATCC 25923	Not Applicable	22 - 29
Enterococcus faecalis ATCC 29212	0.25 - 1	Not Applicable
Streptococcus pneumoniae ATCC 49619	0.12 - 0.5	24 - 30

15 REFERENCES

1. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard – 9th ed., CLSI document M7 A9. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
2. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard – 11th ed. CLSI document M2 A11 (ISBN 1-56238-781-2 [Print]; ISBN 1-56238-782-0 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2012.
3. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing – 24th Informational Supplement. CLSI document M100 S24 (ISBN 1-56238-865-7 [Print]; ISBN 1-56238-866-5 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2014

NDA: 205435/205436 DATE REVIEW COMPLETED: 02/24/2014
Tedizolid Phosphate (Sivextro)

EXECUTIVE SUMMARY

Antibacterial Spectrum of Activity

The Applicant has submitted data from surveillance and other investigator studies to support the claim that Tedizolid (TR-700) demonstrates in vitro activity against selected gram-positive pathogens associated with acute bacterial skin and skin structure infections (ABSSSI).

Staphylococci
Tedizolid has demonstrated activity against staphylococcal isolates from the USA and Europe. The data presented included staphylococcal isolates that tested positive for the Panton-Valentine leukocidin (*pvl*) gene; vancomycin-intermediate and –resistant *S. aureus* (VISA/VRSA) isolates; methicillin-susceptible coagulase –negative staphylococci (MSCoNS); and methicillin-resistant coagulase –negative staphylococci (MRCoNS). The MIC values ranged from as low as 0.12 mcg/ml to 1.0 mcg/ml. Additionally, TR-700 appears to have limited activity against a number of linezolid-resistant *S. aureus*. Against a subset of these isolates, MIC_{90} values of 8 mcg/ml were reported for linezolid-resistant *S. aureus* with the MIC ranging from 0.25-16 mcg/ml.

Streptococci
Tedizolid is active in vitro against *S. pneumonia*, including penicillin susceptible, penicillin intermediate and penicillin resistant *Streptococcus pneumoniae* isolates (MIC_{90} 0.25 mcg/ml). Against beta hemolytic streptococci (*Streptococcus pyogenes* and *Streptococcus agalactiae*) MIC values ranged from 0.06 mcg/ml- 1.0 mcg/ml with an MIC_{90} value of 0.5 mcg/ml. Against the Viridans Group streptococci, the MIC_{90} was 0.25 mcg/ml (range 0.06-0.5 mcg/ml).

Enterococci
The in vitro activity of TR-700 against vancomycin-susceptible and -resistant *E. faecalis* (VSEfa and VREfa) was also evaluated. TR-700 demonstrated MIC values that ranged from 0.5 to 1 mcg/mL. The comparator, linezolid, demonstrated an MIC value of 2 mcg/ml; against vancomycin-susceptible and -resistant *E. faecium* (VSEfm and VREfm) TR-700 demonstrated MIC_{90} values ranging from 0.25-1 mcg/ml.

Mechanism of Action

Tedizolid phosphate (TR-701) is TR-700 linked to a phosphate via an ester bond; the linking of TR-700 to a phosphate, is said to improve the solubility and bioavailability of TR-700. Tedizolid is a member of the oxazolidinone class of drugs and is a protein synthesis inhibitor that interacts with the bacterial ribosome and prevents translation.

The Applicant conducted a number of studies to characterize the mechanism of action of TR-700. Their studies explored the ability of TR-700 to inhibit bacterial translation using either a cell-free system or actively growing bacteria. The data indicate that TR700 inhibits prokaryotic protein synthesis (prokaryotic translation) but not eukaryotic protein translation. However, TR-700 was shown to inhibit mitochondrial protein synthesis at a dose reported to be 17-26 folds lower than linezolid.

Mechanism of Resistance and Resistance Studies

Resistance to oxazolidinone may be mediated through mechanisms that include mutations in genes encoding the 23S rRNA, the ribosomal proteins L3 and L4, and/or the acquisition of the *cfr* methyltransferase gene. In vitro studies have demonstrated the selection of TR-700-resistance in certain staphylococcal and enterococcal strains during serial passages, and these mutants were cross-resistant to linezolid. However, under laboratory conditions, TR-700 demonstrated activity against cfr+ isolates of *S. aureus*. TR-700 maintained a 16- to 32-fold potency advantage over linezolid for all cfr strains tested. The clinical significance of this finding is not known.

Interaction with other antimicrobials

In vitro studies evaluating the fractional inhibitory concentration indices of TR-700 in combination with a wide array of agents showed no apparent antagonism or synergy between TR-700 and other agents against both gram-positive and gram-negative pathogens. Furthermore, combining TR-700 with common antifungal agents also had no impact on the in vitro antibacterial activity of the compounds for the target pathogens

Intracellular Antibacterial Concentration Assessment

The data showed that similar to linezolid, TR-700 could penetrate phagocytic cells, in this case, human macrophages. Studies show that TR-700 exhibited enhanced cellular accumulation that was both pH and temperature dependent. TR-700 exhibited an intracellular to extracellular ratio of 10 to 14 while a ratio of 1 to 2 was observed for linezolid. A dose-dependent activity was also demonstrated with TR-700 against the intracellular pathogens *L. monocytogenes* and *L. pneumophila*.

Animal Studies

The Applicant has provided data from a variety of animal models including 1) staphylococcal systemic infections in mice; 2) enterococcal systemic infections in mice; 3) streptococcal systemic infections in mice; 4) MRSA skin and soft tissue infection in mice; 5) mouse thigh infection model with MRSA and MSSA; 6) rat skin and soft tissue infection; 7) lung infection and epithelial lining fluid exposure in mice; 8) a neutropenic mouse pneumonia model; 9) an *S. aureus* endocarditis model in rabbits; 10) and a mouse *Streptococcus pneumoniae* model. Efficacy was demonstrated in all models tested.

Effect of Granulocytes on the Antibacterial Effect of Tedizolid in a Mouse Thigh Infection Model

Treatment with TR-700 resulted in a significant increase in activity in the presence of granulocytes compared with animals that were granulocytopenic. An examination of both normal and granulocytopenic animals indicated an improvement in the exposure response as a function of the presence of granulocytes. Although the mechanism behind this finding is unclear, it is possible that the generation of reactive oxygen species (ROS) during the phagocytosis response may be contributing to the enhanced activity of TR-700.

Pharmacokinetics/Pharmacodynamics

TR-701 FA has been shown to be rapidly converted by phosphatases to the microbiologically active moiety TR-700. The absolute bioavailability of TR-700 following oral administration of TR-701 FA is said to be >80% and steady-state concentrations is said to be achieved within 3 days and indicate modest drug accumulation of approximately 30% following multiple once-daily oral or IV administration. Following oral administration, peak plasma TR-700 concentrations are achieved within approximately 3 hours after administration. Pharmacokinetic studies have demonstrated that TR-700 rapidly distributes into tissues and penetrates into the interstitial space fluid of adipose and skeletal muscle tissue, resulting in TR-700 exposures in these compartments that were similar to free drug exposure in plasma. Moderate protein binding was observed with TR-700 in human plasma (70% to 90%, primarily to albumin), and binding appeared to be independent of concentration. Pharmacodynamic parameter determinations were conducted using a neutropenic mouse *S. aureus* pneumoniae model. The relationships between microbiologic effect against *S. aureus* and each of the pharmacodynamic parameters, i.e., the percent time above the MIC, the AUC/MIC, and the peak/MIC were examined. The strongest relationship was seen when results were correlated with the 24-h AUC/MIC.

Clinical Studies

The Applicant conducted a Phase 2 and two Phase 3 studies. The Phase 3 studies were randomized, non-inferiority clinical trials comparing the efficacy of Tedizolid (200 mg/day for 6 days) with linezolid (600 mg every 12 hours for 10 days) in adult patients with ABSSSI. In the Tedizolid and Linezolid groups, 95.6% and 98.0% of patients, respectively, had a favorable microbiological response for *Staphylococcus aureus* at the EOT Visit. The favorable response rates for MRSA were 92.6% for the TR-701/FA group and 96.8% for the linezolid group, and for MSSA were 97.7% and 98.9%, respectively. For *Streptococcus pyogenes*, both the Tedizolid and Linezolid groups had a 100% favorable response at EOT Visit.

Tedizolid In Vitro Susceptibility Interpretive Criteria

Applicants Proposed TR-700 MIC and Zone Diameter Interpretive Breakpoints

Organism	MIC (µg/mL)	Zone Diameter[a] (mm)
Staphylococcus aureus (including methicillin-resistant and methicillin susceptible strains) *Staphylococcus haemolyticus* *Staphylococcus lugdunensis*	≤0.5 Susceptible 1 Intermediate ≥2 Resistant	≥19 Susceptible 16-18 Intermediate ≤15 Resistant
Streptococcus pyogenes *Streptococcus agalactiae*	≤0.5 Susceptible ≥1 Nonsusceptible	≥18 Susceptible ≤17 Nonsusceptible
Streptococcus anginosus Group (including *S. anginosus, S. intermedius and S. constellatus*)	≤0.25 Susceptible ≥0.5 Nonsusceptible	≥17 Susceptible ≤16 Nonsusceptible

Enterococcus faecalis (vancomycin-susceptible isolates only)	≤0.5 ≥1	Susceptible Nonsusceptible	≥19 ≤18	Susceptible Nonsusceptible

[a] **20 mcg TR-700 disk.**

INTRODUCTION

The Applicant has elected to develop Tedizolid (TR-700) for the treatment of Acute Bacterial Skin and Skin Structure Infections (ABSSSI) formally referred to as complicated skin and skin structure infections (cSSSI). In 2012, the Applicant submitted a request for designation as a Qualified Infectious Disease Product (QIDP) for tedizolid phosphate in the treatment of ABSSI and HABP/VABP and their requests were granted by the Agency.

Tedizolid is a member of the oxazolidinone class of synthetic antibacterial agents effective against many gram-positive bacteria[1,2,3]. Linezolid is well known among this class and has demonstrated activity against gram-positive pathogens, and is used to treat a wide range of infections including endocarditis, meningitis complicated skin and soft tissue infections and nosocomial pneumoniae caused by methicillin-resistant *Staphylococcus aureus*. The mechanism of oxazolidinone action has been suggested to be inhibition of protein synthesis by the binding to domain V of 23S rRNA[4].

Trius Therapeutics, Inc., is developing TR701, an orally-and IV administered oxazolidinone antibiotic, for the treatment of Acute Bacterial Skin and Skin Structure Infections (ABSSSI) caused by *Staphylococcus aureus*; methicillin-resistant (MRSA and –susceptible (MSSA); (b) (4) *Streptococcus pyogenes* (b) (4) *Streptococcus agalactiae*, *Enterococcus faecalis*, including vancomycin- (b) (4) susceptible (VSE), (b) (4)

Acute Bacterial Skin and Skin Structure Infections (ABSSSI)

Skin and skin structure infections represent one of the most common indications for antibiotic therapy and skin infections. They are common and range from minor skin infections to severe necrotizing infections which may require surgical intervention such wound drainage[5,6]. Skin and skin structure infection are considered complicated when they involves deeper layers of soft tissue such as fascia and muscle tissue. Complicated infections including extensive cellulitis, abscess, traumatic or surgical wound infections, and foot infections in diabetic patients are both severe and complex to treat. Therefore, one of the most important aspects of treating complicated skin infection is the clinical assessment of the severity of infection[5,7]. The etiological agents associated with Acute Bacterial Skin and Skin Structure Infections (ABSSSI) are predominantly *S. aureus* and streptococci, including Group A and Group B β-hemolytic streptococci (*S. pyogenes* and *S. agalactiae*,

respectively). It is also not uncommon to identify *Enterococcus* species, as well as a mixed gram-positive and –negative aerobic and anaerobic bacteria in cSSSI[8,9].

FACTORS INFLUENCING THE MANAGEMENT OF INFECTIONS

The routes of administration, the pharmacokinetic-pharmacodynamic profile of the antibacterial agent and the dosing of the drug are some of the factors that must be considered in the treatment and management of infections. Studies have shown that the majority of bacterial infections are present in the extracellular compartment of tissues rather than in the plasma, and in the interstitial fluid of tissues and other body fluids. Therefore, antibiotic penetration into fluids and tissues at infection sites is valuable in predicting therapeutic outcomes and that successful therapy often relies on the unbound antibiotic concentration at the site of action[10].

For an antibacterial to be effective it is important that the drug is present at the site of the bacterial infection. In some instances, treatment failures have been attributed to antibacterials characterized as being highly protein bound in serum despite achieving concentrations in serum that are above the MIC for the target pathogens[11]. For bacterial pneumonia, the delivery of the treatment agent depends on the availability of the unbound concentrations of antibiotic to the infection area. The availability of unbound drug concentrations may also depend on the molecular size, drug diffusion, and a myriad of host factors. Therefore it is also important to measure the concentration of drug from sites including sputum, bronchial secretions, and whole tissue homogenates to determine drug penetration or accessibility[10]. In the case of cSSSI, it is important to obtain culture specimens for documentation of bacteria and for susceptibility testing to guide treatment[12].

ACTIVITY IN VITRO

ANTIBACTERIAL SPECTRUM OF ACTIVITY:

The Applicant submitted in vitro data from large surveillance and independent studies to support the claim that Tedizolid (TR-700) is active against pathogens associated with ABSSSI. Tedizolid has demonstrated activity against a wide range of gram-positive organisms associated with skin infections including those sought after in the proposed indication. The Applicant has also submitted in vitro data from a number of centers across the world and the results are summarized and tabulated below. The MICs were determined by referenced broth and agar dilution methods with the appropriate quality controls using Clinical Laboratory Standard Institute (CLSI) approved guidelines.

Independent studies:

Activity against Gram-positive Organisms:

In independent studies, the in vitro activity of Tedizolid has been determined during the preclinical and clinical development. In addition, MIC data from the (b) (4) initiative on large numbers of recently collected and geographically diverse clinical isolates from the US and Europe were presented and summarized. Susceptibility testing of the isolates was conducted using Clinical Laboratory Standard Institute (CLSI) approved guidelines.

Activity against *Staphylococcus aureus* (MSSA/MRSA)

The in vitro activity of TR-700 against clinical isolates of methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) is summarized below. Linezolid was used as a comparator. Table 1 shows the summary of TR-700 against clinical isolates of MSSA.

Table 1: MIC Values of TR 700 and Comparators Against Clinical Isolates of MSSA

Organism (No. of strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
MSSA (102)	TR-700	0.25 - 1	0.25	0.5	MCR-08-0701-016[a]
	Linezolid	1 - 2	2	2	
	Levofloxacin	0.12 - >16	0.25	4	
	Cefotaxime	0.03 - 4	2	2	
MSSA (101)	TR-700	0.25 - 1	0.5	0.5	PHA-07-0701-063 Table 1
	Linezolid	1 - 4	2	4	
	Vancomycin	0.25 - 2	1	1	
	Oxacillin	0.12 - 5	0.25	0.5	
MSSA (45), erythromycin^{R}	TR-700	0.5 - 0.5	0.5	0.5	PHA-07-0701-047 Table 1
	Linezolid	2 - 2	2	2	
	Oxacillin	0.25 - 0.5	0.25	0.25	
	Erythromycin	0.25 - >128	>128	>128	
	Clindamycin	0.12 - >128	0.12	0.12	
Organism (No. of strains)	**Antibiotic**	**Minimum Inhibitory Concentration (MIC, µg/mL)**			**Source**
		MIC Range	**MIC_{50}**	**MIC_{90}**	
MSSA (30)	TR-700	0.5 - 1	0.5	0.5	PHA-07-0701-042 Table 1
	Linezolid	2 - 4	4	4	
	Vancomycin	0.5 - 1	0. 5	1	
	Erythromycin	0.5 - >128	0.5	>128	
	Clindamycin	≤0.06 - 1	0.25	0.25	
	Levofloxacin	0.5 - 8	0.5	1	
	Tetracycline	0.25 - 64	0.5	32	
	Gentamicin	0.06 - >128	0.5	128	
	Cotrimoxazole	≤0.06 - 32	0.25	2	
	Oxacillin	0.06 - 0.5	0.5	0.5	
MSSA (202) bacteremia	TR-700	0.12 - 0.5	0.25	0.25	Betriu 2010 Table 1
	Linezolid	≤0.25 - 4	1	2	
	Daptomycin	≤0.12 - 1	0.25	0.5	
	Vancomycin	≤0.5 - 2	1	1	
	Teicoplanin	≤0.5 - 2	≤0.5	1	
MSSA (909)	TR-700	0.06 - 2	0.25	0.5	MCR-11-0701-016 Table 3
	Linezolid	0.5 - 4	2	2	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; MSSA=methicillin-susceptible *S. aureus*; No.=Number; ^{R}=resistant
[a]Derived from raw data from MCR-08-0701-016, as described in TR701-016.0, on file with sponsor.

To summarize, a total of 1389 MSSA isolates were tested across six studies; the MIC_{90} ranged from 0.25-0.5 mcg/ml compared to linezolid, which MIC_{90} was reported to have ranged from 2-4 mcg/ml.

In another analysis, the activity of TR-700 against MRSA was evaluated in 9 independent studies against a total of 1589 isolates. Across the 9 studies, TR-700 had MIC_{90} values that ranged from 0.25 to 1 mcg/ml. The comparator, linezolid, demonstrated MIC_{90} values ranging from 2-4 mcg/ml (Table 2).

Table 2: MIC Values of TR 700 and Comparators Against Clinical Isolates of MRSA

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
MRSA (88)	TR-700	0.25-0.5	0.5	0.5	MCR-08-0701-016[a]
	Linezolid	1 - 4	2	4	
	Levofloxacin	0.12 - >16	8	>16	
	Cefotaxime	2 - >64	16	>64	
MRSA (103)	TR-700	0.25 - 1	0.5	0.5	PHA-07-0701-063 Table 2
	Linezolid	1 - 4	2	4	
	Vancomycin	0. 5 - 2	1	1	
	Oxacillin	4 - >32	32	>32	
CA-MRSA (100)	TR-700	0.25 - 1	0.5	0.5	PHA-07-0701-063 Table 3
	Linezolid	1 - 4	2	4	
	Vancomycin	1 - 4	4	4	
	Oxacillin	4 - >32	32	>32	
MRSA (19)	TR-700	0.25 - 0.25	0.25	0.25	PHA-07-0701-041 Table 1
	Linezolid	2 - 2	2	2	
MRSA (50)	TR-700	0.25 - 0.5	0.5	0.5	PHA-08-0701-003 Table 1 Livermore 2009
	Linezolid	2 - 4	2	2	
MRSA (30)	TR-700	0.5 - 0.5	0.5	0.5	PHA-07-0701-042 Table 2
	Linezolid	2 - 4	2	4	
	Vancomycin	0.5 - 2	1	1	
	Erythromycin	0.5 - >128	>128	>128	
	Clindamycin	0.25 - >128	>128	>128	
	Levofloxacin	0.5 - >128	16	>128	
	Tetracycline	0.5 - 128	64	64	
	Gentamicin	0.25 - >128	64	>128	
	Cotrimoxazole	0.25 - >128	0.5	>128	
	Oxacillin	32 - >128	>128	>128	

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
MRSA (254) bacteremia	TR-700	0.12 - 0.5	0.25	0.5	Betriu 2010 Table 1
	Linezolid	≤0.25 - 16	2	4	
	Daptomycin	≤0.12 - 1	0.5	0.5	
	Vancomycin	≤0.5 - 4	1	2	
	Teicoplanin	≤0.5 - 4	≤0.5	1	
MRSA (26) erythromycin^{R}	TR-700	0.5 - 0.5	0.5	0.5	PHA-07-0701-047 Table 1
MRSA (100)	TR-700	0.25 - 1	0.5	0.5	MCR-12-0701-056
	Linezolid	2 - 4	2	2	
	Daptomycin	0.12 - 1	0.5	1	
	Vancomycin	0.25 - 2	1	1	
	Teicoplanin	0.12 - 16	1	2	
	Oxacillin	16 - >128	128	>128	
MRSA (818)	TR-700	0.06 - 2	0.25	0.5	MCR-11-0701-016 Table 3
	Linezolid	0.5 - 16	2	2	

Abbreviations: CA-MRSA=community-acquired methicillin-resistant *S. aureus*; MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; MRSA=methicillin-resistant *S. aureus*; No.=Number; ^{R}=resistant

[a]Derived from MCR-08-0701-016 raw data, as described in TR701-016.0 (on file with sponsor).

Linezolid-Resistant *S. aureus*

The Applicant also investigated the activity of TR-700 relative to linezolid against vancomycin-intermediate and –resistant *S. aureus* (VISA/VRSA) isolates (Table 3). TR-700 had an MIC range from 0.12-1 mcg/ml against the 25 VISA isolates tested (MIC90 = 1 mcg/ml). Against the 7 VRSA isolates tested the MIC range from 0.25-1 mcg/ml. In contrast, linezolid had an MIC_{90} value of 4 mcg/mL against the 25 evaluated VISA isolates. The linezolid MIC values ranged from 1 to 4 mcg/mL against VRSA.

Table 3: MIC Values of TR-700 and Comparators Against Vancomycin- Intermediate and Resistant Clinical Isolates of *Staphylococcus aureus.*

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
VISA (25)	TR-700	0.12 - 1	0.25	1	PHA-08-0701-017 Table 1 and Table 2
	Linezolid	1 - 4	2	4	
	Vancomycin	2 - 8	4	8	
VRSA (7)	TR-700	0.25 - 1	0.25	-	PHA-08-0701-017 **Table 1** and **Table 2**
	Linezolid	1 - 4	2	-	
	Vancomycin	>32	>32	-	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=Number; VISA=vancomycin-intermediate *S. aureus*; VRSA=vancomycin-resistant *S. aureus*

The in vitro activity of TR-700 was investigated against *S. aureus* isolates deemed to be resistant to linezolid (MIC value ≥8 mcg/mL). There were 4 separate studies conducted to evaluate the activity of TR-700. The data show that TR-700 had higher MIC values than those observed against MSSA/MRSA/VISA/VRSA (Table 4).

Table 4: MIC Values of TR-700 and Comparators Against Linezolid-Resistant Clinical Isolates of *Staphylococcus aureus*

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
LRSA (12)	TR-700	0.25 - 16	4	8	MCR-08-0701-016[a]
	Linezolid	8 - >8	>8	>8	
LRSA (10)	TR-700	1 - 4	2	4	PHA-08-0701-003 Table 1 Livermore 2009
	Linezolid	8 - 32	16	32	
LRSA (5)	TR-700	1 - 16	4	-	PHA-07-0701-063 Table 17
	Linezolid	8 - >32	32	-	
	Vancomycin	0.5 - 2	1	-	
	Oxacillin	≤0.03 - >32	32	-	
LRSA (8)	TR-700	0.5 - 2	1	-	PHA-08-0701-006 Table 7
	Linezolid	8 - 32	8	-	
	Vancomycin	0.5 - 1	1	-	
	Daptomycin	0.25 - 0.5	0.25	-	
	Erythromycin	≤0.25 - >2	>2	-	
	Clindamycin	≤0.25 - >2	0.5	-	
	Tetracycline	≤2 - >8	≤2	-	
	Cotrimoxazole	≤0.5 - >2	≤0.5	-	
	Ciprofloxacin	0.25 - >4	>4	-	
	Gentamicin	≤2 - >8	≤2	-	

Abbreviations: LRSA=linezolid-resistant *S. aureus*; MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=Number
Note: Dashed lines represent no value due to insufficient number of isolates
[a]Derived from MCR-08-0701-016 raw data, as described in TR701-016.0 (on file with Sponsor)

<u>Methicillin-Susceptible and –Resistant Coagulase-Negative Staphylococci</u>

The in vitro activity of TR-700 was investigated against both methicillin-susceptible coagulase –negative staphylococci (MSCoNS) and methicillin-resistant coagulase –negative staphylococci (MRCoNS). Against a total of 165 isolates, TR-700 MIC ranged from 0.12-1mcg/ml with the MIC_{90} values ranging from 0.25-0.5 mcg/ml. Linezolid MIC values were 4- to 8-fold higher with MIC_{90} values ranging from 2 to 4 mcg/ml (Table 5).

Table 5: MIC Values of TR-700 and Comparators Against Clinical Isolates of MSCoNS

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
MSCoNS[a] (46)	TR-700 Linezolid Levofloxacin Cefotaxime	0.12 - 1 0.5 - 4 0.06 - 16 0.03 - 4	0.25 1 0.25 0.5	0.5 2 0.5 2	MCR-08-0701-016 Table 1
MSCoNS (49) 100% *Staphylococcus epidermidis*	TR-700[b] Linezolid Vancomycin Oxacillin	0.12 - 1 0.5 - 4 1 - 4 0.06 - 0.25	0.25 1 2 0.12	0.5 2 2 0.25	PHA-07-0701-063 Table 4
MSCoNS (29)	TR-700 Linezolid Vancomycin Erythromycin Clindamycin Levofloxacin Tetracycline Gentamicin Cotrimoxazole Oxacillin	0.25 - 0.5 1 - 4 0.5 - 2 0.25 - >128 0.12 - >128 0.25 - 32 0.5 - 128 0.06 - 128 ≤0.06 - 32 0.06 - 0.25	0.5 2 1 0.5 0.25 0.5 0.5 0.12 0.25 0.12	0.5 4 1 128 1 0.5 32 64 16 0.25	PHA-07-0701-042 Table 3
MSCoNS (41), bacteremia	TR-700 Linezolid Daptomycin Vancomycin Teicoplanin	0.06 - 0.25 ≤0.25 - 2 ≤0.12 - 0.5 ≤0.5 - 2 ≤0.5 - 8	0.25 1 0.25 2 2	0.25 2 0.5 2 4	Betriu 2010 Table 1

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; MSCoNS=methicillin-susceptible coagulase-negative staphylococci; No.=Number

[a]23 *S. epidermidis*, 9 *S. haemolyticus*, 6 *S. lugdenensis*, 4 *S. hominis*, 3 *S. saprophyticus*, 1 *Staphylococcus* spp.

[b]Only 48 out of 49 isolates tested for TR-700 MIC.

The activity of TR-700 was evaluated across 4 independent studies against a total of 319 MRCoNS isolates. Across the 4 studies, TR-700 had MIC values that ranged from 0.03-4 mcg/ml and MIC_{90} values that ranged from 0.25 to 0.5 mcg/mL. The Applicant reported that in a 2010 study, four isolates (1%) had TR-700 MIC values that exceeded 1 mcg/ml with MIC values of 4 mcg/mL. Relative to TR-700, linezolid MIC values were 4- to 8-fold higher MIC_{90} values ranging from 2 to 4 mcg/ml (Table 6).

Table 6: MIC Values of TR-700 and Comparators Against Clinical Isolates of MRCoNS

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
MRCoNS[a] (58)	TR-700 Linezolid Levofloxacin Cefotaxime	0.12 - 1 0.5 - 8 0.12 - >16 0.5 - >64	0.25 1 8 8	0.5 4 >16 >64	MCR-08-0701-016 Table 1
MRCoNS (72) 100% *Staphylococcus epidermidis*	TR-700 Linezolid Vancomycin Oxacillin	0.12 - 1 0.5 - 4 0.25 - 4 0.5 - >32	0.25 1 2 16	0.5 2 2 >32	PHA-07-0701-063 Table 5

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
MRCoNS (26)	TR-700	0.12 - 0.5	0.5	0.5	PHA-07-0701-042 Table 4
	Linezolid	0.5 - 4	2	2	
	Vancomycin	0.25 - 2	1	2	
	Erythromycin	≤0.06 - >128	64	128	
	Clindamycin	0.12 - >128	0.25	>128	
	Levofloxacin	0.12 - 16	0.5	16	
	Tetracycline	0. 5 - >128	4	128	
	Gentamicin	0.06 - 128	16	64	
	Cotrimoxazole	≤0.06 - 32	2	32	
	Oxacillin	0.5 - >128	4	64	
MRCoNS (163), bacteremia	TR-700	0.03 - 4	0.25	0.25	Betriu 2010 Table 1
	Linezolid	≤0.25 - 256	1	2	
	Daptomycin	≤0.12 - 1	0.25	0.5	
	Vancomycin	≤0.5 - 4	2	2	
	Teicoplanin	≤0.5 - 32	2	8	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; MRCoNS=methicillin-resistant coagulase-negative staphylococci; No.=Number
[a]31 *S. epidermidis*, 10 *S. saprophyticus*, 6 *S. hominis*, 5 *S. haemolyticus*, 5 Staphylococcus spp., 1 *S. lugdenensis*.

In addition, MRCoNS co-resistant to erythromycin were also evaluated (Table 7). The data showed that TR-700 MIC values ranged from 0.12 to 0.25 mcg/ml while linezolid MIC values ranged from 0.5 to 1 mcg/mL.

Table 7: MIC Values of TR-700 and Comparators Against Clinical Isolates of Erythromycin-Resistant MRCoNS

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
MRCoNS[a] (9), erythromycin^{R} *erm*A	TR-700	0.25	0.25	-	PHA-07-0701-047 Table 1
	Linezolid	1	1	-	
	Oxacillin	0.25 - >128	2	-	
	Erythromycin	32 - >128	>128	-	
	Clindamycin	0.06 - >128	>128	-	
MRCoNS[a] (24), erythromycin^{R} *erm*C	TR-700	0.12 - 0.25	0.25	0.25	PHA-07-0701-047 **Table 1**
	Linezolid	0.5 - 1	1	1	
	Oxacillin	0.06 - >128	>128	>128	
	Erythromycin	16 - >128	>128	>128	
	Clindamycin	0.06 - >128	>128	>128	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; MRCoNS=methicillin-resistant coagulase-negative staphylococci; No.=Number; ^{R}=resistant
Note: Dashed lines represent no value due to insufficient number of isolates
[a]species distribution unavailable.

<u>Activity against Linezolid-Resistant Coagulase-Negative Staphylococci</u>

S. lugdenensis and *S. haemolyticus*

The Applicant determined the in vitro antibacterial susceptibility studies to evaluate the activity of TR-700 against 106 *S. lugdenensis* and 103 *S. haemolyticus* (US and European) isolates. For *S. lugdenensis*, 72/106 (67.9%) isolates were from USA and 34/106 (32.1%) were from European countries. For *S. haemolyticus*, 69/103 (67.0%) isolates were from USA and 34/103 (33.0%) were from European countries. Isolates were obtained in 2012-2013 and from the following specimen types (n): wound (50), blood culture (48), skin and skin structure (32), and other (79). All susceptibility tests were conducted in accordance to CLSI methods and the appropriate quality controls were used. The results of the study are shown in Table 8 below.

Table 8. Activity of tedizolid (read at 100% inhibition) and linezolid (read at 80% inhibition) against *S. lugdunensis* and *S. haemolyticus* isolated from patients in United States and European medical centers during 2012 to 2013

	MIC (µg/ml)						
	0.06	0.12	0.25	0.5	1	MIC_{50}	MIC_{90}
Tedizolid (100% read): number of isolates at MIC (cumulative % inhibited)							
All strains (209)	0 (0.0)	61 (29.2)	144 (98.1)	4 (100.0)		0.25	0.25
S. lugdunensis (106)	0 (0.0)	55 (51.9)	50 (99.1)	1 (100.0)		0.12	0.25
S. haemolyticus (103)	0 (0.0)	6 (5.8)	94 (97.1)	3 (100.0)		0.25	0.25
Linezolid (80% read): number of isolates at MIC (cumulative % inhibited)							
All strains (209)	0 (0.0)	0 (0.0)	35 (16.8)	168 (97.1)	6 (100.0)	0.5	0.5
S. lugdunensis (106)	0 (0.0)	0 (0.0)	35 (33.0)	70 (99.1)	1 (100.0)	0.5	0.5
S. haemolyticus (103)	0 (0.0)	0 (0.0)	0 (0.0)	98 (95.2)	5 (100.0)	0.5	0.5

The activity of *S. lugdenensis* and *S. haemolyticus* against comparator agents are shown in Table 9 and 10.

Table 9: Activity of tedizolid and comparator antibacterial agents when tested against 106 isolates of *S. lugdenensis*

Antimicrobial agent	MIC_{50}	MIC_{90}	Range	CLSI[a] %S / %I / %R	EUCAST[a] %S / %I / %R
Tedizolid	0.12	0.25	0.12 – 0.5	-[b]	-
Linezolid	0.5	0.5	0.25 – 1	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
Oxacillin	0.5	1	≤0.25 – >2	98.1 / 0.0 / 1.9	98.1 / 0.0 / 1.9
Gentamicin	≤1	≤1	≤1 – ≤1	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
Levofloxacin	0.25	0.25	≤0.12 – >4	99.1 / 0.0 / 0.9	99.1 / 0.0 / 0.9
Clindamycin	≤0.25	≤0.25	≤0.25 – >2	88.7 / 0.0 / 11.3	88.7 / 0.0 / 11.3
Erythromycin	>16	>16	≤0.12 – >16	80.0 / 4.8 / 16.2	82.8 / 1.0 / 16.2
Tetracycline	≤0.03	>32	0.06 – >32	93.4 / 0.0 / 6.6	93.4 / 0.0 / 6.6
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5 – 4	99.1 / 0.0 / 0.9	99.1 / 0.9 / 0.0
Vancomycin	1	1	≤0.12 – 2	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0

a. Criteria as published by the CLSI [2014] and EUCAST [2014].
b. No breakpoints available

Table 10: Activity of tedizolid and comparator antibacterial agents when tested against 103 isolates of *S. haemolyticus*

Antimicrobial agent	MIC_{50}	MIC_{90}	Range	CLSI[a] %S / %I / %R	EUCAST[a] %S / %I / %R
Tedizolid	0.25	0.25	0.12 – 0.5	-[b]	-
Linezolid	0.5	0.5	0.5 – 1	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
Oxacillin	>2	>2	≤0.25 – >2	29.1 / 0.0 / 70.9	29.1 / 0.0 / 70.9
Gentamicin	≤1	>8	≤1 – >8	55.3 / 14.6 / 30.1	50.5 / 0.0 / 49.5
Levofloxacin	4	>4	≤0.12 – >4	38.8 / 1.0 / 60.2	38.8 / 1.0 / 60.2
Clindamycin	≤0.25	>2	≤0.25 – >2	63.1 / 1.0 / 35.9	63.1 / 0.0 / 36.9
Erythromycin	>16	>16	≤0.12 – >16	17.5 / 1.0 / 81.6	17.5 / 0.0 / 82.5
Tetracycline	1	>32	0.06 – >32	74.5 / 2.0 / 23.5	64.7 / 1.0 / 34.3
Trimethoprim/sulfamethoxazole	≤0.5	>4	≤0.5 – >4	62.1 / 0.0 / 37.9	62.1 / 2.0 / 35.9
Vancomycin	1	2	0.25 – 2	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0

a. Criteria as published by the CLSI [2014] and EUCAST [2014].
b. No breakpoints available

In two additional and separate studies the Applicant conducted antibacterial susceptibility studies to evaluate the activity of TR-700 against linezolid-resistant CoNS clinical isolates (Table 11). A total of 46 isolates were tested and TR-700 demonstrated an MIC_{90} value of 16 mcg/ml in the larger study consisting of 40 isolates. In contrast, linezolid had an MIC_{90} value of >32 mcg/ml also in the larger study consisting of 40 isolates. In summary, TR-700 MIC values ranged from 1- to >32 mcg/ml against linezolid-resistant isolates.

Table 11: MIC Values of TR-700 and Comparators Against Linezolid-Resistant Clinical Isolates of Coagulase-Negative Staphylococci

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
CoNS[a] (6) linezolid[R]	TR-700 Linezolid	2 - 4 32 - 64	4 32	- -	PHA-08-0701-003 Table 1 Livermore 2009
CoNS[a] (40) linezolid[R]	TR-700 Linezolid Vancomycin Daptomycin Erythromycin Clindamycin Tetracycline Cotrimoxazole Ciprofloxacin Gentamicin	1 - >32 8 - >32 1 - 2 ≤0.25 - 1 ≤0.06 - >2 ≤0.25 - >4 ≤2 - >8 ≤0.5 - >2 4 - >4 ≤2 - >8	4 32 2 0.25 >2 1 ≤2 >2 >4 >8	16 >32 2 0.5 >2 >2 >8 >2 >4 >8	PHA-08-0701-006 Table 9

Abbreviations: CoNS=coagulase-negative staphylococci; MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=Number; [R]=resistant
Note: Dashed lines represent no value due to insufficient number of isolates
[a]species distribution unavailable.

Activity against Enterococci

Enterococcus faecalis

The in vitro activity of TR-700 against vancomycin-susceptible *E. faecalis* (VSEfa) was evaluated. A total of 329 isolates were evaluated across 5 independent studies. TR-700 had MIC_{90} values that ranged from 0.5 to 1 mcg/mL compared with linezolid which demonstrated an MIC_{90} value of 2 mcg/ml (Table 12).

Table 12: MIC Values of TR-700 and Comparators Against Clinical Isolates of Vancomycin-Susceptible *Enterococcus faecalis*

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
VSEfa (54)	TR-700	0.25 - 1	0.5	0.5	MCR-08-0701-016 Table 1
	Linezolid	1 - 4	2	2	
	Levofloxacin	1 - >16	1	>16	
	Cefotaxime	0.25 - >64	>64	>64	
VSEfa (73)	TR-700	0.25 - 1	0.5	1	PHA-07-0701-063
	Linezolid	1 - 4	2	2	
	Vancomycin	0.5 - 4	1	2	
	Oxacillin	0.06 - >32	16	>32	
VSEfa (45)	TR-700	0.12 - 0.5	0.25	0.5	PHA-07-0701-042 Table 5
	Linezolid	0.5 - 2	2	2	
	Vancomycin	1 - 4	2	2	
	Teicoplanin	≤0.12 - 0.5	0.25	0.5	
	Erythromycin	0.12 - >128	4	>128	
	Levofloxacin	0.5 - 64	2	64	
	Tetracycline	0.5 - 128	64	64	
	Ampicillin	0.25 - 8	1	4	
VSEfa (10), erythromycin^{R} *ermA*	TR-700	0.5 - 0.5	0.5	0.5	PHA-07-0701-047 Table 2
	Linezolid	2 - 2	2	2	
	Vancomycin	1 - 2	1	2	
	Erythromycin	4 - 128	128	128	
	Clindamycin	8 - >128	>128	>128	
VSEfa (71), erythromycin^{R} *ermB*	TR-700	0.25 - 0.5	0.25	0.5	PHA-07-0701-047 **Table 2**
	Linezolid	2 - 2	2	2	
	Vancomycin	0.5 - 4	1	2	
	Erythromycin	0.12 - >128	128	>128	
	Clindamycin	4 - >128	>128	>128	
VSEfa (76)	TR-700	0.12 - 0.5	0.25	0.5	MCR-11-0701-016 **Table 1**
	Linezolid	0.25 - 2	1	2	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=Number; ^{R}=resistant; VSEfa=vancomycin-susceptible *E. faecalis*

In another study, the activity of TR-700 was evaluated across 3 independent studies against a total of 106 vancomycin-resistant *E. faecalis* (VREfa). TR-700 had an MIC90 value of 0.5 mcg/ml across all evaluated studies and did not exceed 1 mcg/mL. Linezolid MIC values were 4- to 8-fold higher and MIC_{90} values were reported to range from 1 to 2 mcg/ml (Table 13).

Table 13: MIC Values of TR-700 and Comparators Against Clinical Isolates of Vancomycin-Resistant *Enterococcus faecalis*

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
VREfa (45)	TR-700	0.25 - 1	0.5	0.5	MCR-08-0701-016 Table 1
	Linezolid	1 - 4	2	2	
	Levofloxacin	0.5 - >16	>16	>16	
	Cefotaxime	0.25 - >64	>64	>64	
VREfa (49)	TR-700	0.25 - 1	0.5	0.5	PHA-07-0701-063 Table 7
	Linezolid	1 - 4	2	2	
	Vancomycin	4 - >32	>32	>32	
	Oxacillin	4 - >32	>32	>32	
VREfa (12)	TR-700	0.25 - 0.5	0.25	0.5	PHA-07-0701-042 Table 6
	Linezolid	0.5 - 1	1	1	
	Vancomycin	>128 - >128	>128	>128	
	Teicoplanin	32 - 128	64	64	
	Erythromycin	>128 - >128	>128	>128	
	Levofloxacin	16 - 128	64	64	
	Tetracycline	0.5 - 64	32	64	
	Ampicillin	1 - 4	2	4	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=Number; VREfa=vancomycin-resistant *E. faecalis*

Enterococcus faecium

The activity of TR-700 was evaluated in 5 independent studies against a total of 202 vancomycin-susceptible *E. faecium* (VSEfm) isolates. The data showed that across 4 studies, TR-700 had MIC_{90} values ranging from 0.25 to 1 mcg/mL. Linezolid MIC values were 4- to 8-fold higher MIC_{90} values of 2 to 4 mcg/ml across evaluated studies (Table 14).

Table 14: MIC Values of TR-700 and Comparators Against Clinical Isolates of Vancomycin-Susceptible *Enterococcus faecium*

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		Range	MIC_{50}	MIC_{90}	
VSEfm (52)	TR-700	0.25 - 1	0.5	0.5	MCR-08-0701-016 Table 1
	Linezolid	2 - 4	2	2	
	Levofloxacin	0.5 - >16	4	>16	
	Cefotaxime	0.5 - >64	>64	>64	
VSEfm (53)	TR-700	0.25 - 1	0.5	1	PHA-07-0701-063 Table 8
	Linezolid	1 - 4	2	4	
	Vancomycin	0.25 - 4	1	1	
	Oxacillin	2 - >32	>32	>32	
VSEfm (30)	TR-700	0.06 - 0.25	0.25	0.25	PHA-07-0701-042 Table 7
	Linezolid	0.5 - 2	2	2	
	Vancomycin	0.5 - 4	0.5	0.5	
	Teicoplanin	0.25 - 2	0.5	0.5	
	Erythromycin	0.25 - >128	>128	>128	
	Levofloxacin	2 - 128	64	64	
	Tetracycline	0.12 - 32	0.5	1	
	Ampicillin	1 - >128	>128	>128	
VSEfm (8), erythromycin^R *ermA*	TR-700	0.5	0.5	-	PHA-07-0701-047 Table 2
	Linezolid	2	2	-	
	Vancomycin	0.5 - 2	0.5	-	
	Erythromycin	4 - 128	128	-	
	Clindamycin	0.12 - >128	>128	-	
VSEfm (17), erythromycin^R *ermB*	TR-700	0.5	0.5	0.5	PHA-07-0701-047 **Table 2**
	Linezolid	2	2	2	
	Vancomycin	0.5 - 4	0.5	2	
	Erythromycin	128	128	128	
	Clindamycin	16 - >128	>128	>128	
VSEfm (42)	TR-700	0.03 - 0.5	0.25	0.5	MCR-11-0701-016 Table 3
	Linezolid	0.12 - 2	2	2	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=Number; ^{R}=resistant; VSEfm=vancomycin-susceptible *E. faecium*
Note: Dashed lines represent no value due to insufficient number of isolates

The activity of TR-700 was evaluated across 4 independent studies against a total of 161 vancomycin-resistant *E. faecium* (VREfm). TR-700 had an MIC range from 0.06-4 mcg/ml, with MIC_{90} values ranging from 0.25 to 0.5 mcg/mL. However, relative to TR-700, linezolid MIC values ranged from 0.5-32 mcg/ml, with MIC_{90} values ranging from 1 to 4 mcg/mL (Table 15).

Table 15: MIC Values of TR-700 and Comparators Against Clinical Isolates of Vancomycin-Resistant *Enterococcus faecium*

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		Range	MIC_{50}	MIC_{90}	
VREfm (52)	TR-700	0.25 - 2	0.5	0.5	MCR-08-0701-016 Table 1
	Linezolid	1 - >8	2	4	
	Levofloxacin	1 - >16	>16	>16	
	Cefotaxime	>64 - >64	>64	>64	
VREfm (51)	TR-700	0.12 - 1	0.5	0.5	PHA-07-0701-063 Table 9
	Linezolid	1 - 4	2	2	
	Vancomycin	8 - >32	>32	>32	
	Oxacillin	>32	>32	>32	
VREfm (29)	TR-700	0.06 - 0.25	0.12	0.25	PHA-07-0701-042 Table 8
	Linezolid	0.5 - 1	1	1	
	Vancomycin	64 - >128	128	>128	
	Teicoplanin	2 - 64	16	64	
	Erythromycin	64 - >128	128	>128	
	Levofloxacin	16 - 128	64	128	
	Tetracycline	≤0.06 - 128	0.25	128	
	Ampicillin	64 - >128	>128	>128	
VREfm (29)	TR-700	0.25 - 4	0.25	0.5	MCR-11-0701-016 Table 3
	Linezolid	1 - 32	2	2	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=Number; VREfm=vancomycin-resistant *E. faecium*

Linezolid resistant Enterococci

To address the potential for cross-resistance of TR-700 with linezolid-resistant strains, a total of 117 linezolid-resistant clinical isolates of enterococci (29% *E. faecalis*, 71% *E. faecium*) were evaluated across 4 independent studies, and the results are summarized in Table 16 below. TR-700 had MIC_{90} values of 2 to 4 mcg/mL. In the submission, the Applicant reported that with the exception of 1 isolate with a TR-700 MIC value of 16 mcg/mL, all evaluated isolates had TR-700 MIC values ≤8 mcg/mL. Linezolid had MIC_{90} values of 16 to 32 mcg/mL.

Table 16: MIC Values of TR-700 and Comparators Against Linezolid Non- Susceptible Clinical Isolates of Enterococci

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		Range	MIC_{50}	MIC_{90}	
Enterococcus faecalis (16) linezolid[NS]	TR-700	0.5 - 8	2	4	PHA-08-0701-006 Table 4
	Linezolid	4 - 32	8	32	
	Vancomycin	1 - >16	1	>16	
	Daptomycin	0.5 - 2	1	1	
	Erythromycin	2 - >8	>8	>8	
	Clindamycin	>2 - >8	>2	>2	
	Tetracycline	≤0.25 - >8	>8	>8	
	Cotrimoxazole	≤0.5 - >2	>2	>2	
	Ciprofloxacin	1 - >4	>4	>4	
	Gentamicin	8 - >8	>8	>8	
Enterococcus faecium (55) linezolid[NS]	TR-700	0.5 - 4	1	2	PHA-08-0701-006 Table 2
	Linezolid	4 - 32	8	16	
	Vancomycin	0.5 - >16	>16	>16	
	Daptomycin	0.12 - 4	1	2	
	Erythromycin	≤0.25 - >2	>2	>2	
	Clindamycin	≤0.25 - >2	>2	>2	
	Tetracycline	≤2 - >8	≤2	>8	
	Cotrimoxazole	≤0.5 - >2	>2	>2	
	Ciprofloxacin	>2 - >4	>2	>2	
	Gentamicin	4 - >8	>8	>8	
Enterococci (36) linezolid[R] *E. faecalis* (9) *E. faecium* (27)	TR-700	1 - 16	2	4	PHA-08-0701-003 Table 1 Livermore 2009
	Linezolid	8 - 64	16	32	
Enterococci (10) linezolid[R] *E. faecalis* (9), *E. faecium* (1)	TR-700	2 - 8	4	4	PHA-07-0701-063 Table 17
	Linezolid	16 - >32	16	32	
	Vancomycin	1 - >32	32	>32	
	Oxacillin	2 - >32	16	>32	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=Number; [NS]= Nonsusceptible; [R]=resistant

Activity against Streptococci

Streptococcus pneumoniae

TR-700 activity against *S. pneumoniae* was evaluated in 2 studies consisting of 243 isolates. The analysis summarized in Table 12 focused on the activity of TR-700 relative to linezolid and the impact of the penicillin susceptibility phenotype on TR-700 activity (Table 17). Against penicillin-susceptible *S. pneumoniae* (PSSP), TR-700 had an MIC_{90} value of 0.25 mcg/ml. Linezolid showed MIC_{90} values that ranged from 1 to 2 mcg/mL. Against penicillin-intermediate *S. pneumoniae* (PISP), TR-700 had MIC_{90} values ranging from 0.25 to 0.5 mcg/mL. Linezolid had MIC_{90} values ranging from 1 to 2 mcg/mL. TR-700 had MIC_{90} values of 0.25 mcg/mL against penicillin-resistant *S. pneumoniae* (PRSP), compared with MIC_{90} values ranging from 1 to 2 mcg/ml observed with linezolid.

Table 17: MIC Values of TR-700 and Comparators Against Clinical Isolates of Penicillin-Susceptible, Intermediate, or Resistant *Streptococcus pneumoniae*

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		Range	MIC_{50}	MIC_{90}	
PSSP (53)	TR-700	0.03 - 0.5	0.25	0.25	MCR-08-0701-016 Table 1
	Linezolid	0.12 - 2	1	2	
	Levofloxacin	0.25 - 4	1	1	
	Cefotaxime	0.015 – 0.25	0.015	0.03	
PSSP (38)	TR-700	0.06 - 0.5	0.25	0.25	PHA-07-0701-063 Table 10
	Linezolid	0.25 - 1	1	1	
	Vancomycin	0.12 - 1	0.25	0.5	
	Oxacillin	0.06 - 0.5	0.06	0.12	
PISP (26)	TR-700	0.12 - 0.5	0.25	0.5	MCR-08-0701-016 **Table 1**
	Linezolid	0.5 - 4	1	2	
	Levofloxacin	0.5 - 1	1	1	
	Cefotaxime	0.03 - 1	0.12	0.5	
PISP (37)	TR-700	0.06 - 0.5	0.25	0.25	PHA-07-0701-063 Table 11
	Linezolid	0.5 - 1	1	1	
	Vancomycin	0.25 - 0.5	0.25	0.5	
	Oxacillin	0.5 - 16	4	8	
PRSP (54)	TR-700	0.12 - 0.5	0.25	0.25	MCR-08-0701-016 **Table 1**
	Linezolid	0.5 - 2	1	2	
	Levofloxacin	0.5 - 2	1	1	
	Cefotaxime	0.5 - 8	1	8	
PRSP (35)	TR-700	0.06 - 0.5	0.25	0.25	PHA-07-0701-063 Table 12
	Linezolid	0.25 - 2	1	1	
	Vancomycin	0.12 - 1	0.25	0.5	
	Oxacillin	8 - 32	16	16	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; PISP=penicillin-intermediate *S. pneumonia*; PRSP=penicillin-resistant *S. pneumonia*; PSSP=penicillin-susceptible *S. pneumonia*; No.=number

In another study *S. pneumoniae* isolates were evaluated regardless of susceptibility to penicillin and TR-700 had an MIC_{90} value of 0.25 mcg/mL compared to the linezolid MIC_{90} value of 1 mcg/ml (Table 18). In summary, the activity of TR-700 against *S. pneumoniae* was greater than that observed with linezolid. Neither agent appeared to be affected by resistance to penicillin.

Table 18: MIC Values of TR-700 and Comparators Against *Streptococcus pneumoniae* Clinical Isolates of Mixed Penicillin Susceptibility

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		Range	MIC_{50}	MIC_{90}	
Streptococcus pneumoniae (30) 17% PSSP, 47% PISP, 37% PRSP	TR-700	0.12 - 0.5	0.25	0.25	PHA-07-0701-042 Table 9
	Linezolid	0.5 - 2	1	1	
	Penicillin	0.015 - 2	1	2	
	Cefotaxime	0.015 - 2	1	2	
	Erythromycin	0.25 - >128	>128	>128	
	Levofloxacin	1 - 32	2	2	
	Clindamycin	0.25 - >128	>128	>128	
	Tetracycline	≤0.06 - 32	16	32	
	Cotrimoxazole	0.5 - 128	16	64	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; PISP=penicillin-intermediate *Streptococcus pneumonia*; PRSP=penicillin-resistant *Streptococcus pneumonia*; PSSP=penicillin-susceptible *Streptococcus pneumonia;* No.=number

Beta-Hemolytic Streptococci (*Streptococcus pyogenes* and *Streptococcus agalactiae*)

The activity of TR-700 and linezolid evaluated in two studies against 268 *S. pyogenes* and 127 *S. agalactiae* is summarized in Table 19 and Table 20 below. Against *S. pyogenes*, TR-700 MIC_{50} MIC_{90} values ranged from 0.25 to 0.5 mcg/mL, compared to an MIC_{90} value of 2 mcg/ml observed with linezolid. Against *S. agalactiae,* TR-700 had an MIC_{90} value of 0.5 mcg/mL, compared MIC_{90} values of 2 mcg/ml observed with linezolid.

Table 19: MIC Values of TR-700 and Comparators Against *Streptococcus pyogenes* Clinical Isolates

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		Range	MIC_{50}	MIC_{90}	
Streptococcus pyogenes (102)	TR-700	0.06 - 0.5	0.25	0.5	PHA-07-0701-063 Table 13
	Linezolid	0.06 - 2	1	2	
	Vancomycin	0.25 - 1	0.5	0.5	
	Oxacillin	≤0.03 - 0.25	0.06	0.06	
S. pyogenes (15)	TR-700	0.06 - 0.25	0.12	0.25	PHA-07-0701-042 Table 10
	Linezolid	1 - 2	1	2	
	Penicillin	≤0.008 - 0.015	0.015	0.015	
	Cefotaxime	≤0.06 - 0.03	0.015	0.03	
	Erythromycin	0.12 - 0.25	0.12	0.25	
	Clindamycin	0.12 - 0.25	0.12	0.25	
	Levofloxacin	0.5 - 4	1	4	
S. pyogenes (135) erythromycin^{S}	TR-700	0.06 - 0.25	0.12	0.25	MCR-11-0701-016 Table 3
	Linezolid	0.5 - 1	0.5	1	
S. pyogenes (16) erythromycin^{R}	TR-700	0.06 - 0.25	0.12	0.25	MCR-11-0701-016 **Table 3**
	Linezolid	0.5 - 1	0.5	1	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=Number

Table 20: MIC Values of TR-700 and Comparators Against *Streptococcus agalactiae* Clinical Isolates

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		Range	MIC_{50}	MIC_{90}	
Streptococcus agalactiae (52)	TR-700	0.06 - 1	0.25	0.5	PHA-07-0701-063 Table 14
	Linezolid	1 - 2	2	2	
	Vancomycin	0.25 - 1	0.5	0.5	
	Oxacillin	0.12 - 16	0.5	0.5	
S. agalactiae (15)	TR-700	0.12 - 0.5	0.25	0.5	PHA-07-0701-042 Table 11
	Linezolid	1 - 2	2	2	
	Penicillin	0.03 - 0.06	0.06	0.06	
	Cefotaxime	0.03 - 0.06	0.06	0.06	
	Erythromycin	0.25 - >128	0.5	>128	
	Clindamycin	0.25 - >128	0.25	>128	
	Levofloxacin	1 - 2	1	2	
S. agalactiae (41) erythromycin^{S}	TR-700	0.12 - 0.25	0.12	0.25	MCR-11-0701-016 Table 3
	Linezolid	0.5 - 1	1	1	
S. agalactiae (19) erythromycin^{R}	TR-700	0.12 - 0.25	0.12	0.25	MCR-11-0701-016 **Table 3**
	Linezolid	0.5 - 1	1	1	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=Number; ^{R}=resistant; ^{S}=susceptible

Viridans Group Streptococci

TR-700 activity was evaluated against 30 isolates of viridans group streptococci in one study (**Table 21**). An MIC_{90} value of 0.25 mcg/ml was observed for TR-700 while linezolid an MIC_{90} value of 2 mcg/mL. Similar to its activity against beta-hemolytic streptococci, TR-700 had 8-fold greater activity than linezolid.

Table 21: MIC Values of TR-700 and Comparators Against Clinical Isolates of Viridans Group Streptococci

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		Range	MIC_{50}	MIC_{90}	
Viridans Group Streptococci (30)	TR-700	0.06 - 0.5	0.25	0.25	MCR-08-0701-016 Table 1
	Linezolid	0.5 - 2	2	2	
	Levofloxacin	0.25 - 2	1	2	
	Cefotaxime	0.015 - 2	0.12	0.5	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=Number

Other Bacteria

In another study, TR-700 activity was also evaluated against *Corynebacterium jeikeium* and *Listeria monocytogenes* (Table 22a) Against *C. jeikeium*, TR-700 had an MIC_{90} value of 0.5 mcg/mL; an MIC_{90} value of 1 mcg/ml was observed with linezolid. Against *L. monocytogenes*, a TR-700 MIC_{90} value of 0.25 mcg/ml was observed compared to an MIC_{90} value of 2 mcg/ml observed with linezolid.

Table 22a: MIC Values of TR-700 and Comparators Against Miscellaneous Aerobic Gram-Positive Clinical Isolates

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
Corynebacterium jeikeium (12)	TR-700	0.25 - 0.5	0.25	0.5	MCR-08-0701-016 Table 1
	Linezolid	0.5 - 1	1	1	
	Levofloxacin	16 - >16	>16	>16	
	Cefotaxime	8 - 32	32	32	
Listeria monocytogenes (33)	TR-700	0.25 - 0.5	0.25	0.25	MCR-08-0701-016 **Table 1**
	Linezolid	2	2	2	
	Levofloxacin	1 - 2	1	1	
	Cefotaxime	2 - 32	32	32	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=number

TR-700 activity was evaluated against 143 *Haemophilus influenzae* that included both beta-lactamase-positive and beta-lactamase-negative, ampicillin-resistant isolates (Table 22b). TR-700 had MIC_{90} values ranging from 4 to 32 mcg/mL. Linezolid had MIC_{90} values of >8 mcg/mL. Against 113 *Moraxella catarrhalis* isolates, TR-700 had MIC_{90} values of ranging from 1 and 4 mcg/mL.

Table 22b: MIC Values of TR-700 and Comparators Against Fastidious Aerobic Gram-Negative Clinical Isolates

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
Haemophilus influenzae (32) Beta-lactamase-negative	TR-700	4 - 32	8	16	MCR-08-0701-016 Table 1
	Linezolid	4 - >8	>8	>8	
	Levofloxacin	0.12 - 0.12	0.12	0.12	
	Cefotaxime	0.008 - 0.03	0.008	0.015	
Haemophilus influenzae (42) Beta-lactamase-positive	TR-700	4 - 32	8	32	MCR-08-0701-016 **Table 1**
	Linezolid	8 - >8	>8	>8	
	Levofloxacin	0.12 - 0.12	0.12	0.12	
	Cefotaxime	0.008 - 0.03	0.015	0.015	
Haemophilus influenzae (25) BLNAR	TR-700	2 - 16	8	16	MCR-08-0701-016 **Table 1**
	Linezolid	8 - >8	>8	>8	
	Levofloxacin	0.12 - 0.12	0.12	0.12	
	Cefotaxime	0.03 - 2	0.5	0.5	
Haemophilus influenzae (19)	TR-700	1 - 32	8	16	PHA-07-0701-063 Table 15
	Linezolid	8 - 32	16	32	
Haemophilus influenzae (25) 76% ampicillin^{R}	TR-700	2 - 16	2	4	PHA-07-0701-042 Table 13
	Linezolid	4 - 16	8	16	
	Ampicillin	0.5 - >128	>128	>128	
	Cefuroxime	0.25 - >128	1	>128	
	Azithromycin	2 - 4	4	4	
	Cotrimoxazole	≤0.06 - 32	4	32	
	Levofloxacin	0.015 - >8	0.03	0.06	
	Tetracycline	0.25 - 32	0.5	8	
Moraxella catarrhalis (50)	TR-700	2 - 4	4	4	MCR-08-0701-016 **Table 1**
	Linezolid	8 - 16	8	8	
	Levofloxacin	0.03 – 0.06	0.06	0.06	
	Cefotaxime	0.03 - 2	0.25	1	
Moraxella catarrhalis (36)	TR-700	0.5 - 4	2	4	PHA-07-0701-063 Table 16
	Linezolid	2 - 8	8	8	
Moraxella catarrhalis (27)	TR-700	0.5 - 2	1	1	PHA-07-0701-042 Table 12
	Linezolid	2 - 8	4	4	
	Penicillin	0.03 - 32	16	32	
	Cefaclor	0.25 - 32	2	8	
	Erythromycin	0.12 - 0.5	0.25	0.5	
	Cotrimoxazole	1 - 2	1	1	
	Levofloxacin	0.06	0.06	0.06	
	Tetracycline	0.25 - 16	0.5	0.5	
	Gentamicin	0.12 - 0.5	0.25	0.5	

Abbreviations: BLNAR=Beta-lactamase-negative, ampicillin resistant; MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; ^{R}=resistant; No.=number

The in vitro activity of TR-700 was evaluated activity against atypical respiratory pathogens (Table 20). TR-700 had MIC values of 0.5 to 1 mcg/ml against *Legionella pneumophila* (n=8), 8 mcg/ml against *Mycoplasma pneumoniae* (n=3), and 1 mcg/ml against *Chlamydophila pneumoniae* (n=1). In contrast, linezolid had MIC values of 4 to 8 mcg/ml against *L. pneumophila*, >32 mcg/ml against *M. pneumoniae*, and 2 mcg/ml against *C. pneumoniae* (n=1). TR-700 activity against urogenital pathogens was also evaluated (Table 22c). Against *Neisseria gonorrhoeae* (n=50), TR-700 had an MIC90 value of 4 mcg/ml compared to an MIC_{90} value of 32 mcg/ml for linezolid. Against a single strain of *Chlamydia psittaci* and *Chlamydia trachomatis*, TR-700 had an MIC value of 2 mcg/ml compared to 32 mcg/ml observed with linezolid.

Table 22c: MIC Values of TR-700 and Comparators Against Clinical Isolates of Atypical Respiratory Tract Pathogens and Urogenital Pathogens

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
Legionella pneumophila (8) 1 ea. from serogroup 1-8	TR-700	0.5 - 1	0.5	-	PHA-07-0701-045 Table 2
	Linezolid	4 - 8	8	-	
	Erythromycin	0.015 - 0.25	0.12	-	
	Rifampin	≤0.008 - 0.015	0.015	-	
	Moxifloxacin	≤0.008 - 0.06	0.015	-	
Mycoplasma pneumoniae (3)	TR-700	8 - 8	-	-	PHA-07-0701-045 Table 1
	Linezolid	>32 - >32	-	-	
	Clarithromycin	≤0.015 - 0.03	-	-	
	Moxifloxacin	0.25 - 0.25	-	-	
Neisseria gonorrhoeae (50)	TR-700	0.5 - >128	2	4	PHA-09-0701-018
	Linezolid	8 - >128	16	32	
	Penicillin	≤0.06 - >2	1	>2	
	Ceftriaxone	≤0.002 - >4	0.015	0.06	
Chlamydia pneumoniae (1)	TR-700	1 - 1	-	-	PHA-09-0701-024 Table 1
	Linezolid	2-2	-	-	
Chlamydia psittaci (1)	TR-700	2 - 2	-	-	
	Linezolid	32 - 32	-	-	
Chlamydia trachomatis (1)	TR-700	2 - 2	-	-	
	Linezolid	32 - 32	-	-	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=number
Note: Dashed lines represent no value due to insufficient number of isolates

TR-700 was inactive at the highest concentration tested (64 mcg/mL) against other clinically prevalent gram-negative pathogens, such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, as shown in Table 22d. TR-700 and linezolid MIC values were greater than or equal to 64 mcg/ml against these pathogens.

Table 22d: MIC Values of TR-700 and Comparators Against Other Aerobic Gram-Negative Clinical Isolates

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
Escherichia coli (11)	TR-700	>64 - >64	>64	>64	MCR-12-0701-057 Table 1
	Linezolid	>64 - >64	>64	>64	
	Imipenem	0.06 - 0.12	0.06	0.12	
Klebsiella pneumoniae (11)	TR-700	>64 - >64	>64	>64	
	Linezolid	64 - >64	>64	>64	
	Imipenem	0.06 - 16	0.25	8	
Proteus mirabilis (11)	TR-700	>64 - >64	>64	>64	
	Linezolid	64 - >64	64	>64	
	Imipenem	0.5 - 4	2	4	
Enterobacter cloacae (11)	TR-700	>64 - >64	>64	>64	
	Linezolid	>64 - >64	>64	>64	
	Imipenem	0.12 - >16	0.5	2	
Citrobacter freundii (11)	TR-700	>64 - >64	>64	>64	
	Linezolid	>64 - >64	>64	>64	
	Imipenem	0.25 - 0.5	0.5	0.5	
Serratia marcescens (11)	TR-700	>64 - >64	>64	>64	
	Linezolid	>64 - >64	>64	>64	
	Imipenem	0.25 - 8	1	2	
Pseudomonas aeruginosa (11)	TR-700	>64 - >64	>64	>64	
	Linezolid	>64 - >64	>64	>64	
	Imipenem	0.25 - 16	1	16	
Acinetobacter baumannii (11)	TR-700	>64 - >64	>64	>64	
	Linezolid	>64 - >64	>64	>64	
	Imipenem	0.06 - 16	4	16	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=number

Activity of TR-700 against an array of *Mycobacterium* spp. and *Norcardia brasiliensis* is summarized in Table

22e. TR-700 had MIC values of ≤0.06 to 2 mcg/ml against *Mycobacterium avium* (n=7), and an MIC_{90} value of 0.5 mcg/ml against *Mycobacterium tuberculosis* (n=115). Linezolid had MIC values ranging from 0.5 to 8 mcg/ml against *M. avium* (n=7) an MIC_{90} value of 2 mcg/ml against *M. tuberculosis* (n=20). Against 31 *N. brasiliensis* isolates, TR-700 had an MIC_{90} value of 1 mcg/mL. This was comparable to linezolid, which had an MIC_{90} value of 1 mcg/mL.

Table 22e: MIC Values of TR-700 and Comparators Against Clinical Isolates of *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Nocardia brasiliensis*

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
Mycobacterium avium (7)	TR-700	≤0.06 - 2	2	-	PHA-07-0701-044 Table 1
	Linezolid	0.5 - 8	4	-	
	Rifampin	0.5 - 16	2	-	
	Isoniazid	4 - 32	8	-	
	Ofloxacin	1 - 32	8	-	
	Ethambutol	4 - 8	4	-	
	Clarithromycin	0.25 - 8	1	-	
Mycobacterium tuberculosis (20)	TR-700	≤0.12 - 1	0.25	0.5	PHA-07-0701-044 Table 2
	Linezolid	0.5 - 4	1	2	
	Rifampin	≤0.12 - 64	32	64	
	Isoniazid	≤0.12 - >64	4	>64	
	Ethambutol	1 - >64	32	64	
Mycobacterium tuberculosis (95) 26% isoniazid & rifampin^{R}	TR-700	0.12 - 0.5	0.25	0.5	Vera-Cabrera 2006 Table 1
Nocardia brasiliensis (31)	TR-700	0.5 - 2	1	1	Vera-Cabrera 2006 **Table 1**
	Linezolid	0.12 - 2	0.5	1	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; ^{R}=resistant; No.=number
Note: Dashed lines represent no value due to insufficient number of isolates

Table 23 summarizes the TR-700 activity against evaluated isolates of gram-positive anaerobic bacteria. TR-700 was active against the evaluated *Clostridium* spp. including *Clostridium difficile* (MIC_{90} value of 1 mcg/mL), *Clostridium perfringens* (MIC_{90} values ranging from 0.25 to 2 mcg/mL), and *Clostridium* spp. (non-*perfringens*; MIC_{90} of 0.25 mcg/mL). Against *Clostridium* spp., linezolid MIC values were typically 4-fold higher than those of TR-700. TR-700 was also evaluated against *Peptostreptococcus* spp. including *P. anaerobius* (MIC_{90} value of 0.5 mcg/mL), *P. micros* (MIC_{90} value of 0.5 mcg/mL), and *Peptostreptococcus* spp. (MIC_{90} value of 0.25 mcg/mL). Against *Peptostreptococcus* spp., linezolid MIC values were typically 4-fold higher than those of TR-700.

Table 23: MIC Values of TR-700 and Comparators Against Anaerobic Gram-Positive Clinical Isolates

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
Clostridium difficile (15)	TR-700	0.25 - 1	1	1	PHA-08-0701-033 Table 1
	Linezolid	2 - 4	4	4	
	Metronidazole	0.25 - 1	0.5	1	
	Vancomycin	0.25 - 2	1	2	
Clostridium perfringens (10)	TR-700	0.25 - 2	0.5	2	PHA-07-0701-063 Table 21
	Linezolid	2 - 8	2	4	
	Metronidazole	1 - >32	1	>32	
	Imipenem	0.06 - 1	0.12	0.5	
Clostridium perfringens (15)	TR-700	0.12 - 0.25	0.25	0.25	PHA-07-0701-042 Table 15
	Linezolid	1 - 2	2	2	
	Metronidazole	1 - 4	4	4	
	Clindamycin	≤0.06 - 2	1	2	
	Imipenem	≤0.06 - 0.12	≤0.06	≤0.06	
	Vancomycin	0.5 - 2	0.5	0.5	
Clostridium spp. (15) non-perfringens	TR-700	≤0.06 - 0.25	0.25	0.25	PHA-07-0701-042 Table 16
	Linezolid	0.5 - 4	2	4	
	Metronidazole	0.12 - 16	4	8	
	Clindamycin	≤0.06 - >128	1	>128	
	Imipenem	≤0.06 - 4	1	4	
	Vancomycin	0.25 - 8	4	8	
Peptostreptococcus anaerobius (10)	TR-700	0.12 - 0.5	0.25	0.5	PHA-07-0701-063 **Table 21**
	Linezolid	0.5 - 8	1	2	
	Metronidazole	≤0.06 - 1	0.5	1	
	Imipenem	≤0.03 - 1	0.06	1	
Peptostreptococcus micros (10)	TR-700	0.12 - 1	0.25	0.5	PHA-07-0701-063 **Table 21**
	Linezolid	0.5 - 4	1	2	
	Metronidazole	≤0.06 - >32	≤0.06	>32	
	Imipenem	≤0.03 - 0.06	≤0.03	≤0.03	

Table 23: (*Continued*) MIC Values of TR-700 and Comparators Against Anaerobic Gram-Positive Clinical Isolates

Organism (No. of Strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
Peptostreptococcus spp. (59)	TR-700	0.03 - 0.25	0.06	0.25	PHA-07-0701-042 Table 14
	Linezolid	0.25 - 2	0.5	1	
	Metronidazole	≤0.06 - >4	1	2	
	Clindamycin	≤0.06 - >128	0.5	64	
	Imipenem	≤0.06 - 1	≤0.06	0.12	
	Vancomycin	≤0.12 - 1	0.25	0.5	
Gram-positive rods (13)	TR-700	0.06 - 0.5	0.06	0.5	PHA-07-0701-042 Table 17
	Linezolid	≤0.06 - 4	0.5	2	
	Metronidazole	0.25 - >128	>128	>128	
	Clindamycin	≤0.06 - 4	≤0.06	2	
	Imipenem	≤0.06 - 2	0.12	2	
	Vancomycin	0.25 - >32	0.5	>32	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=number

The in vitro activity of TR-700 activity against gram- negative anaerobic bacteria isolates was also evaluated (Table 24). TR-700 had MIC values ranging from 2 to 32 mcg/ml against *Bacteroides spp*.

Table 24: MIC Values of TR-700 and Comparators Against Anaerobic Gram-Negative Clinical Isolates

Organism (No. of strains)	Antibiotic	Minimum Inhibitory Concentration (MIC, µg/mL)			Source
		MIC Range	MIC_{50}	MIC_{90}	
Bacteroides fragilis (10)	TR-700	2 - 4	4	4	PHA-07-0701-063 Table 21
	Linezolid	4	4	4	
	Metronidazole	0.12 - >32	0.12	1	
	Imipenem	0.12 - 1	0.12	0.5	
Bacteroides fragilis (30)	TR-700	1 - 4	2	2	PHA-07-0701-042 Table 18
	Linezolid	2 - 4	4	4	
	Metronidazole	≤0.06 - >128	128	>128	
	Clindamycin	≤0.06 - 0.5	0.12	0.5	
	Imipenem	≤0.06 - 4	0.25	1	
Bacteroides ovatus (10)	TR-700	≤0.06 - 8	2	8	PHA-07-0701-063 **Table 21**
	Linezolid	0.5 - 8	8	8	
	Metronidazole	0.5 - >32	1	1	
	Imipenem	0.06 - 0.5	0.25	0.5	
Bacteroides thetaiotaomicron (10)	TR-700	2 - 4	2	2	PHA-07-0701-063 **Table 21**
	Linezolid	4 - 8	4	8	
	Metronidazole	0.5 - >32	1	1	
	Imipenem	0.12 - 4	0.5	1	
Bacteroides thetaiotaomicron (15)	TR-700	1 - 2	2	2	PHA-07-0701-042 Table 19
	Linezolid	4	4	4	
	Metronidazole	2 - 4	4	4	
	Clindamycin	2 - >128	8	>128	
	Imipenem	0.12 - 2	0.25	2	
Bacteroides vulgatus (10)	TR-700	1 - 8	2	4	PHA-07-0701-063 **Table 21**
	Linezolid	2 - 4	2	2	
	Metronidazole	0.12 - 0.5	0.25	0.25	
	Imipenem	0.12 – 0.5	0.25	0.5	
Bacteroides spp. (15)	TR-700	1 - 32	2	4	PHA-07-0701-042 Table 20
	Linezolid	1 - 32	2	4	
	Metronidazole	≤0.25 - 4	4	4	
	Clindamycin	4 - >128	>128	>128	
	Imipenem	≤0.06 - 2	0.5	1	

Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; No.=number

[redacted] (b) (4) **Database**

Studies in this section describe the activity of TR-700 and comparators against target pathogens collected in a single, large prospective surveillance study. More than 6,800 recent clinical isolates were prospectively collected from 49 labs across the US (40 labs) and Europe (9 labs) in 2011 and 2012. All isolates were tested at a central reference laboratory [redacted] (b) (4) under highly controlled testing conditions (CLSI M7-A8 2009, CLSI M100-S21 2011). The surveillance data provide the FDA with the most up-to-date and comprehensive evaluation of the activity of TR-700 against clinical isolates of the target pathogens at the time of this submission. These data also compare the potency of TR-700 to linezolid and other comparators, and evaluate any variation in activity between isolates from the US and those from Europe.

***Staphylococci* isolates**

S. aureus

Tables 25, Table 26 and Figure 1 show the in vitro activity of TR-700 in the US and Europe collected between 2011 and 2012. An MIC value of 0.5 mcg/ml was reported for both the US and European isolates. Linezolid had an MIC value of 2 mcg/ml.

Table 25: Antibacterial Activity of TR-700 and Comparators Against *Staphylococcus aureus* in the US (2011-2012)

Antibiotic	Phenotype (N)	Minimum Inhibitory Concentration (MIC, µg/mL)			% Susceptible
		Range	MIC_{50}	MIC_{90}	
TR-700	Overall (3,743)	≤0.015 - 1	0.25	0.5	ND
	MSSA (2,152)	≤0.015 - 0.5	0.25	0.25	ND
	MRSA (1,591)	≤0.015 - 1	0.25	0.5	ND
Linezolid	Overall	≤0.25 - >4	2	2	99.9
Clindamycin	Overall	≤0.03 - >4	0.12	>4	83.1
Daptomycin	Overall	≤0.06 - 4	0.5	0.5	99.8
Erythromycin	Overall	≤0.12 - >8	>8	>8	34.1
Gentamicin	Overall	≤0.06 - >16	0.25	0.5	98.3
Levofloxacin	Overall	≤0.03 - >32	0.25	32	62.0
Oxacillin	Overall	≤0.06 - >4	0.5	>4	57.5
Tigecycline	Overall	≤0.015 - 0.5	0.06	0.12	100.0
Cotrimoxazole	Overall	≤0.5 - >4	≤0.5	≤0.5	98.6
Vancomycin	Overall	≤0.25 - 2	0.5	1	100.0

Source: Derived from MCR-13-0701-096, Table 18 and MCR-13-0701-096 Raw Data, as described in TR701-017.0 (on file with Sponsor)
Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; MRSA=methicillin-resistant *S. aureus*; MSSA=methicillin-susceptible *S. aureus*; ND=interpretive criteria not defined; N=number

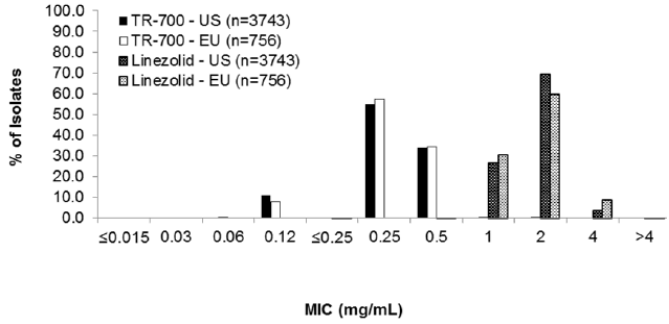
Table 26: Antibacterial Activity of TR-700 and Comparators Against *Staphylococcus aureus* in Europe (2011-2012)

Antibiotic	Phenotype (N)	Minimum Inhibitory Concentration (MIC, µg/mL)			% Susceptible
		MIC Range	MIC_{50}	MIC_{90}	
TR-700	Overall (756)	0.06 - 2	0.25	0.5	ND
	MSSA (577)	0.06 - 1	0.25	0.5	ND
	MRSA (179)	0.06 - 2	0.25	0.5	ND
Linezolid	Overall	≤0.25 - >4	2	2	99.7
Clindamycin	Overall	≤0.03 - >4	0.12	2	89.8
Daptomycin	Overall	≤0.06 - 1	0.25	0.5	100.0
Erythromycin	Overall	≤0.12 - >8	0.5	>8	64.8
Gentamicin	Overall	≤0.06 - >16	0.25	>16	89.2
Levofloxacin	Overall	≤0.03 - >32	0.12	16	71.7
Oxacillin	Overall	≤0.06 - >4	0.25	>4	76.3
Tigecycline	Overall	≤0.015 - 0.5	0.12	0.25	100.0
Cotrimoxazole	Overall	≤0.5 - >4	≤0.5	≤0.5	98.9
Vancomycin	Overall	≤0.25 - 2	0.5	1	100.0

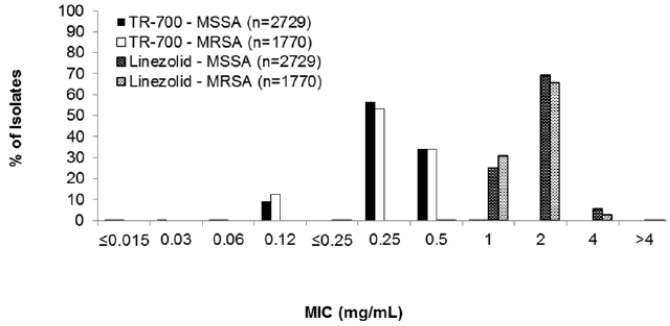
Source: Derived from MCR-13-0701-096, **Table 18** and MCR-13-0701-096 Raw Data, as described in TR701-017.0 (on file with Sponsor)
Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; MRSA=methicillin-resistant *S. aureus*; MSSA=methicillin-susceptible *S. aureus* ND=interpretive criteria not defined; N=number

Figure 1: MIC Distribution of TR-700 and Linezolid Against *Staphylococcus aureus* by Geographic Origin and by Phenotype

A: geography



B: phenotype



Source: Derived from MCR-13-0701-096, Table 18 and MCR-13-0701-096 Raw Data, as described in TR701-017.0 (on file with Sponsor).
Abbreviations: MIC=minimum inhibitory concentration; MRSA=methicillin-resistant *S. aureus*; MSSA=methicillin-susceptible *S. aureus*; US=United States; EU=Europe
Note: Staphylococci were tested withTR-700 in the range of 0.015-16 µg/mL and with linezolid in the range of 0.25-4 µg/mL.

S. epidermidis

Against *S. epidermidis*, TR-700 had an overall MIC_{90} value of 0.25 mcg/ml against US isolates and an overall MIC_{90} value of 0.5 mcg/ml against European isolates (Tables 27, 28 and Figure 2). Linezolid had MIC_{90} value of 1 mcg/ml against *S. epidermidis*. TR-700 activity was within one dilution of the weighted average MIC value observed across independent studies profiling the activity of TR-700 against coagulase-negative staphylococci during preclinical development (MSCoNS and MRCoNS). Similar results observed for TR-700 with *S. epidermidis* in the US and Europe was observed with non-*epidermidis* coagulase-negative staphylococci in the US and Europe.

Table 27: Antibacterial Activity of TR-700 and Comparators Against *Staphylococcus epidermidis* in the US (2011-2012)

Antibiotic	Phenotype (N)	Minimum Inhibitory Concentration (MIC, µg/mL)			% Susceptible
		MIC Range	MIC_{50}	MIC_{90}	
TR-700	Overall (290)	≤0.015 - 4	0.12	0.25	ND
	MSSE (82)	≤0.015 - 1	0.12	0.25	ND
	MRSE (208)	0.06 - 4	0.12	0.25	ND
Linezolid	Overall	≤0.25 - >4	1	1	99.0
Clindamycin	Overall	≤0.03 - >4	0.12	>4	59.0
Daptomycin	Overall	≤0.06 - 1	0.5	0.5	100.0
Erythromycin	Overall	≤0.12 - >8	>8	>8	26.2
Gentamicin	Overall	≤0.06 - >16	≤0.06	>16	74.8
Levofloxacin	Overall	≤0.03 - >32	4	>32	41.4
Oxacillin	Overall	≤0.06 - >4	4	>4	27.9
Tigecycline	Overall	≤0.015 - 0.5	0.12	0.25	ND
Cotrimoxazole	Overall	≤0.5 - >4	2	>4	53.8
Vancomycin	Overall	≤0.25 - 2	1	2	100.0

Source: Derived from MCR-13-0701-096, Table 18 and MCR-13-0701-096 Raw Data, as described in TR701-017.0 (on file with Sponsor).
Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; MRSE=methicillin-resistant *S. epidermidis*; MSSE=methicillin-susceptible *S. epidermidis*; ND=interpretive criteria not defined

Table 28: Antibacterial Activity of TR-700 and Comparators Against *Staphylococcus epidermidis* in Europe (2011-2012)

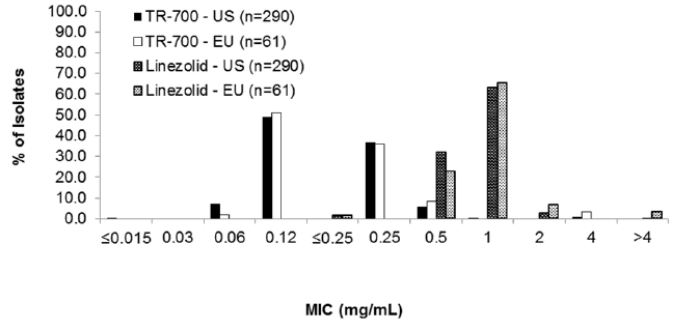
Antibiotic	Phenotype (N)	Minimum Inhibitory Concentration (MIC, µg/mL)			% Susceptible
		MIC Range	MIC_{50}	MIC_{90}	
TR-700	Overall (61)	0.06 - 4	0.12	0.5	ND
	MSSE (12)	0.12 - 0.25	0.12	0.25	ND
	MRSE (49)	0.06 - 4	0.25	0.5	ND
Linezolid	Overall	≤0.25 - >4	1	1	96.7
Clindamycin	Overall	0.06 - >4	0.12	>4	65.6
Daptomycin	Overall	0.12 - 1	0.5	1	100.0
Erythromycin	Overall	≤0.12 - >8	>8	>8	36.1
Gentamicin	Overall	≤0.06 - >16	16	>16	36.1
Levofloxacin	Overall	0.12 - >32	4	>32	29.5
Oxacillin	Overall	≤0.06 - >4	>4	>4	19.7
Tigecycline	Overall	0.03 - 0.5	0.12	0.25	ND
Cotrimoxazole	Overall	≤0.5 - >4	2	>4	50.8
Vancomycin	Overall	0.5 - 2	1	2	100.0

Source: Derived from MCR-13-0701-096, **Table 18** and MCR-13-0701-096 Raw Data, as described in TR701-017.0 (on file with Sponsor)
Abbreviations: MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; MRSE=methicillin-resistant *S. epidermidis*; MSSE=methicillin-susceptible *S. epidermidis*; ND=interpretive criteria not defined

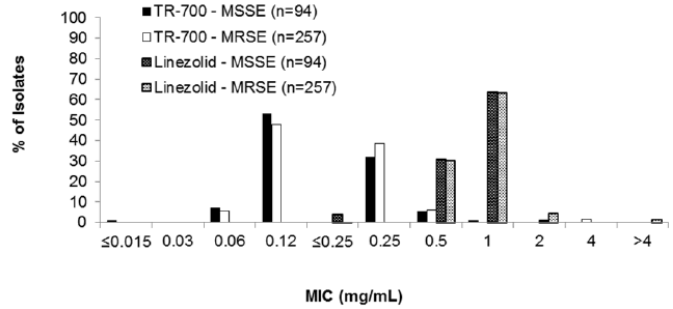
Based on MIC distribution (Figure 2), the activity of TR-700 in the US and Europe was nearly identical, as was the activity against MSSE relative to MRSE. By MIC distribution the increased activity of TR-700 relative to linezolid is also apparent.

Figure 2: MIC Distribution of TR-700 and Linezolid Against *Staphylococcus epidermidis* by Geographic Origin and by Phenotype

A: geography



B: phenotype



Source: Derived from MCR-13-0701-096, Table 18 and MCR-13-0701-096 Raw Data, as described in TR701-017.0 (on file with Sponsor).
Abbreviations: MIC=minimum inhibitory concentration; MRSE=methicillin-resistant *S. epidermidis*; MSSE=methicillin-susceptible *S. epidermidis*; US=United States; EU=Europe
Note: Staphylococci were tested withTR-700 in the range of 0.015-16 µg/mL and with linezolid in the range of 0.25-4 µg/mL.

Enterococcus

Enterococcus faecalis

TR-700 surveillance data against US and European isolates are shown in Tables 29 and 30. Figure 3 shows a graphical representation of the MIC distribution of TR-700 against US and European *E. faecalis* isolates. The data indicated that TR-700 had an overall MIC_{90} value of 0.5 mcg/ml against both US and European isolates. Additionally the activity was identical against sub- populations of VSEfa and VREfa in the US. In Europe, TR-700 showed an MIC_{90} value of 0.5 mcg/ml against VREfa. Linezolid had an MIC_{90} value of 2 mcg/ml against *E. faecalis* overall.

Table 29: Antibacterial Activity of TR-700 and Comparators Against *Enterococcus faecalis* in the US (2011-2012)

Antibiotic	Phenotype (N)	Minimum Inhibitory Concentration (MIC, µg/mL)			% Susceptible
		MIC Range	MIC_{50}	MIC_{90}	
TR-700	Overall (527)	≤0.015 - 1	0.25	0.5	ND
	VSEfa (510)	≤0.015 - 1	0.25	0.5	ND
	VREfa (17)	0.12 - 0.5	0.25	0.5	ND
Linezolid	Overall	≤0.25 - 2	1	2	100.0
	VSEfa	≤0.25 - 2	1	2	100.0
	VREfa	0.5 - 2	1	2	100.0
Ampicillin	Overall	≤0.12 - >16	1	1	99.4
Daptomycin	Overall	≤0.06 - 4	1	2	100.0
Gentamicin (HLAR)	Overall	≤500 - >500	≤500	>500	67.4
Streptomycin (HLAR)	Overall	≤1000 - >1000	≤1000	>1000	73.4
Levofloxacin	Overall	≤0.03 - >32	1	>32	66.8
Tigecycline	Overall	≤0.015 - 0.25	0.06	0.12	100.0
Vancomycin	Overall	≤0.25 - >32	1	2	96.6

Source: Derived from MCR-13-0701-096, Table 18 and MCR-13-0701-096 Raw Data, as described in TR701-017.0 (on file with Sponsor).
Abbreviations: HLAR=high-level aminoglycoside resistance screen; MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; VREfa=vancomycin-resistant *E. faecalis*; VSEfa=vancomycin-susceptible *E. faecalis*; ND=interpretive criteria not defined; N=number

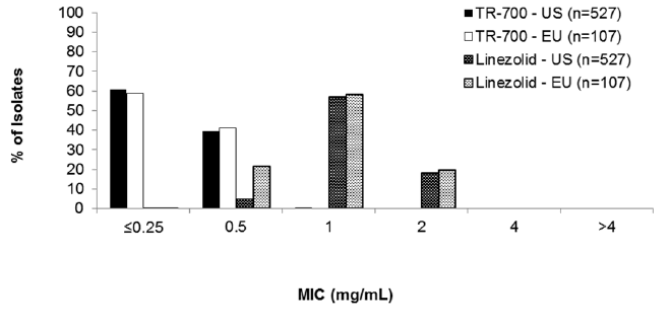
Table 30: Antibacterial Activity of TR-700 and Comparators Against *Enterococcus faecalis* in Europe (2011-2012)

Antibiotic	Phenotype (N)	Minimum Inhibitory Concentration (MIC, µg/mL)			% Susceptible
		Range	MIC_{50}	MIC_{90}	
TR-700	Overall (107)	0.06 - 0.5	0.25	0.5	ND
	VSEfa (96)		0.25	0.5	ND
	VREfa (11)		0.5	0.5	ND
Linezolid	Overall	0.5 - 2	1	2	100.0
	VSEfa		1	2	100.0
	VREfa		1	1	100.0
Ampicillin	Overall	≤0.12 - >16	1	1	99.1
Daptomycin	Overall	0.12 - 4	1	2	100.0
Gentamicin (HLAR)	Overall	≤500 - >500	≤500	>500	59.8
Streptomycin (HLAR)	Overall	≤1000 - >1000	≤1000	>1000	65.4
Levofloxacin	Overall	0.12 - >32	1	>32	70.1
Tigecycline	Overall	0.03 - 0.12	0.06	0.12	100.0
Vancomycin	Overall	≤0.25 - >32	1	>32	88.8

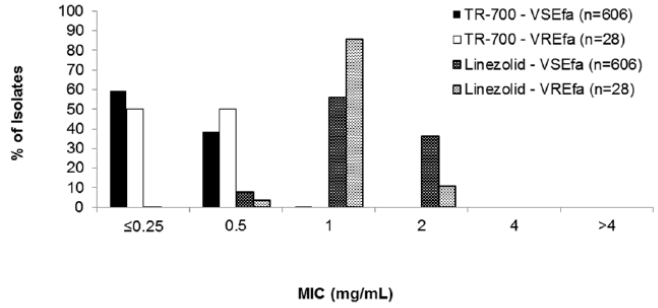
Source: Derived from MCR-13-0701-096, **Table 18** and MCR-13-0701-096 Raw Data, as described in TR701-017.0 (on file with Sponsor)
Abbreviations: HLAR=high-level aminoglycoside resistance screen; MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; VREfa=vancomycin-resistant *E. faecalis*; VSEfa=vancomycin-susceptible *E. faecalis*; ND=interpretive criteria not defined; N=number

Figure 3: MIC Distribution of TR-700 and Linezolid Against *Enterococcus faecalis* by Geographic Origin and by Phenotype

A: geography



B: phenotype



Source: Derived from MCR-13-0701-096, Table 18 and MCR-13-0701-096 Raw Data, as described in TR701-017.0 (on file with Sponsor)
Abbreviations: MIC=minimum inhibitory concentration; VREfa=vancomycin-resistant *E. faecalis*; VSEfa=vancomycin-susceptible *E. faecalis*; US=United States; EU=Europe
Note: Enterococci were tested withTR-700 in the range of 0.015-16 µg/mL and with linezolid in the range of 0.25-4 µg/mL.

Enterococcus faecium

Against *E. faecium*, a TR-700 MIC_{90} value of 0.5 mcg/ml was reported against US and European isolates. Moreover, linezolid had an MIC_{90} value of 2 mcg/ml against *E. faecium* overall (Table 31 and 32). Figure 4 shows a graphical representation of the MIC distribution of TR-700 against US and European *E. faecium* isolates.

Table 32: Antibacterial Activity of TR-700 and Comparators Against *Enterococcus faecium* in the US (2011-2012)

Antibiotic	Phenotype (N)	Minimum Inhibitory Concentration (MIC, µg/mL)			% Susceptible
		Range	MIC_{50}	MIC_{90}	
TR-700	Overall (176)	0.12 - 2	0.25	0.5	ND
	VSEfm (57)		0.25	0.5	ND
	VREfm (119)		0.25	0.5	ND
Linezolid	Overall	0.5 - >4	2	2	99.4
	VSEfm	0.5 - 2	2	2	100.0
	VREfm	1 - >4	2	2	99.2
Ampicillin	Overall	≤0.12 - >16	>16	>16	14.8
Daptomycin	Overall	≤0.06 - >4	2	4	96.6
Gentamicin (HLAR)	Overall	≤500 - >500	≤500	≤500	90.3
Streptomycin (HLAR)	Overall	≤1000 - >1000	≤1000	>1000	68.2
Levofloxacin	Overall	0.25 - >32	>32	>32	14.2
Tigecycline	Overall	≤0.015 - 0.5	0.06	0.12	100
Vancomycin	Overall	≤0.25 - >32	>32	>32	32.4

Source: Derived from MCR-13-0701-096, Table 18 and MCR-13-0701-096 Raw Data, as described in TR701-017.0 (on file with Sponsor).
Abbreviations: HLAR=high-level aminoglycoside resistance screen; MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; VREfm=vancomycin-resistant *E. faecium;* VSEfm=vancomycin-susceptible *E. faecium*; ND=interpretive criteria not defined; N=number

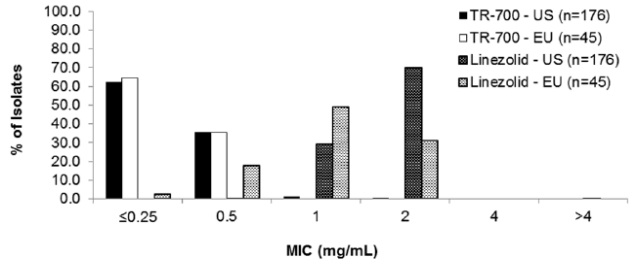
Table 32: Antibacterial Activity of TR-700 and Comparators Against *Enterococcus faecium* in Europe (2011-2012)

Antibiotic	Phenotype (N)	Minimum Inhibitory Concentration (MIC, µg/mL)			% Susceptible
		MIC Range	MIC_{50}	MIC_{90}	
TR-700	Overall (45)	0.12 - 0.5	0.25	0.5	ND
	VSEfm (31)		0.25	0.5	ND
	VREfm (14)		0.25	0.5	ND
Linezolid	Overall	≤0.25 - 2	2	2	100.0
	VSEfm	≤0.25 - 1	2	2	100.0
	VREfm	1 - 2	2	2	100.0
Ampicillin	Overall	0.25 - >16	>16	>16	22.2
Daptomycin	Overall	≤0.06 - 4	2	4	100.0
Gentamicin (HLAR)	Overall	≤500 - >500	>500	>500	48.9
Streptomycin (HLAR)	Overall	≤1000 - >1000	>1000	>1000	26.7
Levofloxacin	Overall	0.25 - >32	>32	>32	15.6
Tigecycline	Overall	≤0.015 - 0.25	0.06	0.12	100
Vancomycin	Overall	≤0.25 - >32	1	>32	66.7

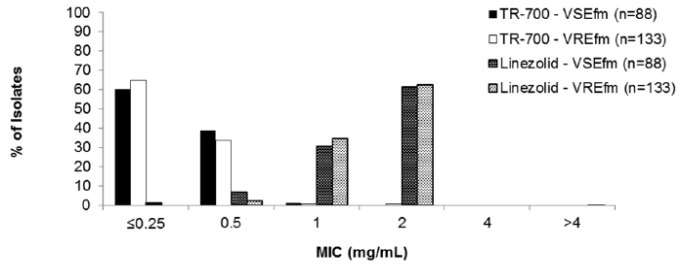
Source: Derived from MCR-13-0701-096, **Table 18** and MCR-13-0701-096 Raw Data, as described in TR701-017.0 (on file with Sponsor).
Abbreviations: HLAR=high-level aminoglycoside resistance screen; MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; VREfm=vancomycin-resistant *E. faecium*; VSEfm=vancomycin-susceptible *E. faecium*; ND=interpretive criteria not defined; N=number

Figure 4: MIC Distribution of TR-700 and Linezolid Against *E. faecium* by Geographic Origin and by Phenotype

A: geography



B: phenotype



Source: Derived from MCR-13-0701-096, Table 18 and MCR-13-0701-096 Raw Data, as described in TR701-017.0 (on file with Sponsor).
Abbreviations: MIC=minimum inhibitory concentration; VREfm=vancomycin-resistant *E. faecium*; VSEfm=vancomycin-susceptible *E. faecium*; US=United States; EU=Europe
Note: Enterococci were tested withTR-700 in the range of 0.015-16 µg/mL and with linezolid in the range of 0.25-4 µg/mL.

Conclusions

The data presented above demonstrated the in vitro activity of TR-700 (with linezolid as an active comparator) against gram-positive pathogens. TR-700 is consistently more effective in vitro than linezolid against these organisms, including against isolates with the following resistant phenotypes; methicillin-resistant staphylococci and vancomycin-resistant enterococci. The activity of TR-700 against target pathogens was consistent across the several profiling studies conducted during development and a large recent surveillance study conducted in the US and Europe.

MECHANISM OF ACTION

Tedizolid (TR-700) is a member of the oxazolidinone class of antibacterial agents. TR-700 is a protein synthesis inhibitor that interacts with the bacterial ribosome and prevents translation. The Applicant conducted a number of studies to characterize the mechanism of action of TR-700. Their studies explored the ability of TR-700 to inhibit bacterial translation using either a cell-free system of actively growing bacteria. TR-700 and TR-701 was evaluated in their effects on the inhibition of prokaryotic and eukaryotic protein synthesis in vitro.

The inhibition of protein synthesis was evaluated in a coupled transcription - translation assay that use pBEST circular plasmid DNA encoding for luciferase reporter gene and an *E. coli* according to methods provided by manufacturer. In this system the *in vitro* synthesis of luciferase was monitored by its enzymatic activity and the concentration of compound resulting in 50% of inhibition of luciferase synthesis was determined from plots of concentration vs. % inhibition of luciferase synthesis. Table 33 shows the IC_{50} data of the test compounds used in the study. Please note that the active compound TR-700 demonstrated a significantly lower IC_{50} that that of chloramphenicol and linezolid (these are compounds that are known protein synthesis inhibitors).

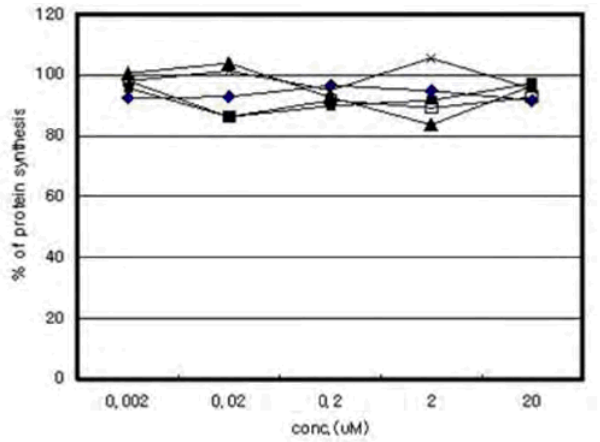
Table 33: IC50s of test compounds for protein synthesis inhibition

Drugs	IC_{50} (µM)
TR-700	0.9567
TR-701	3.4957
Linezolid	3.2965
Chloramphenicol	1.266
Ampicillin	>20

Source: PHA-07-0701-039 Table 1
Abbreviations: TR-700=protein synthesis inhibitor, binds to the bacterial 50S ribosomal subunit; biologically active moiety of TR-701

In another study, protein synthesis inhibition was also examined in a eukaryotic system using a rabbit reticulocyte coupled transcription/translation assay. The data showed that neither TR-700 nor any of the other compounds tested inhibited eukaryotic translation (Figure 5).

Figure 5: Inhibition of In vitro protein synthesis by TR-700, linezolid and controls determined by in vitro coupled transcription and translation assay on prokaryotic and eukaryotic systems.



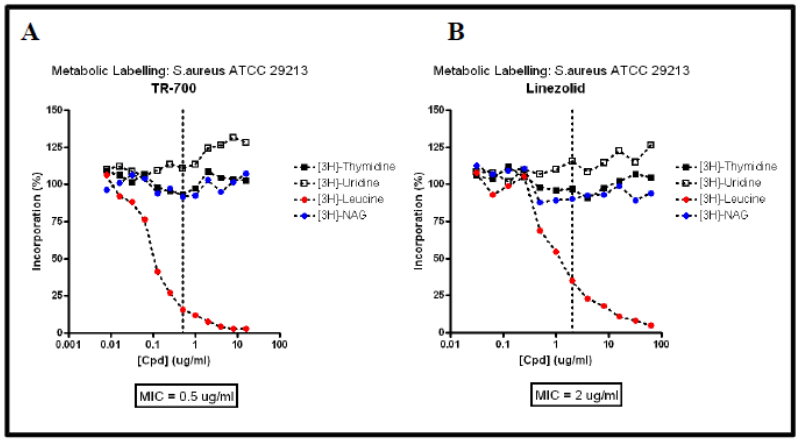
Source: PHA-07-0701-039 Figure 1
Abbreviations: TR-700=protein synthesis inhibitor, binds to the bacterial 50S ribosomal subunit; biologically active moiety of TR-701
Symbols: □ TR-700; ♦ TR-701; ■ linezolid; ▲ chloramphenicol; × ampicillin

The Applicant examined the inhibition of macromolecular pathways leading to DNA, RNA, protein, and cell wall synthesis. TR-700 inhibition of macromolecule synthesis was studied in actively growing *S. aureus* ATCC

29213. TR-700 was compared to 5 control antibiotics specific for selected metabolic pathways: linezolid and tetracycline (protein synthesis) ciprofloxacin (DNA synthesis), rifampicin (RNA synthesis) and vancomycin (cell wall synthesis).

Macromolecular synthesis in the presence of increasing doses of antibacterial agent was monitored by measuring the incorporation of radiolabeled precursors of DNA ([3H]- Thymidine), RNA ([3H]-Uridine), protein synthesis ([3H]-Leucine), and for cell wall synthesis ([3H]-N-acetylglucosamine, (NAG)). Inhibition of a particular pathway is indicated by decreasing incorporation of the labeled precursor with increasing compound concentration. The data indicate that TR-700 specifically inhibited protein synthesis in an actively growing culture of *S. aureus* as shown in Figure 6A. Little to no inhibition of DNA, RNA, or cell wall synthesis was observed. Figure 6B shows that linezolid also demonstrated selective inhibition of protein synthesis; indicating that both compounds act similarly.

Figure 6: Specific Inhibition of Protein Macromolecular Synthesis by TR-700 and Linezolid in *Staphylococcus aureus* ATCC 29213



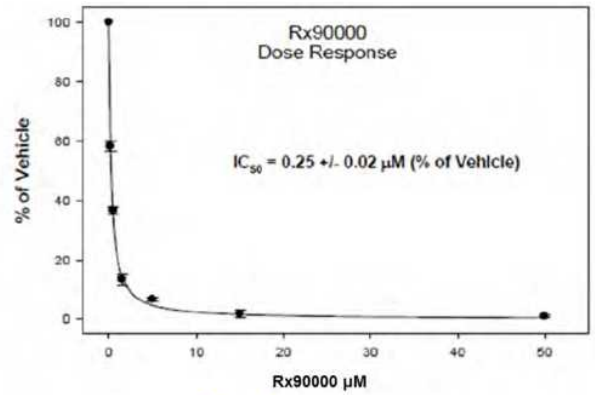
Source: PHA-08-0701-034 Figure 2
Abbreviations: ATCC=American Type Culture Collection; MIC=minimum inhibitory concentration; NAG=N-acetylglucosamine; Cpd=compound; TR-700=protein synthesis inhibitor, binds to the bacterial 50S ribosomal subunit; biologically active moiety of TR-701
Note: MIC values are indicated by the dashed line

Studies that examined the effects of TR-700, TR-701 (and the comparator linezolid) on mitochondrial protein synthesis were also conducted using intact mitochondria isolated from normal rat heart. In this study, the rate of mitochondrial protein synthesis was calculated for each test compound by following the time course of [35S]- methionine incorporation into mitochondrial protein at 20, 40, and 60 minutes of incubation using a filter paper disc assay. The 50% inhibitory concentration (IC_{50}) for each test compound was computed and plotted on a dose response curve.

In Study 1, the IC_{50} for TR-700 was 0.37±0.04 mcM, while linezolid showed an IC_{50} of 6.2±2.0 mcM. The time course for all treatments was linear over time. Similar results were obtained in Study 2: the IC_{50} for TR-700 was 0.25±0.02 mcM (Figure 7), while linezolid showed an IC_{50} of 6.5±0.8 mcM (Figure 8). The inhibition time course for TR-701 (Study 2 only; Rx900001=TR-701) was non-linear with moderate inhibition observed at the 20 minute time-point, and much higher inhibition at the later time points. Figure 9 shows the dose-response effect of TR-701 at 20 minutes as compared to the last 40 minutes of the study. The dose response curve for

TR-701 using the 20 minute time-point gave an IC_{50} of 28.9±6.6 mcM. Assays with TR-701 in this system were consistent with a slow conversion of TR-701 to TR-700 over the course of the assay within the assay mixture.

Figure 7: Dose Response Curve of TR-700 (Rx90000) Inhibition of Mitochondrial Protein Synthesis



Source: SPH-08-0701-028 Figure 6
Abbreviations: Rx90000=TR-700; TR-700=protein synthesis inhibitor, binds to the bacterial 50S ribosomal subunit; biologically active moiety of TR-701

Figure 8 Dose Response Curve of Linezolid Inhibition of Mitochondrial Protein Synthesis

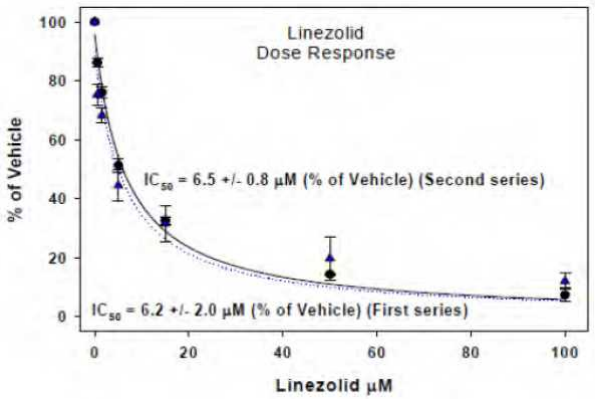
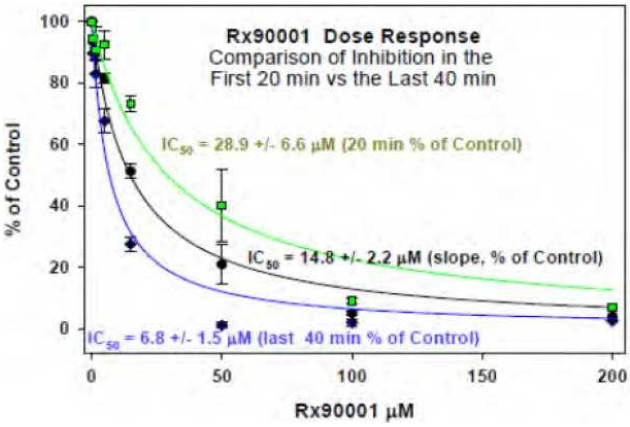


Figure 9: Dose Response Curve of TR-701 (Rx90001) Inhibition of Mitochondrial Protein Synthesis



In summary, results of both studies were similar. TR-700 had an IC_{50} on isolated rat heart mitochondrial protein synthesis that was approximately 17- to 26-fold lower than linezolid.

Assessment of Bactericidal Activity

The bactericidal activities of TR-700 were determined against bacterial isolates. Studies were conducted in several independent laboratories and for the purpose of this review; the Minimum Bactericidal Concentration (MBC) was defined as the lowest concentration of antibacterial agent that killed ≥ 99.9 % of the starting inoculum. The term "bactericidal" was defined as an MBC/MIC ratio of ≤ 4. Assessment of bactericidal activity was limited to gram-positive pathogens (staphylococci, enterococci, and streptococci) only, for which TR-700 has demonstrated in vitro activity.

Minimum Bactericidal Concentration

MBC values for TR-700 against species of staphylococci, enterococci, and streptococci were determined and are summarized in Table 34, Table 35 and Table 36 below. TR-700 MBC:MIC ratios were indicative of bactericidal activity (≤4) for the majority of *S. pneumoniae* isolates (25/33), for a subset of *S. aureus* isolates (27/82), and for 1 *E. faecium* isolate (1/34). TR-700 had MBC activity consistent with that of a bacteriostatic agent (MBC:MIC ratios >4) for *S. pyogenes*, *S. agalactiae* and the majority of *S. aureus* (55/82) and enterococci (67/68). Similar results were observed with linezolid across the evaluated species and phenotypes.

Table 35: Summary of MBC Data (mcg/mL) for TR-700 and Linezolid Against Staphylococci

Organism	Type	Drug	MBC range	MBC_{50}	MBC_{90}	MBC:MIC ratio ≤4 (%)
Staphylococcus. aureus	all (n=82)	TZD	0.5 - >32	4	16	27 (32.9)
		LZD	2 - >8	>8	>8	41[a] (51.9)
	Methicillin^S (n=25)	TZD	1 - 32	16	32	1 (4.0)
		LZD	4 - >8	>8	>8	3 (12.0)
	Methicillin^R (n=57)	TZD	0.5 - >32	2	16	26 (45.6)
		LZD	2 - >8	8	>8	38[a] (70.4)
Coagulase-negative staphylococci[b]	all (n=32)	TZD	2 - >32	16	32	0 (0.0)
		LZD	2 - >8	>8	>8	3[c] (10.0)
	Methicillin^S (n=11)	TZD	2 - 32	16	32	0 (0.0)
		LZD	2 - >8	>8	>8	3[c] (30.0)
	Methicillin^R (n=21)	TZD	2 - >32	16	32	0 (0.0)
		LZD	8 - >8	>8	>8	0[c] (0.0)

Source: MCR-08-0701-016
Abbreviations: LZD=linezolid; MBC=minimum bactericidal concentration; Methicillin^R=methicillin-resistant; Methicillin^S=methicillin-susceptible; MIC=minimum inhibitory concentration; MRSA=methicillin-resistant *S. aureus*; TZD=TR-700; TR-700=protein synthesis inhibitor, binds to the bacterial 50S ribosomal subunit; biologically active moiety of TR-701
[a]3 MRSA isolates that exhibited undefined (>) MBC values within 4-fold of the MIC value were excluded from analysis.
[b]Consisted of 3 unspeciated coagulase-negative staphylococci, 15 *S. epidermidis*, 4 *S. haemolyticus*, 5 *S. hominis*, 1 *S. lugdenensis*, and 4 *S. saprophyticus*.
[c]1 Methicillin^S isolate and 1 Methicillin^R *S. saprophyticus* isolate that exhibited undefined (>) MBC values within 4-fold of the MIC value were excluded from analysis .

Table 36: Summary of MBC Data (mcg/mL) for TR-700 and Linezolid Against Enterococci

Organism	Type	Drug	MBC range	MBC_{50}	MBC_{90}	MBC:MIC ratio ≤4 (%)
Enterococcus faecalis	all (n=34)	TZD	>8 - >32	>16	>16	0 (0.0)
		LZD	>8 - >8	>8	>8	0[a] (0.0)
	VSEfa (n=15)	TZD	>8 - >16	>16	>16	0 (0.0)
		LZD	>8 - >8	>8	>8	0[a] (0.0)
	VREfa (n=19)	TZD	>8 - >32	>16	>32	0 (0.0)
		LZD	>8 - >8	>8	>8	0[a] (0.0)
Enterococcus faecium	all (n=34)	TZD	1 - >16	>16	>16	1 (2.9)
		LZD	4 - >8	>8	>8	1[b] (3.0)
	VSEfm (n=14)	TZD	>8 - >16	>16	>16	0 (0.0)
		LZD	>8 - >8	>8	>8	0[b] (0.0)
	VREfm (n=20)	TZD	1 - >16	>16	>16	1 (5.0)
		LZD	4 - >8	8	>8	1 (5.0)

Source: MCR-08-0701-016
Abbreviations: LZD=linezolid; MBC=minimum bactericidal concentration; MIC=minimum inhibitory concentration; TR-700=protein synthesis inhibitor, binds to the bacterial 50S ribosomal subunit; biologically active moiety of TR-701; TZD=TR-700; VREfa=vancomycin-resistant *E. faecalis*; VSEfa=vancomycin-susceptible *E. faecalis*; VREfm=vancomycin-resistant *E. faecium*; VSEfm=vancomycin-susceptible *E. faecium*
Note: Ranges and $MIC_{50/90}$ reported incorrectly in MCR-08-0701-016, correctly reported above
[a]1 VSEfa isolate and 1 VREfa isolate that exhibited undefined (>) MBC values within 4-fold of the MIC value were excluded from analysis.
[b]1 VREfm isolate that exhibited an undefined (>) MBC value within 4-fold of the MIC value was excluded from analysis.

Table 37: Summary of MBC Data (mcg/mL) for TR-700 and Linezolid Against Streptococci

Organism	Type	Drug	MBC range	MBC_{50}	MBC_{90}	MBC:MIC ratio ≤4 (%)
Streptococcus pneumoniae	all (n=33)	TZD	0.5 - 16	1	2	25 (75.8)
		LZD	2 - >8	4	8	24 (72.7)
	PSSP (n=12)	TZD	0.5 - 8	1	4	8 (66.7)
		LZD	2 - >8	4	8	8 (66.7)
	PISP (n=10)	TZD	0.5 - 16	1	1	9 (90.0)
		LZD	2 - >8	4	8	8 (80.0)
	PRSP (n=11)	TZD	1 - 2	1	2	8 (72.7)
		LZD	4 - 8	4	8	8 (72.7)
Beta-hemolytic streptococci[a]	all (n=22)	TZD	>4 - >16	>8	>16	0 (0.0)
		LZD	>8 - >8	>8	>8	0[b] (0.0)
	SPY (n=11)	TZD	>4 - >8	>8	>8	0 (0.0)
		LZD	>8 - >8	>8	>8	0[b] (0.0)
	SAG (n=11)	TZD	>8 - >16	>8	>16	0 (0.0)
		LZD	>8 - >8	>8	>8	0 (0.0)

Source: **MCR-08-0701-016**
Abbreviations: CLSI= Clinical and Laboratory Standards Institute; LZD=linezolid; MBC=minimum bactericidal concentration; MIC=minimum inhibitory concentration; PISP=penicillin-intermediate *S. pneumoniae*; PRSP=penicillin-resistant *S. pneumoniae* [applying oral penicillin breakpoints, CLSI M100-S22 2012]; PSSP=penicillin-susceptible *S. pneumoniae*; SAG=*S. agalactiae;* SPY=*S. pyogenes*; TZD=TR-700; TR-700=protein synthesis inhibitor, binds to the bacterial 50S ribosomal subunit; biologically active moiety of TR-701
[a]Ranges and $MIC_{50/90}$ values derived from MCR-08-0701-016, Appendix A.
[b]1 SPY isolate that exhibited an undefined (>) MBC value within 4-fold of the MIC value was excluded from analysis.

Time-kill Kinetic Analysis.

The time-kill kinetics of TR-700 were evaluated primarily for *S. aureus* and enterococci across several independent studies (PHA-07-0701-060, PHA-08-0701-029, PHA-09-0701-017, and CPH-12-0701-051). The log_{10} kill observed with various concentrations of TR-700 is summarized in Table 38 below.

Table 38: Change in Log10 CFU/mL Relative to Starting Inocula Over Time for TR-700 and Linezolid Against Gram-positive Pathogens

Organism	Drug	MIC (µg/mL)	Fold MIC Evaluated	ΔLog_{10} (CFU/mL)[a]	Time point (hr)[b]	Source
Staphylococcus aureus MSSA Smith	TZD	0.5	1X	-1.1	24	PHA-07-0701-060 Table 1
			2X	-1.8		
			4X	-2.3		
			8X	-2.2		
	LZD	2	1X	0.7		
			2X	-2.6		
			4X	-2.6		
			8X	-2.4		
	TZD	0.5	1X	-0.2	24	PHA-09-0701-017 Table 2
			2X	-2.7		
			4X	-2.4		
	LZD	2	1X	-0.7		
			2X	-2.2		
			4X	-1.6		
	TZD	0.5	1X	0.9	24	PHA-09-0701-017 Table 3
			3X	-1.8		
			9X	-1.9		
			12X	-2.3		
	LZD	2	1X	1.4		
			3X	-0.2		
			9X	-1.5		
			12X	-1.9		
S. aureus MSSA Smith	TZD	0.5	1X	1.3	48	PHA-09-0701-017 **Table 3**
			3X	-2.4		
			9X	-2.4		
			12X	-3.2		
	LZD	2	1X	1.7		
			3X	-0.6		
			9X	-2.2		
			12X	-1.8		
S. aureus MSSA 97-3-2923	TZD	0.5	1X	0.3	24	PHA-07-0701-060 **Table 1**
			2X	-1.1		
			4X	-1.7		
			8X	-1.7		
	LZD	2	1X	1.8		
			2X	-0.9		
			4X	-1.4		
			8X	-1.8		

Table 38: (*Continued*) Change in Log10 CFU/mL Relative to Starting Inocula Over Time for TR-700 and Linezolid Against Gram-positive Pathogens

Organism	Drug	MIC (µg/mL)	Fold MIC Evaluated	ΔLog_{10} (CFU/mL)[a]	Time point (hr)[b]	Source
S. aureus MSSA 97-4-2022	TZD	0.5	1X	1.8	24	PHA-07-0701-060 Table 1
			2X	0.6		
			4X	-0.4		
			8X	-1.2		
	LZD	2	1X	2.0		
			2X	0.9		
			4X	-1.0		
			8X	-1.4		
S. aureus MRSA 98-11-R782	TZD	0.5	1X	-1.1	24	PHA-07-0701-060 **Table 1**
			2X	-1.8		
			4X	-2.3		
			8X	-2.2		
	LZD	2	1X	0.7		
			2X	-2.6		
			4X	-2.6		
			8X	-2.4		
S. aureus MRSA 98-12-P85	TZD	0.5	1X	1.0	24	PHA-07-0701-060 **Table 1**
			2X	-0.7		
			4X	-1.1		
			8X	-1.4		
	LZD	2	1X	1.0		
			2X	-0.7		
			4X	-1.1		
			8X	-1.4		
S. aureus MRSA M126	TZD	0.5	1X	-0.1	24	PHA-07-0701-060 **Table 1**
			2X	-0.8		
			4X	-1.8		
			8X	-1.9		
	LZD	2	1X	0.7		
			2X	-0.7		
			4X	-1.7		
			8X	-2.2		
S. aureus MRSA ATCC 33591	TZD	0.25	1X	2.8[a]	24	PHA-08-0701-029
			2X	1.0[a]		
			4X[b]	-0.1[a]		
			8X	-1.6[a]		
			16X	-1.6[a]		

Table 38: (*Continued*) Change in Log_{10} CFU/mL Relative to Starting Inocula Over Time for TR-700 and Linezolid Against Gram-positive Pathogens

Organism	Drug	MIC (µg/mL)	Fold MIC Evaluated	ΔLog_{10} (CFU/mL)[a]	Time point (hr)[b]	Source
Enterococcus faecium 00-4-U563	TZD	0.5	1X	0.1	24	PHA-07-0701-060 Table 2
			2X	-0.1		
			4X	-0.1		
			8X	-0.5		
	LZD	2	1X	0.0		
			2X	-0.1		
			4X	-0.5		
			8X	-0.8		
E. faecium 00-4-U535	TZD	0.5	1X	0.4	24	PHA-07-0701-060 **Table 2**
			2X	0.3		
			4X	0.1		
			8X	0.0		
	LZD	2	1X	0.4		
			2X	0.2		
			4X	0.0		
			8X	0.0		
E. faecium VRE 99-4-C159	TZD	0.25	1X	0.7	24	PHA-07-0701-060 **Table 2**
			2X	0.1		
			4X	0.1		
			8X	-0.1		
	LZD	1	1X	0.6		
			2X	0.1		
			4X	-0.1		
			8X	-0.2		
E. faecium VRE 98-4-5932	TZD	0.25	1X	0.6	24	PHA-07-0701-060 **Table 2**
			2X	0.1		
			4X	-0.1		
			8X	-0.3		
	LZD	1	1X	0.5		
			2X	0.1		
			4X	-0.2		
			8X	-0.2		
E. faecium VRE 98-4-1074	TZD	0.25	1X	0.8	24	PHA-07-0701-060 **Table 2**
			2X	0.7		
			4X	0.1		
			8X	0.1		
	LZD	1	1X	0.8		
			2X	0.6		
			4X	0.1		
			8X	-0.1		

Table 38: (*Continued*) Change in Log_{10} CFU/mL Relative to Starting Inocula Over Time for TR-700 and Linezolid Against Gram-positive Pathogens

Organism	Drug	MIC (µg/mL)	Fold MIC Evaluated	ΔLog_{10} (CFU/mL)[a]	Time point (hr)[b]	Source
Streptococcus pneumoniae PRSP 98-3-3588	TZD	0.125	1X	-0.2	7	PHA-07-0701-060 Table 3
			2X	-0.7		
			4X	-0.9		
			8X	-1.3		
	LZD	0.5	1X	-0.6		
			2X	-0.7		
			4X	-1.0		
			8X	-1.6		
S. pneumoniae PRSP 00-4-R456	TZD	0.125	1X	0.7	7	PHA-07-0701-060 **Table 3**
			2X	-0.1		
			4X	-0.7		
			8X	-1.7		
	LZD	0.5	1X	0.7		
			2X	0.3		
			4X	-0.9		
			8X	-1.5		

Abbreviations: ATCC=American Type Culture Collection; CFU=colony-forming unit; hr=hour; Linezolid^R=linezolid-resistant; LZD=linezolid; MRCoNS=methicillin-resistant coagulase-negative staphylococci; MIC=minimum inhibitory concentration; MRSA=methicillin-resistant *S. aureus*; MSSA=methicillin-susceptible *S. aureus;* PRSP=penicillin-resistant *S. pneumoniae;* TR-700=protein synthesis inhibitor, binds to the bacterial 50S ribosomal subunit; biologically active moiety of TR-701; TZD=TR-700; VRE=vancomycin-resistant enterococci
[a]Change in mean log_{10} CFU/mL determined from time-kill assays performed in triplicate.
[b]4X time point determined at 22 hr for MRSA ATCC 33591, PRSP strains evaluated at 7 hr due to autolysis after prolonged incubation.

The time-kill kinetics of TR-700 and linezolid were also evaluated against *S. aureus* Smith strain (known their ability to exhibit serologically distinct capsules) in several independent studies with similar results. Against the Smith strain, at concentrations at or above 4X the MIC, both TR-700 and linezolid exhibited an approximate 2-log_{10} kill after 24 hours, which was below the 3-log_{10} kill threshold that is achieved with bactericidal agents. The degree of killing observed with TR-700 and linezolid against the Smith strain was comparable when evaluated in the presence or absence of human serum (Table 39).

Table 39: Growth of *S. aureus* ATCC 13709 (MSSA) Exposed to TR-700 or Linezolid in the Presence or Absence of 50% Human Serum

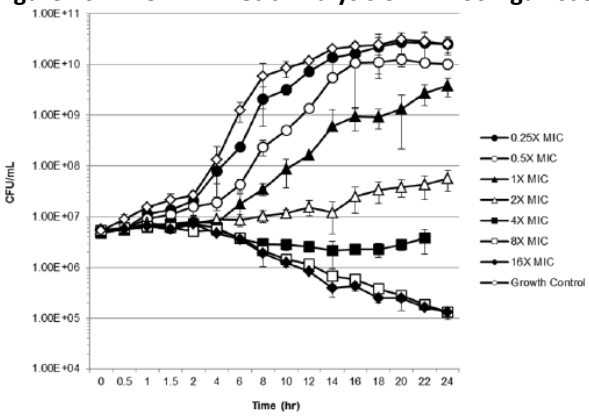
TZD MIC Concentration	TZD (ΔLog_{10} CFU/mL)	TZD+ser (ΔLog_{10} CFU/mL)	LZD MIC Concentration	LZD+ser (ΔLog_{10} CFU/mL)
0x	3.6	3.6	0x	3.6
1x (0.5 µg/mL)	-0.2	0.5	1x (2 µg/mL)	-0.7
2x (1 µg/mL)	-2.7	-0.6	2x (4 µg/mL)	-2.2
4x (2 µg/mL)	-2.4	-2.5	4x (8 µg/mL)	-1.6

Source: PHA-09-0701-017 Table 2
Abbreviations: ATCC=American Type Culture Collection; CFU=colony forming units; LZD=linezolid; MIC=minimum inhibitory concentration; ser=50% human serum; TR-700=protein synthesis inhibitor, binds to the bacterial 50S ribosomal subunit; biologically active moiety of TR-701; TZD=TR-700

Against three evaluated MRSA isolates (Figure 10, Figure 11, and Figure 12), a bacteriostatic effect was observed, with log_{10} reductions not exceeding 2 throughout the course of the experiment. Against the remaining strains of *S. aureus*, including several MRSA isolates and one isolate of coagulase-negative staphylococci, a 1 to 2 log10 kill was generally observed for both TR-700 and linezolid at concentrations 2X or above the MIC, consistent with activity of a bacteriostatic agent rather than a bactericidal agent. Against the

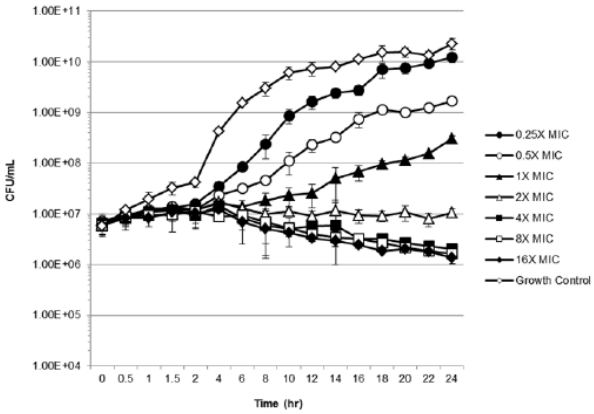
evaluated isolates of *E. faecalis* and *E. faecium*, including vancomycin-resistant isolates, the decrease in viable bacteria rarely exceeded 1-$\log_{10}$ at 24 hours for both TR-700 and linezolid, regardless of the concentration evaluated. Against the 2 evaluated isolates of penicillin-resistant *S. pneumoniae*, a <2-$\log_{10}$ kill was observed with both TR-700 and linezolid at 7 hours at 2X, 4X, and 8X the MIC.

Figure 10 Time-kill Kinetic Analysis of TR-700 Against *S. aureus* ATCC 33591 (Methicillin-Resistant)



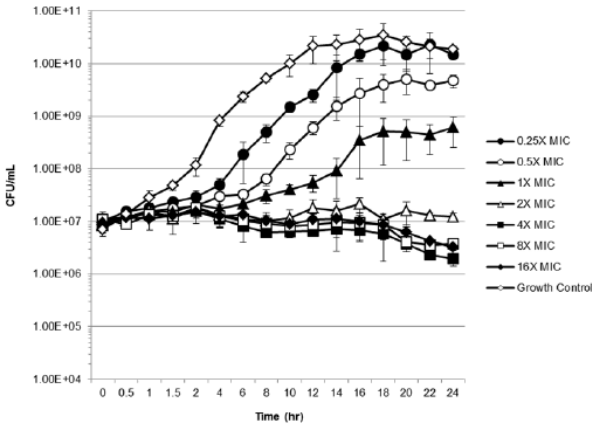
Source: Derived from PHA-08-0701-029, Tables 4-7, as described in TR701-019.0, on file with sponsor.
Abbreviations: ATCC=American Type Culture Collection; CFU=colony forming units; hr=hour; MIC=minimum inhibitory concentration; TR-700=protein synthesis inhibitor, binds to the bacterial 50S ribosomal subunit; biologically active moiety of TR-701: TZD=TR-700

Figure 11 Time-kill Kinetic Analysis of TR-700 Against *Staphylococcus aureus* CM05 (Methicillin- and Linezolid-Resistant)



Source: Derived from PHA-08-0701-029 Tables 8-11, as described in TR701-019.0, on file with sponsor.
Abbreviations: CFU=colony forming units; hr=hour; MIC=minimum inhibitory concentration; TR-700=protein synthesis inhibitor, binds to the bacterial 50S ribosomal subunit; biologically active moiety of TR-701; TZD=TR-700

Figure 12 Time-kill Kinetic Analysis of TR-700 Against *Staphylococcus aureus* NRS271 (Methicillin- and Linezolid-Resistant)



Source: Derived from PHA-08-0701-029 Tables 12-15, as described in TR701-019.0, on file with sponsor.
Abbreviations: CFU=colony forming units; hr=hour; MIC=minimum inhibitory concentration; TR-700=protein synthesis inhibitor, binds to the bacterial 50S ribosomal subunit; biologically active moiety of TR-701; TZD=TR-700

Summary and Conclusions

A common method for the estimation of the potential activity of an antibiotic is to compare the MICs of the bacteria with the achievable antibiotic concentration. Factors such as inoculum size, test medium, pharmacologic response, post antibiotic effect and pharmacokinetic properties of the antibiotic can affect the achievable antibiotic concentrations. Moreover, highly protein bound antibiotics may have minimal bactericidal activity despite good MICs or MBCs. Therefore, the measurement of bacterial activity in the presence of serum against becomes a useful predictor of antibacterial effect in vitro.

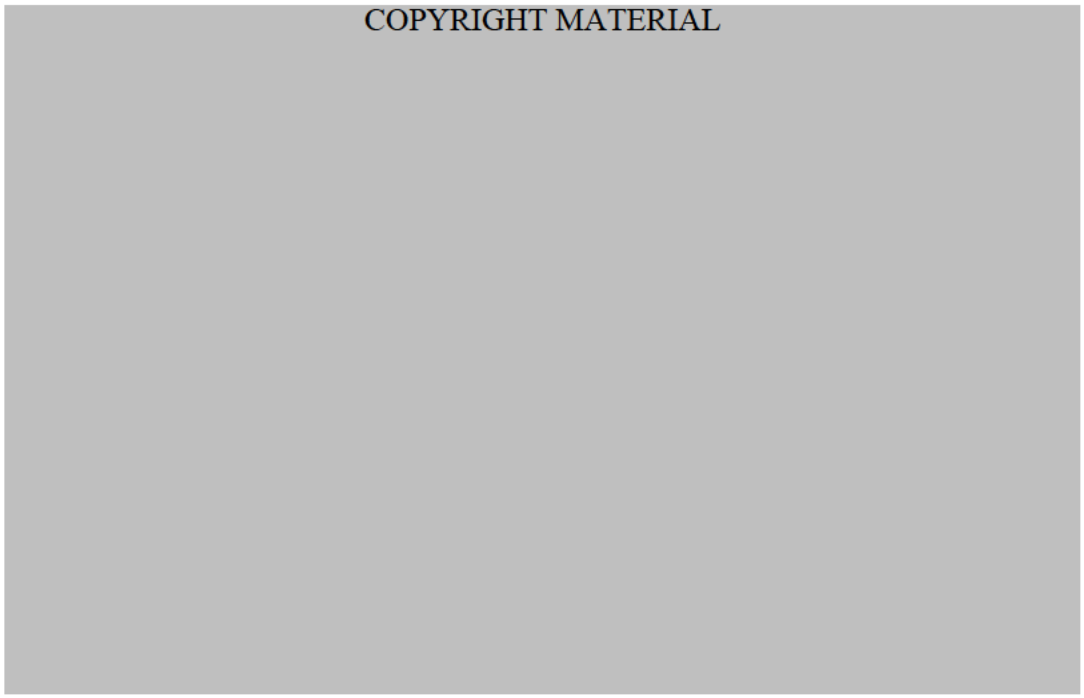
MBC analysis indicated that both TR-700 and linezolid were bactericidal against a subset of *S. aureus* and the majority of *S. pneumoniae* isolates examined. Bacteriostatic activity was observed against the majority of *S. aureus* isolates, enterococci, and other streptococci including *S. pyogenes* and *S. agalactiae*. In contrast, by time-kill kinetic analysis, little bactericidal activity was observed for either TR-700 or linezolid against evaluated staphylococci, despite the proportion of isolates with MBC values indicating bactericidal activity. Furthermore, against the two isolates of *S. pneumoniae* evaluated by time-kill kinetic analysis, a ≥3-$\log_{10}$ kill indicative of bactericidal activity was not achieved with either TR-700 or linezolid, despite MBC values ≤4 observed with 75.7% and 72.8% of evaluated strains for these agents, respectively. Time-kill kinetic analysis of enterococci confirmed the bacteriostatic activity of both TR-700 and linezolid against these species suggested by MBC analysis.

Intracellular Antibacterial Concentration Assessment

The intracellular accumulation of TR-700 within human macrophages (THP-1) and human endothelial cells and the activity of intracellular TR-700 against phagocytized *S. aureus*, *L. monocytogenes*, and *L. pneumophila* were investigated. The data showed that similar to linezolid, TR-700 could penetrate phagocytic cells, in this case, human macrophages. However, TR-700 exhibited enhanced cellular accumulation that was both pH and

temperature dependent (Figure 13). TR-700 exhibited an intracellular to extracellular ratio of 10 to 14 while a ratio of 1 to 2 was observed for linezolid.

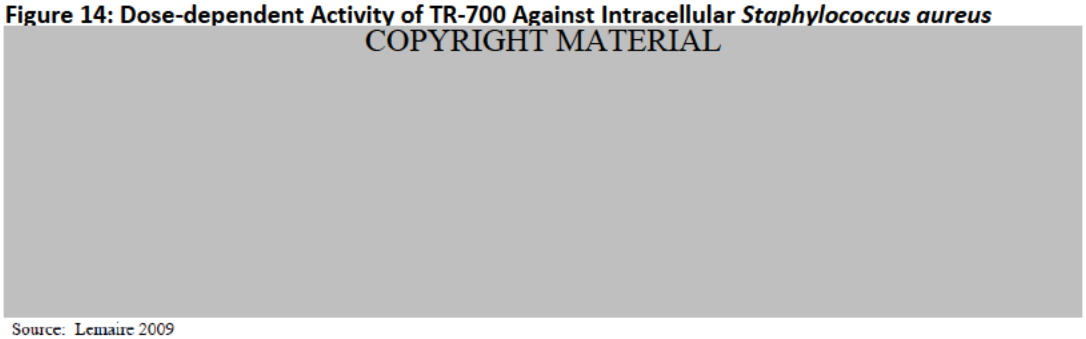
Figure 13: Intracellular Accumulation of TR-700 and Linezolid Within THP-1 Human Macrophages



Source: Lemaire 2009 (A: accumulation over time, B: accumulation after 0.5 hours at varied pH, C: accumulation after 2 hours at varied temperature)
Abbreviations: Cc/Ce=apparent cellular to extracellular concentration ratio; LNZ=Linezolid; THP-1=human macrophages

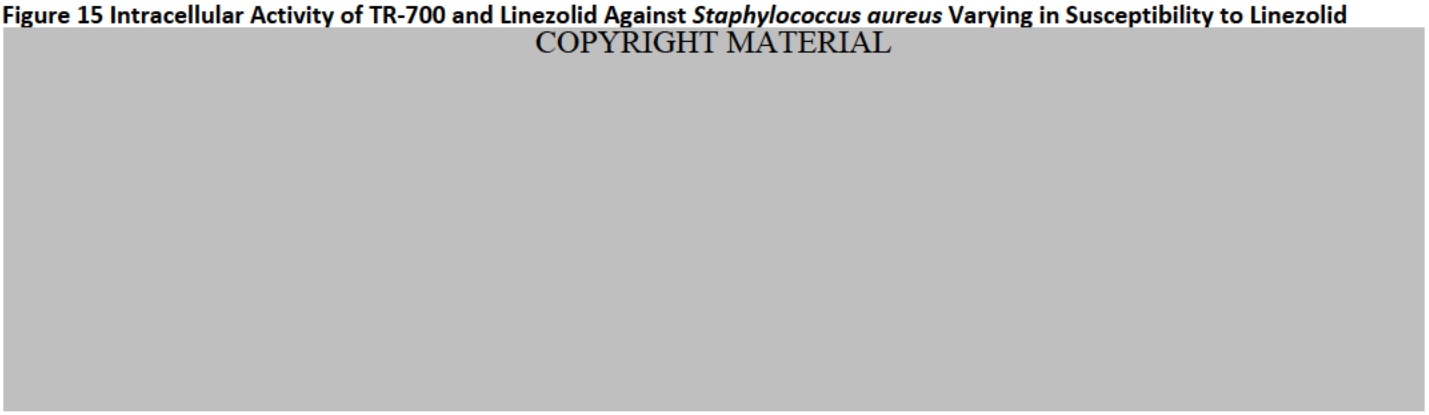
The Applicant conducted additional studies to show that intracellular TR-700 exhibited dose-dependent activity against phagocytized *S. aureus* (Figure 14), including linezolid-resistant isolates (Figure 15).

Figure 14: Dose-dependent Activity of TR-700 Against Intracellular *Staphylococcus aureus*



Source: Lemaire 2009
Abbreviations: CFU=colony-forming unit; HUVEC=human umbilical vein endothelial cells; LNZ=Linezolid; THP-1=human macrophages
Note: the panel designated broth represents the activity observed with extracellular drug; strain *S. aureus* ATCC 25923 was evaluated in this experiment; results represent means ± SD of 3 independent experiments

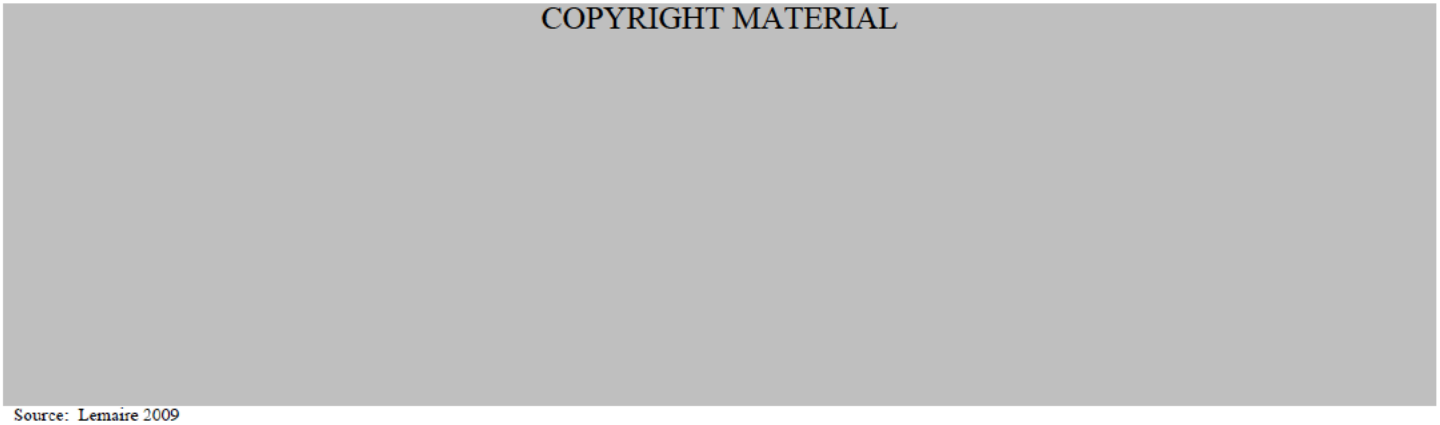
Figure 15 Intracellular Activity of TR-700 and Linezolid Against *Staphylococcus aureus* Varying in Susceptibility to Linezolid



Source: Lemaire 2009
Abbreviations: ATCC=American Type Culture Collection; CFU=colony-forming unit; NRS=Isolate from Network on Antimicrobial Resistance in *S. aureus* (NARSA)
Note: ATCC 25923, NRS 384, and SA 238 are linezolid-susceptible isolates of *S. aureus*, CM05 and SA 238 L are linezolid-resistant isolates of *S. aureus*, results represent means ± SD of 3 independent experiments

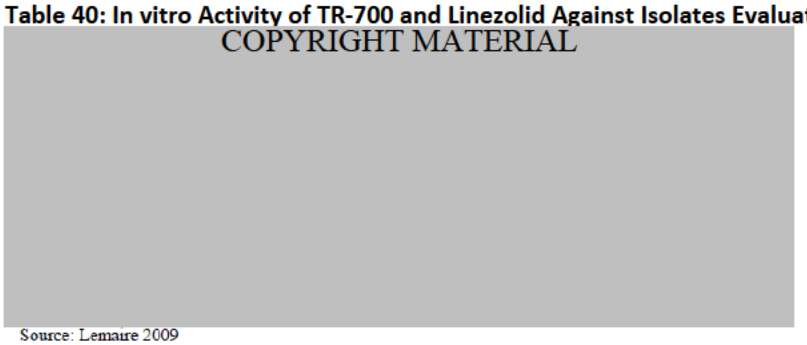
Moreover, dose-dependent activity was also demonstrated with TR-700 against intracellular pathogens *L. monocytogenes* and *L. pneumophila* (Figure 16). This activity was approximately 1-$\log_{10}$ decrease over time at concentrations approaching the extracellular MIC as determined by broth microdilution (Table 40). Such activity reflects the bacteriostatic mode of action described for the compounds belonging to the oxazolidinone class of antibacterial agents.

Figure 16: Dose-dependent Activity of TR-700 and Linezolid Against *L. monocytogenes* and *L. pneumophila*



Source: Lemaire 2009
Abbreviations: CFU=colony-forming unit
Note: Intracellular (THP-1) *L. monocytogenes* strain EGC and *L. pneumophila* strain ATCC 33153 were evaluated; results represent means ± SD of 3 independent experiments

Table 40: In vitro Activity of TR-700 and Linezolid Against Isolates Evaluated Intracellularly



Source: Lemaire 2009
Abbreviations: ATCC=American Type Culture Collection; MIC=minimum inhibitory concentration; NRS=Isolate from Network on Antimicrobial Resistance in *S. aureus* (NARSA)
[a]Strain from Trius Therapeutics (San Diego, CA).
[b]Strain from Peter Appelbaum (Kosowska-Shick 2006).
[c]USA300 PFGE-type community-acquired MRSA.
[d]Strain from P. Berche (Hopital Necker, Paris, France).

Conclusions

The data submitted by the Applicant demonstrate that TR-700 accumulated within human THP-1 macrophages and HUVEC cells and exhibited concentration-dependent bacteriostatic activity against the evaluated gram-positive organisms, including *S. aureus* strains that were resistant to linezolid. The accumulation of TR-700, at an intracellular to extracellular concentration ratio of 10 to 14, exceeded the 1 to 2 ratio for linezolid.

MECHANISMS OF RESISTANCE

Resistance to oxazolidinone may be mediated through mechanisms that include mutations in genes encoding the 23S rRNA, the ribosomal proteins L3 and L4, and/or the acquisition of the *cfr* methyltransferase gene. In the studies detailed below, TR-700 resistance was evaluated in vitro using both spontaneous mutation frequency and serial passage methods with staphylococci, enterococci, and streptococci.

Spontaneous Mutation Frequency (S. aureus)

The frequency of spontaneous mutations that confer reduced susceptibility to TR-700 and linezolid was evaluated in 6 strains of *S. aureus* including 3 MRSA isolates. Results from this study are summarized in Table 41 below. Among *S. aureus*, no TR-700- resistant mutants were observed when selection was performed at 2X the MIC value, resulting in mutation frequencies $<10^{-11}$. In contrast, when selection was performed using linezolid at 2X the MIC value, mutants occurred at frequencies between 10^{-9} to 10^{-10} in 5 of the 6 evaluated *S. aureus* isolates. In a second study, the mutation frequency of TR-700 and linezolid for *S. aureus* ATCC 29213 (MSSA) and ATCC 33591 (MRSA) was determined at 2X the MIC value for 12 replicates. TR-700 mutants occurred at a median frequency of 1.1×10^{-10} for ATCC 29213 and 1.9×10^{-10} for ATCC 33591, and linezolid mutants occurred at the median frequencies of 2.0×10^{-9} and 3.0×10^{-9} for the same strains, respectively (**Table 41**). The genetic basis of the elevated TR-700 and linezolid MIC values consisted of mutations in either the 23S rRNA genes or the *rplC* gene resulting in a mutant L3 protein. In a third study (PHA-13-0701-074), spontaneous mutation frequencies of TR-700 were determined against *S. aureus* ATCC 29213 and a USA300 PFGE-type

MRSA strain. No mutants were observed with TR-700 at 4X, 8X, and 16X the MIC resulting in mutation frequencies of $<4.5\times10^{-10}$ (**Table 41**). Taken together, data from these studies illustrate the low potential for spontaneous resistance to develop among *S. aureus* to TR-700, and further show that the frequency of spontaneous TR-700 resistance is lower than that observed with linezolid.

Table 41: Spontaneous Mutation Frequency of TR-700 and Linezolid in *Staphylococcus aureus* (MSSA/MRSA)

Isolate	Antibiotic Selection	Inoculum (CFU)	Mutation Frequency		Source
			TR-700	Linezolid	
Staphylococcus aureus ATCC 29213 (MSSA)	2X MIC	~ 1×10^{10}	1.1×10^{-10a}	2.0×10^{-9a}	Locke 2009
	4X MIC	2.2×10^{9}	$<4.5\times10^{-10b}$	ND	PHA-13-0701-074 Table 2
	8X MIC	2.2×10^{9}	$<4.5\times10^{-10b}$	ND	
	16X MIC	2.2×10^{9}	$<4.5\times10^{-10b}$	ND	
S. aureus DR3 (MSSA)	2X MIC	6.0×10^{10}	$<1.7\times10^{-11}$	3.3×10^{-10}	PHA-07-0701-049 Table 1
S. aureus DR5 (MSSA)		4.2×10^{10}	$<2.4\times10^{-11}$	5.7×10^{-9}	
S. aureus DR10 (MSSA)		2.7×10^{10}	$<3.7\times10^{-11}$	8.2×10^{-9}	
S. aureus ATCC 33591 (MRSA)	2X MIC	~ 1×10^{10}	1.9×10^{-10a}	3.0×10^{-9a}	Locke 2009
S. aureus DR1 (MRSA)	2X MIC	3.0×10^{10}	$<3.3\times10^{-11}$	3.7×10^{-10}	PHA-07-0701-049 **Table 1**
S. aureus DR3 (MRSA)		4.0×10^{10}	$<2.5\times10^{-11}$	$<2.5\times10^{-11}$	
S. aureus DR5 (MRSA)		3.0×10^{10}	$<3.3\times10^{-11}$	2.3×10^{-8}	
S. aureus USA300-0114 (MRSA)	4X MIC	2.2×10^{9}	$<4.5\times10^{-10b}$	ND	PHA-13-0701-074 **Table 2**
	8X MIC	2.2×10^{9}	$<4.5\times10^{-10b}$	ND	
	16X MIC	2.2×10^{9}	$<4.5\times10^{-10b}$	ND	

Abbreviations: CFU=colony-forming units; MIC=minimum inhibitory concentration; ND=not determined; MSSA=methicillin-susceptible *S. aureus*; MRSA=methicillin-resistant *S. aureus*
[a]Results shown represent the median frequency observed for 12 replicates.
[b]No growth; mutation frequency calculated as <1/inoculum.

Spontaneous Mutation Frequency (Enterococci)

In another study, the development of spontaneous resistance was evaluated in 5 *E. faecalis* and 5 *E. faecium* strains, including vancomycin-resistant isolates. Among the evaluated enterococci, no TR-700-resistant mutants were observed at 2X the MIC value resulting in mutation frequencies $<10^{-10}$. In contrast, at 2X the MIC, linezolid mutants occurred at frequencies between 10^{-8} to 10^{-9} for 3 of the 5 evaluated *E. faecium* isolates and 10^{-9} for 4 of the 5 evaluated *E. faecalis* isolates. Since the genetic basis of these mutants was not determined, and an increase in MIC was not confirmed by broth microdilution, it is difficult to draw conclusions about mutations arising under these conditions. In a second study, no resistance was observed for TR-700 at 4X, 8X, and 16X the MIC against *E. faecalis* ATCC 29212 resulting in a mutation frequency of $<9.1\times10^{-10}$ (mutation frequency calculated as <1/inoculum). In a third study (PHA-13-0701-076), spontaneous mutation frequencies in the presence of TR-700 were determined for *E. faecalis* ATCC 29212 (vancomycin-susceptible) and *E. faecalis* ATCC 700802 (V583; vancomycin-resistant). No spontaneous mutants were observed with either TR-700 or linezolid at 4X, 8X, and 16X the MIC resulting in mutation frequencies of $<10^{-11}$. Similar to *S. aureus*, enterococci exhibited a low potential for spontaneous resistance in the presence of TR-700 (Table 42). In the single study where the spontaneous mutation frequency was evaluated at 2X the MIC, mutants were readily observed with linezolid for several enterococcal isolates at a frequency of 10^{-9} but were not observed with TR-700 ($<10^{-10}$).

Table 42: Spontaneous Mutation Frequency of TR-700 and Linezolid in Enterococci (VSE/VRE)

Isolate	Antibiotic Selection	Inoculum (CFU)	Mutation Frequency		Source
			TR-700	Linezolid	
Enterococcus faecalis ATCC 29212 (VSE)	4X MIC	1.1×10^{9}	$<9.1\times10^{-10}$[a]	ND	PHA-13-0701-074 Table 2
	8X MIC	1.1×10^{9}	$<9.1\times10^{-10}$[a]	ND	
	16X MIC	1.1×10^{9}	$<9.1\times10^{-10}$[a]	ND	
	4X MIC	1.8×10^{10}	$<5.7\times10^{-11}$	$<5.7\times10^{-11}$	PHA-13-0701-076 Table 2
	8X MIC	1.8×10^{10}	$<5.7\times10^{-11}$	$<5.7\times10^{-11}$	
	16X MIC	1.8×10^{10}	$<5.7\times10^{-11}$	$<5.7\times10^{-11}$	
E. faecalis DR1 (VSE)	2X MIC	8.4×10^{9}	$<1.2\times10^{-10}$	3.1×10^{-9}	PHA-07-0701-049 Table 2
E. faecalis DR3 (VSE)		4.0×10^{9}	$<2.5\times10^{-10}$	3.8×10^{-9}	
E. faecalis DR5 (VSE)		1.7×10^{10}	$<5.9\times10^{-11}$	1.2×10^{-9}	
E. faecalis ATCC 700802 (VRE)	4X MIC	1.7×10^{10}	$<6.1\times10^{-11}$	$<6.1\times10^{-11}$	PHA-13-0701-076 **Table 2**
	8X MIC	1.7×10^{10}	$<6.1\times10^{-11}$	$<6.1\times10^{-11}$	
	16X MIC	1.7×10^{10}	$<6.1\times10^{-11}$	$<6.1\times10^{-11}$	
E. faecalis DR3 (VRE)	2X MIC	1.5×10^{10}	$<6.7\times10^{-11}$	$<6.7\times10^{-11}$	PHA-07-0701-049 **Table 2**
E. faecalis DR5 (VRE)		1.9×10^{10}	$<5.1\times10^{-11}$	5.0×10^{-9}	
Enterococcus faecium DR1 (VSE)	2X MIC	5.0×10^{9}	$<2.0\times10^{-10}$	9.8×10^{-9}	PHA-07-0701-049 **Table 2**
E. faecium DR7 (VSE)		1.0×10^{10}	$<1.0\times10^{-10}$	2.4×10^{-9}	
E. faecium DR9 (VSE)		1.0×10^{10}	$<1.0\times10^{-10}$	$<1.0\times10^{-10}$	
E. faecium DR16 (VRE)	2X MIC	1.2×10^{10}	$<8.3\times10^{-11}$	$<8.3\times10^{-11}$	PHA-07-0701-049 **Table 2**
E. faecium DR17 (VRE)		2.0×10^{10}	$<5.0\times10^{-11}$	1.0×10^{-9}	

Abbreviations: CFU=colony-forming units; MIC=minimum inhibitory concentration; ND=not determined; VSE=vancomycin susceptible enterococci; VRE=vancomycin-resistant enterococci
[a]No growth; mutation frequency calculated as <1/inoculum.

In another study, the tendency for resistance to develop spontaneously to TR-700 among Group A and Group B streptococci was evaluated. In this study the spontaneous mutation frequency for TR-700 and linezolid at 4X, 8X, and 16X the MIC value was determined for 2 isolates of *S. pyogenes* and 2 isolates of *S. agalactiae*. No TR-700 or linezolid mutants were observed at the evaluated concentrations resulting in mutation frequencies of $<10^{-10}$ for both agents against the tested isolates (Table 43). As with enterococci and staphylococci, there was a low propensity for resistance to develop spontaneously to TR-700 among beta-hemolytic streptococci.

Table 43: Spontaneous Mutation Frequency of TR-700 and Linezolid in Beta-hemolytic Streptococci

Isolate	Antibiotic Selection	Inoculum (CFU)	Mutation Frequency		Source
			TR-700	Linezolid	
Streptococcus pyogenes ATCC 49399	4X MIC	9.6×10^{9}	$<1.0\times10^{-10}$	$<1.0\times10^{-10}$	PHA-13-0701-076 Table 2
	8X MIC	9.6×10^{9}	$<1.0\times10^{-10}$	$<1.0\times10^{-10}$	
	16X MIC	9.6×10^{9}	$<1.0\times10^{-10}$	$<1.0\times10^{-10}$	
S. pyogenes MMX 3929	4X MIC	1.5×10^{10}	$<6.7\times10^{-11}$	$<6.7\times10^{-11}$	
	8X MIC	1.5×10^{10}	$<6.7\times10^{-11}$	$<6.7\times10^{-11}$	
	16X MIC	1.5×10^{10}	$<6.7\times10^{-11}$	$<6.7\times10^{-11}$	
Streptococcus agalactiae ATCC 13813	4X MIC	3.2×10^{9}	$<3.1\times10^{-10}$	$<3.1\times10^{-10}$	
	8X MIC	3.2×10^{9}	$<3.1\times10^{-10}$	$<3.1\times10^{-10}$	
	16X MIC	3.2×10^{9}	$<3.1\times10^{-10}$	$<3.1\times10^{-10}$	
S. agalactiae MMX 4114	4X MIC	1.7×10^{10}	$<6.0\times10^{-11}$	$<6.0\times10^{-11}$	
	8X MIC	1.7×10^{10}	$<6.0\times10^{-11}$	$<6.0\times10^{-11}$	
	16X MIC	1.7×10^{10}	$<6.0\times10^{-11}$	$<6.0\times10^{-11}$	

Abbreviations: ATCC=American Type Culture Collection; CFU=colony-forming units; MIC=minimum inhibitory concentration

Development of Resistance by Serial Passage

In the clinical setting, resistance develops during repeated exposure to an antibacterial over a course of treatment. The capacity for resistance to develop to TR-700 among target organisms during repeated exposure was evaluated in several studies using multiple methodologies, which are summarized by pathogen below.

Serial Passage *S. aureus*

The development of resistance in a methicillin-resistant strain of *S. aureus* (MRSA DR1) was evaluated during serial passage in the presence of either TR-700 or linezolid. In this study, mutants were initially selected based on growth on agar plates containing either TR-700 or linezolid at 2X the MIC. The starting inoculum was 10^8 CFU/mL. These mutants were subsequently passaged on agar plates containing either TR-700 or linezolid at higher concentrations (4X, 8X, 16X MIC, etc.). When growth was observed during the subsequent passages, the result was reported alphanumerically using a D or L to denote selection with TR-700 or linezolid, respectively, and the corresponding multiple of the MIC used during selection. For example, D4 indicates growth after selection with TR-700 at 4X the MIC. The results, including confirmatory MIC by agar dilution of the selected mutants and determination of point mutations in 23S rRNA, are summarized in Table 44. Taken together, the results show that TR-700-resistant mutants were selected during serial passage, and that these mutants were cross-resistant to linezolid. Additionally, the 4-fold potency advantage of TR-700 versus linezolid was maintained against these strains. The data also show that TR-700 demonstrated an 8-fold activity advantage versus mutants selected on linezolid. Mutants selected during passage with linezolid had a different 23S rRNA point mutation (G2576T) than mutants selected with TR-700 (T2500A). The copy number of the 23S rRNA mutations or presence of additional mutations among ribosomal proteins L3 and L4 were not evaluated in this study.

Table 44: MIC and Point Mutation Results for Laboratory Generated Oxazolidinone-Resistant Mutants of *Staphylococcus aureus*

Strain	Treated passage	MIC (µg/ml)		Point mutation 23S rRNA
		Linezolid	TR-700	
Staphylococcus aureus DR1	Control[a]	2	0.5	--
	D1-D1	4	1	T2500A
	D1-D1-D4	16	4	T2500A
	D1-D1-D8	32	8	T2500A
	D1-D1-D4-L32	64	16	T2500A
	L4-L8	16	2	G2576T
	L4-L8-L16	16	2	G2576T
	L4-L8-L16	16	2	G2576T

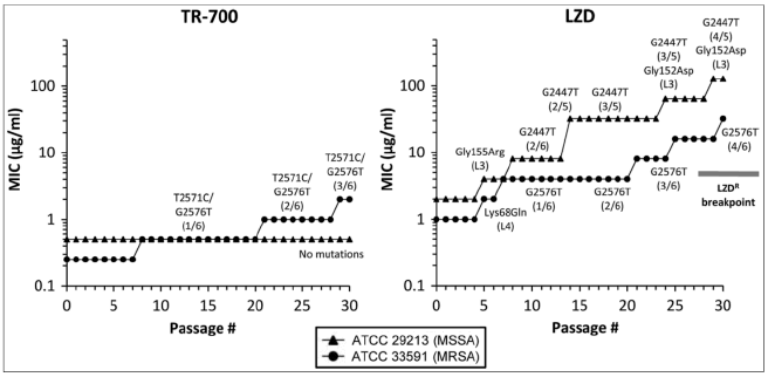
Source: PHA-07-0701-051 Table 2
Abbreviations: DA-7157=TR-700; MIC=minimum inhibitory concentration
Notes: For each treated passage, D refers to DA-7157 and L refers to Linezolid. The number following D or L indicates the µg/ml concentration that showed growth during a three-day passage.
[a]Control cultured without antibiotics.

In another experiment, the Applicant evaluated resistance development during serial passage of *S. aureus* ATCC 29213 (MSSA) and ATCC 33591 (MRSA) using antibiotic gradient agar plates containing a range of concentrations of either TR-700 or linezolid. After each passage, colonies from the leading edge of growth (the highest concentration of drug with visible growth) were cultured in liquid media and used to initiate the

subsequent passage. Isolated colonies from each passage were evaluated by PCR for mutations in target genes (23S rRNA and ribosomal protein genes *rplC*, *rplD*, and *rplV*) and underwent susceptibility testing to determine the MIC value. The results are summarized in Figure 17.

With strain ATCC 29213 (MSSA), no changes in TR-700 MIC value were apparent during 30 passages, while with linezolid the MIC value increased 64-fold from 2 to 128 mcg/mL. These linezolid-resistant isolates harbored mutations in the gene encoding ribosomal protein L3 and/or a G2447T mutation in multiple copies of 23S rRNA. For strain ATCC 33591 (MRSA), 30 passages resulted in an 8-fold increase in the TR-700 MIC value from 0.25 to 2 mcg/mL, with an accumulation of a double mutation (T2571C/G2576T) in multiple copies of 23S rRNA. In contrast, the linezolid MIC value increased 32-fold for the MRSA strain from 1 to 32 mcg/ml during passage, with mutations in the gene encoding ribosomal protein L4 and the accumulation of a G2576T mutation in multiple copies of 23S rRNA.

Figure 17 Activity of TR-700 and Linezolid Against *Staphylococcus aureus* During Serial Passage and Mutational Analysis



Source: Locke 2009 Figure 2
Abbreviations: LZD=linezolid; L3=ribosomal protein L3 encoded by *rplC*; L4=ribosomal protein L4 encoded by *rplD*; MSSA=methicillin-susceptible *S. aureus*; MRSA=methicillin-resistant *S. aureus;* LZD^R=linezolid resistant; ATCC=American Type Culture Collection
Note: 23S rRNA mutations designated by change in the gene base and position number (*E. coli* numbering) (eg, G2576T) and number of copies impacted is shown below as a ratio (ie, 1/6=mutation present in 1 of 6 23S rRNA alleles).

In another experiment, several strains of MSSA and MRSA were passaged in the presence of TR-700. Linezolid was included as a comparator for a subset of these strains. Broth microdilution MIC panels were inoculated per CLSI guidelines at 10^5 CFU/mL. The MIC value was recorded, and a fresh MIC panel was inoculated with cells from the well with the highest concentration of drug that permitted growth (ie, the highest sub-inhibitory concentration). This process was repeated 14 times, resulting in 15 passages of the bacteria. To evaluate the stability of the mutants, the cells were subsequently passaged 3 times in antibiotic-free medium followed by evaluation of the MIC value. The resulting MIC values during serial passage are shown in Table 45 below. For both TR-700 and linezolid, there was no observed increase in MIC value over 15 passages. However, the relatively small inoculum used in this experiment does not confer the sensitivity required to detect resistance at the rate of $\leq 10^{-10}$, typical for TR-700 in other studies.

Table 45: MIC Values of TR-700 and Linezolid During Serial Passage of *Staphylococcus aureus*

		MIC (µg/mL)															
Organism	Drug	Baseline	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Reversion
S. aureus ATCC 29213 (MSSA)	TR-700	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	linezolid	2	2	2	2	2	4	2	2	2	2	4	4	2	8	4	2
S. aureus 025-6337A (MSSA)	TR-700	0.25	0.25	0.25	0.25	0.25	0.5	0.5	0.25	0.25	0.25	0.5	0.5	0.25	0.5	0.5	0.25
S. aureus USA300-0114 (CA-MRSA) 707J	TR-700	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	linezolid	2	2	2	2	2	2	2	2	2	2	2	4	2	2	2	2
S. aureus USA400 (CA-MRSA) 713J	TR-700	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.25
S. aureus USA100 (HA-MRSA) 706J	TR-700	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Source: PHA-13-0701-074 Table 1
Abbreviations: CA=community-acquired; HA=hospital-acquired; MIC=minimum inhibitory concentration; MSSA=methicillin-susceptible *S. aureus*; MRSA=methicillin-resistant *S. aureus*

Serial Passage Enterococci

The investigation into the development of resistance in a vancomycin-susceptible (strain DR8) and vancomycin-resistant (strain DR9) *E. faecium* was examined using serial passage in the presence of either TR-700 or linezolid. Mutants were initially selected in this study based on growth on agar plates containing TR-700 or linezolid at 2X the MIC value. The starting inoculum was 10^8 CFU/mL. These mutants were subsequently passaged on agar plates containing TR-700 or linezolid at higher concentrations (4X, 8X, 16X MIC, etc). Where growth was observed during passage, the result was reported alphanumerically using a D or L to denote selection with TR-700 or linezolid, respectively, and the corresponding multiple of the MIC value used during selection. For example, D4 indicates growth after selection with TR-700 at 4X the MIC value.

The results of the selected mutants, including confirmatory MIC by agar dilution, and corresponding point mutation in 23S rRNA, are summarized in Table 46. The copy number of the 23S rRNA mutations was not evaluated in this study. The results for serial passage of the vancomycin-susceptible DR8 strain show that TR-700 mutants were selected which were cross-resistant to linezolid. MIC values increased up to 16-fold and up to 64-fold, for TR-700 and linezolid, respectively. Mutants were also selected during passage of DR8 with linezolid with MIC values increasing 4-fold; however, these mutants maintained low TR-700 MIC values (0.5-1 mcg/mL). For *E. faecium* (DR8), selection with TR-700 resulted in a T2504A 23S rRNA gene mutation, and selection with linezolid resulted in a G2576T mutation. For the vancomycin-resistant strain of *E. faecium* (DR9), selection with linezolid resulted in up to a 16-fold change in MIC for linezolid and up to 4-fold change in MIC for TR-700. The G2576T mutation was detected in these mutants.

Table 46: MIC and Point Mutation Results for Laboratory Generated Oxazolidinone-Resistant Mutants of *Enterococcus faecium*

Strain	Treated passage	MIC (µg/mL)		Point mutation 23S rRNA
		Linezolid	TR-700	
Enterococcus faecium DR8	Control[a]	2	0.5	--
	L2	4	0.5	G2576T
	L2	4	1	G2576T
	L2-L4-L8	8	1	G2576T
	L2-L4-L8-L16	8	0.5	G2576T
	L2-L4-L8-L16	8	1	G2576T
	D1-D1-D2-D4	32	2	T2504A
	D1-D1-D2-D4	64	8	T2504A
	D1-D1-D2-D4-D16	128	8	T2504A
	D1-D1-D2-D4-D16	128	8	T2504A
VR *E. faecium* DR9	Control[a]	2	0.5	--
	L2-L4-L8	16	2	G2576T
	L2-L4-L8	16	2	G2576T
	L2-L4-L8-L16	16	2	G2576T
	L2-L4-L16-L32	32	2	G2576T
	L2-L4-L16-L32	32	2	G2576T
	L2-L4-L16-L32	32	2	G2576T

Source: PHA-07-0701-050 Table 2
Abbreviations: D=DA 7157; DA 7157=TR-700; L=Linezolid; MIC=minimum inhibitory concentration; VR=Vancomycin resistant
Notes: For each treated passage, D refers to DA-7157 and L refers to linezolid. The number following D or L indicates the µg/ml concentration that showed growth during a three-day passage.
[a]Control cultured without antibiotics.

In separate studies, a vancomycin-susceptible and resistant *E. faecalis* was passed in the presence of TR-700 or linezolid. In these studies, broth microdilution MIC panels were inoculated per CLSI guidelines at 10^5 CFU/mL. The MIC was recorded and a fresh MIC panel was inoculated at 10^5 CFU/mL with cells from the well with the highest concentration of drug that permitted growth (sub-inhibitory concentration). This process was repeated 14 times resulting in 15 passages of the bacteria. To evaluate the stability of resistance mutations the cells were subsequently passaged 3 times in antibiotic-free medium and the MIC value was determined as shown in Table 47.

Table 47: MIC Values of TR-700 and Linezolid During Serial Passage of *Enterococcus faecalis*

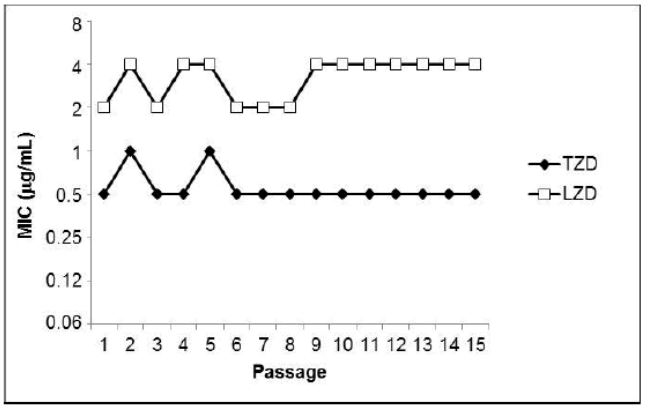
		MIC (µg/mL)															
Organism	Drug	Baseline	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Reversion
Enterococcus faecalis ATCC 29212	TR-700	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	linezolid	2	2	2	2	2	2	2	2	2	2	2	2	4	2	4	2
E. faecalis 003-4732A (VAN A)	TR-700	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Source: PHA-13-0701-074 Table 1
Abbreviations: MIC=minimum inhibitory concentration; VAN A=*vanA* vancomycin resistant genotype; ATCC=American Type Culture Collection

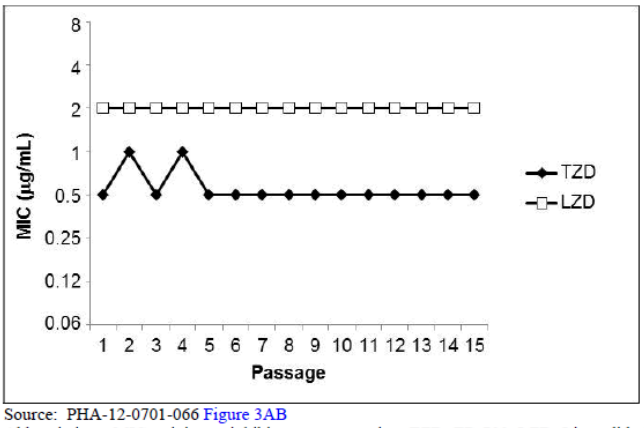
The changes in TR-700 and linezolid MIC values during passage in study PHA-12-0701-066 are shown in Figure 18. Over 15 passages, there was no observed increase in TR-700 or linezolid MIC values against the evaluated *E. faecalis* in either of the studies. However, the relatively small inoculum used in this experiment does not confer the sensitivity required to detect resistance at the rate of $\leq 10^{-10}$, typical for TR-700 in other studies.

Figure 18: Activity of TR-700 and Linezolid Against *Enterococcus faecalis* During Serial Passage

A: *Enterococcus faecalis* ATCC 29212



B: *E. faecalis* ATCC 700802 (V583; vancomycin-resistant)



Source: PHA-12-0701-066 Figure 3AB
Abbreviations: MIC=minimum inhibitory concentration; TZD=TR-700; LZD=Linezolid; ATCC=American Type Culture Collection

Serial Passage Beta-hemolytic Streptococci

In additional separate studies conducted by the Applicant (PHA-13-0701-074 and PHA-12-0701-066), Group A and Group B streptococci were serially passaged in the presence of TR-700 or linezolid. As described above, broth microdilution MIC panels were inoculated per CLSI guidelines at 10^5 CFU/mL. The MIC was recorded, and a fresh MIC panel was inoculated with cells from the well with the highest concentration of drug that permitted growth (ie, highest sub-inhibitory concentrations tested). This process was repeated 14 times, resulting in 15 passages of the bacteria. To evaluate the stability of any mutants in study PHA-13-0701-074, after the final passage in antibiotic media, the cells were passaged 3 times in antibiotic-free medium and the MIC was determined. The resulting MIC values from study PHA-13-0701-074 for the evaluated *S. pyogenes* are shown in Table 48.

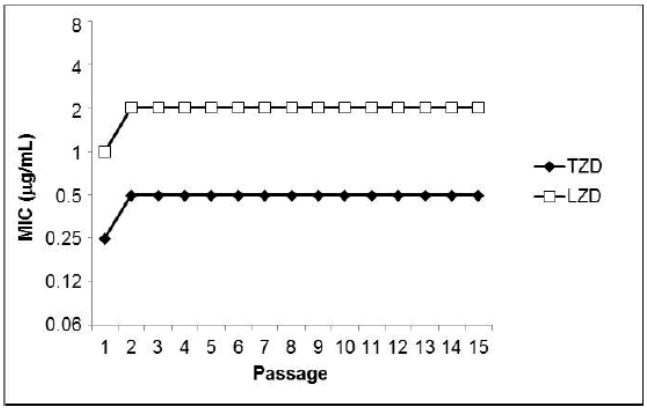
Table 48: MIC Values of TR-700 During Serial Passage of *Streptococcus pyogenes*

		MIC (µg/mL)															
Organism	**Drug**	**Baseline**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**	**Reversion**
S. pyogenes 30-2666X *ermA*, D-test pos	TR-700	0.25	0.25	0.25	0.25	0.25	0.12	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.25

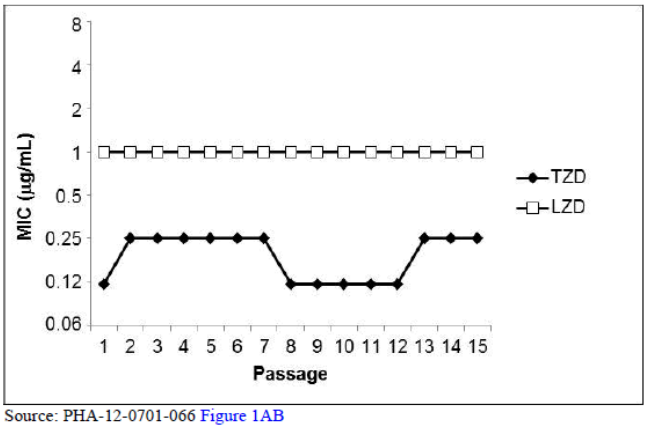
Source: PHA-13-0701-074 Table 1
Abbreviation: MIC=minimum inhibitory concentration; *S. pyogenes*=*Streptococcus pyogenes*
Note: *ermA*: *ermA* methylase gene positive; D-test positive for inducible clindamycin resistance

The change in TR-700 and linezolid MIC value during passage in study PHA-12-0701-066 against 2 strains of *S. pyogenes* and 2 strains of *S. agalactiae* are shown in Figure 19 and Figure 20, respectively. Over 15 serial passages, there were no observed increases in TR-700 or linezolid MIC values for the evaluated streptococcal isolates in either of the studies. However, the relatively small inoculum used in this experiment does not confer the sensitivity required to detect resistance at the rate of $\leq 10^{-10}$, typical for TR-700 in other studies.

Figure 19: Antibacterial Activity of TR-700 and Linezolid Against *Streptococcus pyogenes* During Serial Passage
A: *Streptococcus pyogenes* ATCC 49399



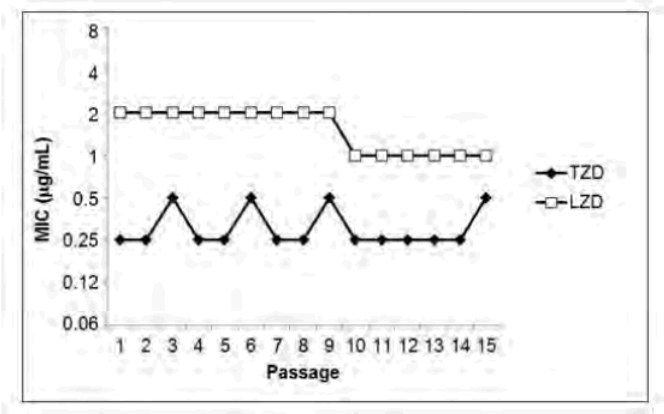
B: *S. pyogenes* MMX 3929



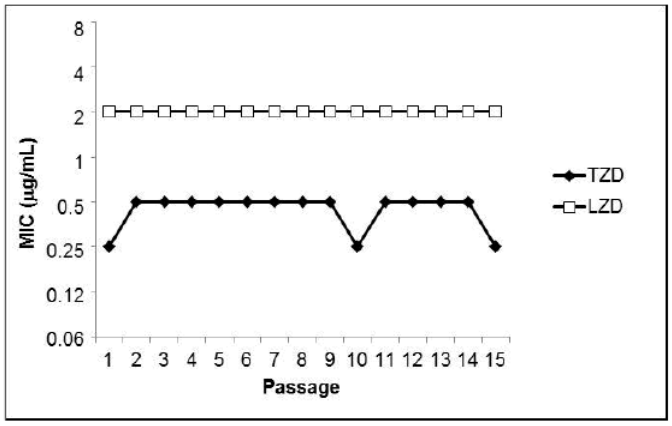
Source: PHA-12-0701-066 Figure 1AB
Abbreviations: MIC=minimum inhibitory concentration; TZD=TR-700; LZD=Linezolid; ATCC=American Type Culture Collection

Figure 20: Antibacterial Activity of TR-700 and Linezolid Against *Streptococcus agalactiae* During Serial Passage

A: *Streptococcus agalactiae* ATCC 13813



B: *S. agalactiae* MMX 4114



Source: PHA-12-0701-066 Figure 2A/B
Abbreviations: MIC=minimum inhibitory concentration; TZD=TR-700; LZD=Linezolid

Summary of Serial Passage Studies

In summary, it was noted that TR-700-resistant mutants emerged during serial passage in a subset of the studies described above in *S. aureus* and *E. faecium,* but not in streptococci. Oxazolidinone mutants with elevated MIC values were observed. It was noted that mutants were generated when *S. aureus* and *E. faecium* were used as the testing strains where the starting inocula were 10^8 CFU/mL, and in a study using gradient plate methodologies in which the starting inoculum was larger. All laboratory mutants and clinical strains with an increase in MIC to TR-700 also showed an elevated MIC to linezolid. However, the data showed that not all mutants and strains with an elevated linezolid MIC demonstrated an increase in the MIC of TR-700.

Structure-Activity Relationships (SAR) and Resistance:

In these series of experiments (with the premise that oxazolidinones shares a common mechanism of action) the Applicant hypothesized that structural differences may lead to unique antibacterial activities against wild-type isolates and isolates possessing different classes of resistance mechanisms. A review of the structure-activity relationships (SAR) among a structurally-diverse group of oxazolidinones demonstrates how unique structural features of TR-700 may result in its increased activity relative to linezolid against linezolid-resistant *S. aureus*. Five oxazolidinones were used to explore SAR trends (**Figure 21**). Compounds included linezolid, TR-700 and three TR-700 analogs: (b) (4)

Figure 21: Oxazolidinone Structures Used to Explore SAR Trends

The 5 oxazolidinones (Figure 21) were tested against isogenic *S. aureus* strains to evaluate SAR trends for *S. aureus* with ribosomal mutations (ie, 23S rRNA, or ribosomal proteins L3 or L4) (Table 49). Each of the mutations resulted in ≥2-fold increase in MIC values for all compounds tested. Potency against all wild-type and isogenic strains appear to correlate strongly (b) (4)
From most to least potent, compounds were ranked as follow: compound **2**, compounds **1**/**3**/TR-700, linezolid. Among the three (b) (4) compounds (TR-700, compounds **1** and **3**) all were equipotent against the wild-type ATCC strains, and only minor potency differences were present between the ribosomal mutants. The (b) (4) (compound **1**) was 2-fold less potent than TR-700 against the NRS127 and G2447T and G2576T mutants, while the (b) (4) (compound **3**), was 2-fold more potent against the G2447T mutant than TR-700. Linezolid had the lowest potency against all strains tested and had fold increases in MIC values greater than or equal to TR-700.

Table 49: MIC Values for *Staphylococcus aureus* Ribosomal Mutants

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Source: Locke 2010
Abbreviations: ATCC=American Type Culture Collection; MIC=minimum inhibitory concentration; LZD=linezolid; NARSA=Network on Antimicrobial Resistance in *S. aureus*; VAN=vancomycin
[a]ATCC 29213 and ATCC 33591 isogenic mutant panels were generated through selection in the presence of LZD and/or TR-700. NRS127 is a LZD^R clinical isolate.
[b]Mutations in 23S rRNA genes (as well as mutant allele copy number) or ribosomal proteins L3 or L4 are shown.
[c]MIC values (broth microdilution, CLSI) were performed against the oxazolidinone panel (Figure 37) and vancomycin (VAN).

Population analysis and Global spread for cfr+ S. aureus

It is noted that Cfr methyltransferase confers resistance to many 50S ribosomal subunit targeted antibacterial compounds, via methylation of a specific region of the 23S rRNA petidyl transferase center. The Applicant used a panel of *cfr+ S. aureus* strains, including isogenic wild-type laboratory (RN4220 and 29213) and clinical (CM05 and 42262) strains, to investigate oxazolidinone SAR trends in the presence of the Cfr methytransferase (Table 50). Contrary to activity versus the ribosomal mutants (and isogenic parent strains thereof), linezolid and Compound 2, (b) (4) lost potency (2- to 8-fold) in the presence of Cfr methylation. Each of the three (b) (4) compounds however, retained potency in the presence of Cfr methylation. Linezolid, which has an acetamide C-5 group and lacks a D-ring, had the greatest loss of potency against the *cfr+* strains. TR-700 maintained a 16- to 32-fold potency advantage over linezolid for all *cfr* strains tested. Reports of environmental, veterinary, and clinical isolates of *Staphylococcus, Streptococcus,* and *Enterococcus* species with the *cfr* gene are being reported with increasing frequency from multiple sites in North America, South America, Europe, and Asia, as shown in **Figure 22.**

Figure 22: Global Spread of the *cfr* Gene

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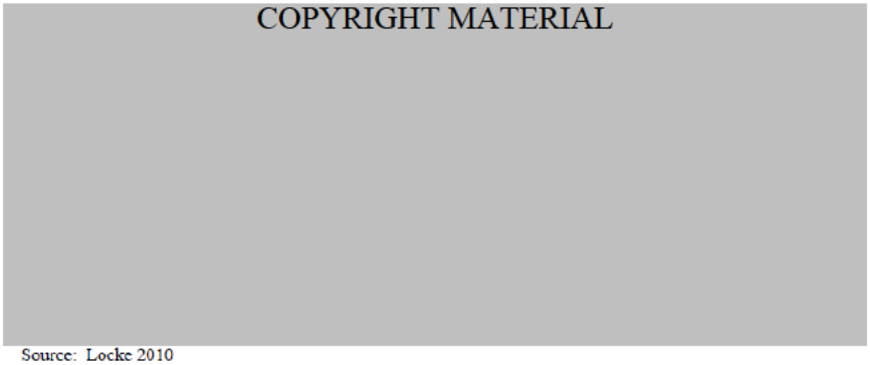
Source: Publications listed in Appendix 13
Note: Each dot represents a strain or group of strains from published work. The year(s) and city/country of isolation are indicated adjacent to the dot. The source of isolation for clinical (brown), veterinary (dark blue) and environmental (green) is shown.

Table 50: MIC Values for *cfr+ Staphylococcus aureus*



Source: Locke 2010
Abbreviations: *cfr*=chloramphenicol-florfenicol resistance gene; MIC=minimum inhibitory concentration; LZD=linezolid; CMPD=Compound; VAN=vancomycin
[a]MIC values (broth microdilution, CLSI) were performed against the oxazolidinone panel (Figure 37) and vancomycin (VAN).
[b]The pLXM1 *cfr*-containing plasmid is isogenic to empty pLI50 vector.
[c]CM05Δ is isogenic to the CM05 clinical *cfr+* strain, but lacks *cfr* and one copy of *ermB*.
[d]29213 + p42262 was generated through transformation of ATCC 29213 with the p42262 *cfr*-containing plasmid isolated from strain 42262.
[e]42262 is a clinical *cfr* isolate from a 2008 hospital outbreak in Madrid, Spain.

Summary:

The SAR trends observed here appear largely conserved for isolates possessing mutations in 23S rRNA and ribosomal proteins L3 and L4. It was noted that this general class of chromosomally-encoded mutations led to some level of reduced susceptibility to all oxazolidinones and the increases in MIC values for linezolid are greater than or equal to those observed for TR-700 in these strains. TR-700 demonstrated ≥4-fold more effective than linezolid against wild-type and linezolid-resistant *S. aureus* with ribosomal mutations. It is hypothesized that much of the antibacterial activity of TR-700 is due to the methyltetrazole D-ring and pyrid-3-yl C-ring, structural features lacking in linezolid.

The SAR data for *cfr+* strains also revealed a trend towards loss of activity for oxazolidinones possessing the C-5 acetamide group. The Applicant stated that TR-700 analog (b) (4) (compound **2**) experienced a 2- to 4-fold increase in MIC against *cfr+* strains. Overall, these SAR data reveal that different structural features of the oxazolidinones may contribute to the activity against linezolid-susceptible and linezolid-resistant *S. aureus*. Activity against all wild-type and resistant strains is aided by the presence and optimization of D-ring substituents. For TR-700, the presence of a highly optimized methyltetrazole D-ring and a hydroxymethyl A-ring C-5 substituent appear to offer a configuration that maintains antibacterial activity advantages over linezolid for isolates of *S. aureus* tested.

Susceptibility Test Methods and Detection of Resistant Organisms

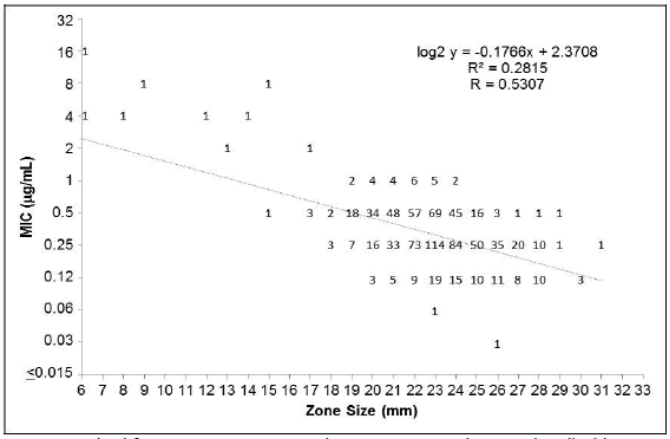
The Applicant evaluated the activity of TR-700 by a variety of CLSI antibacterial susceptibility test methods including broth microdilution, disk diffusion and agar dilution.

Disk Content Studies

A study was conducted to determine the appropriate disk mass for use in disk diffusion testing of TR-700 against target pathogens (MCR-08-0701-016). Given the increased in vitro activity of TR-700 relative to linezolid (tested with a disk mass of 30 mcg), The Applicant evaluated TR-700 disk masses of 2, 5, 10, and 20 mcg that were made in-house at a reference laboratory (b) (4) In this study, 338 *Staphylococcus* spp. (234 *S. aureus*, 104 coagulase-negative staphylococci), 202 *Enterococcus* spp. (99 *E. faecalis*, 103 *E. faecium*), 133 *S. pneumoniae*, and 200 beta-hemolytic streptococci were tested for susceptibility to TR-700 and linezolid using concurrent inocula by broth microdilution and disk diffusion test methods in accordance with CLSI testing guidelines (CLSI M2 2006 and CLSI M7 2006). Disk diffusion zone diameters for TR-700 versus staphylococci were read using reflected and transmitted light. Zone diameters for all other genera were read using reflected light as specified by CLSI methodology. The disk diffusion data presented below for testing TR-700 and staphylococci summarizes results obtained by reading with transmitted light only. Using transmitted light during reading is specified by CLSI for testing in-class comparator linezolid against staphylococci, and the quality control ranges developed for disk diffusion testing of TR-700 against staphylococci are based on reading with transmitted light. Ultimately, the 20 mcg TR-700 disk mass was selected for the manufacture of commercially prepared disks to be used for all subsequent disk diffusion testing of TR-700 during clinical development.

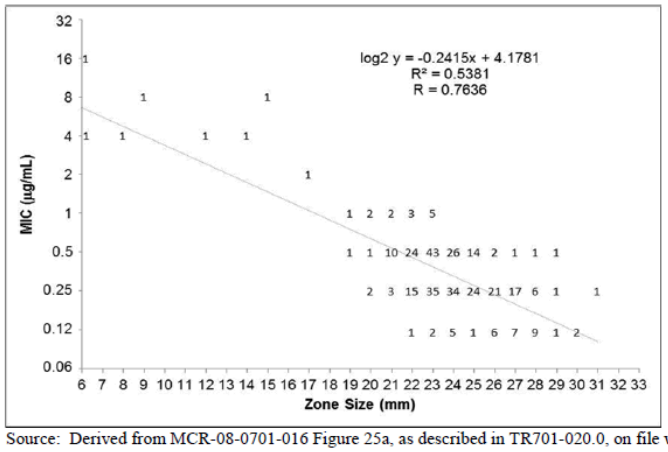
Summary of disk diffusion data in this section is limited to the 20 mcg mass; data on the other evaluated disk masses is located in Study Report MCR-08-0701-016. The 20 mcg disk mass was selected because of the observed correlation between broth MIC values and disk zones. Additionally, isolates with elevated TR-700 MIC values exhibited disk zones of <15 mm, which were easily distinguished from isolates with lower MIC values where disk zones were typically >19 mm. Figure 23, Figure 24, Figure 25, Figure 26, and Figure 27 illustrate the correlation between broth microdilution MIC values and disk zone diameters for the TR-700 20 mcg disk overall and by pathogen.

Figure 23: Overall Broth MIC Value Versus Disk Zone Diameter Correlation for TR-700 (20 mcg) Against Evaluated Target Pathogens (n = 873)



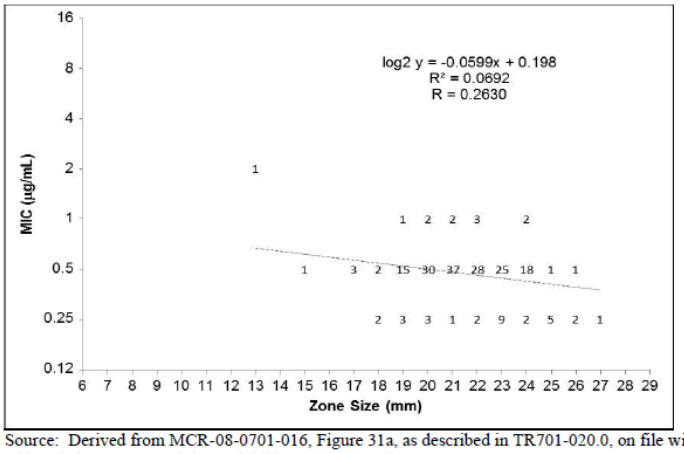
Source: Derived from MCR-08-0701-016 Figures 25a, 31a, and 36a, as described in TR701-020.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration

Figure 24: Broth MIC Value Versus Disk Zone Diameter Correlation for TR-700 (20 mcg) Against *Staphylococcus* spp. (n = 338)



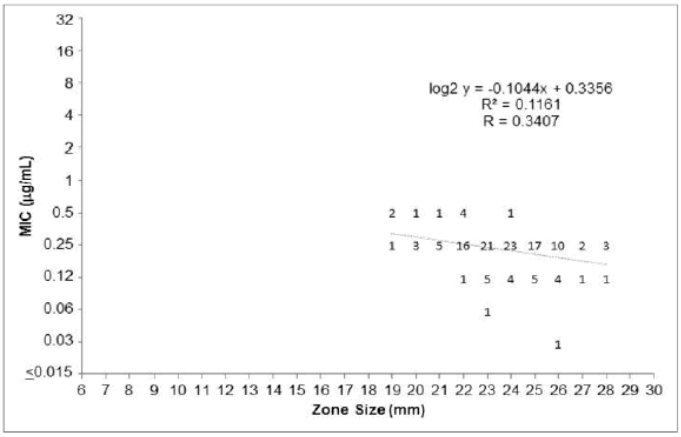
Source: Derived from MCR-08-0701-016 Figure 25a, as described in TR701-020.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration

Figure 25: Broth MIC Value Versus Disk Zone Diameter Correlation for TR-700 (20 mcg) Against *Enterococcus* spp. (n = 202)



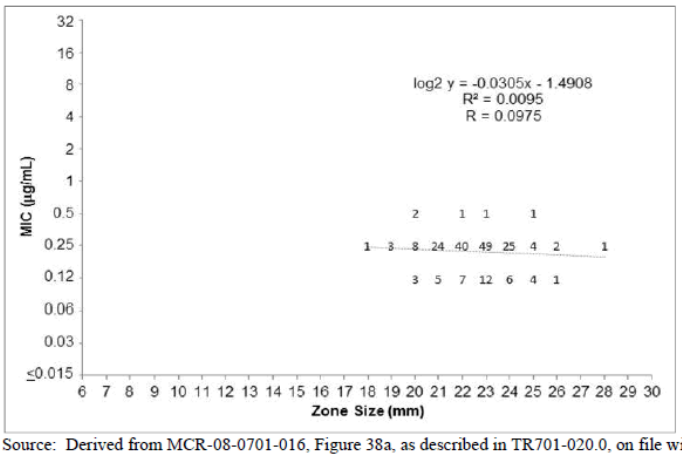
Source: Derived from MCR-08-0701-016, Figure 31a, as described in TR701-020.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration

Figure 26: Broth MIC Value Versus Disk Zone Diameter Correlation for TR-700 (20 mcg) Against *Streptococcus pneumoniae* (n = 133)



Source: Derived from MCR-08-0701-016 Figure 37a, as described in TR701-020.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration

Figure 27: Broth MIC Value Versus Disk Zone Diameter Correlation for TR-700 (20 mcg) Against Beta-Hemolytic Streptococci (n = 200



Source: Derived from MCR-08-0701-016, Figure 38a, as described in TR701-020.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration

COMPARISON OF AGAR AND BROTH DILUTION METHODS:

Broth Microdilution MIC versus Agar Dilution MIC

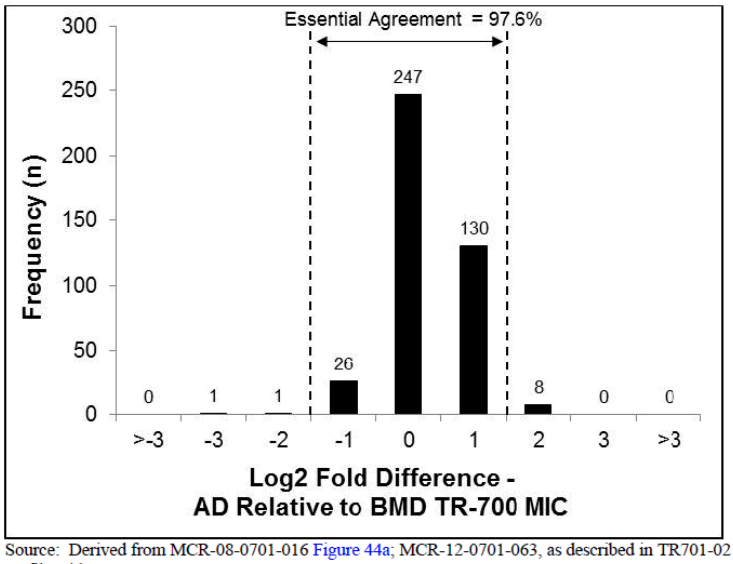
The antibacterial breakpoint may be defined as the drug concentration that differentiates between dissimilar populations of microorganisms, and isolates are subsequently classified as susceptible, intermediate or resistant. It is also important to note that susceptibility breakpoints may differ by region. Methods using zone diameters to classify bacteria as susceptible or resistant to antibiotics depend on clinically meaningful MICs, a representative sample of bacteria, adequate and reproducible methods for determining MICs and zone diameters and a method for the relation of zone diameters to MICs[29].

The Applicant compared the broth microdilution MIC and agar dilution MIC values in two separate studies (MCR-08-0701-016, MCR-12-0701-063). In study MCR-08-0701-016, this evaluation included testing inocula of 104 *S. aureus*, 105 enterococci, and 106 *S. pneumoniae* simultaneously by broth microdilution and agar dilution in accordance with CLSI guidelines (CLSI M7 2006). Broth microdilution was conducted with frozen 96-well MIC trays prepared at a reference laboratory (b) (4) The same analysis was conducted in study MCR-12-0701-063 with 98 beta-hemolytic streptococci (50 *S. pyogenes*, 40 *S. agalactiae*, and 8 Group C/F/G streptococci).

Results for the comparison of TR-700 broth microdilution MIC values with agar dilution MIC values are shown in Figure 27 and Table 49. As shown, 97.6% of TR-700 agar dilution MIC values were at or within 1 doubling-dilution of broth microdilution MIC values; 59.8% were identical. By organism, the Applicant reported that essential agreement was observed for TR-700 between agar and broth microdilution methods for 100% of *S. aureus*, 99.0% of enterococci, 98.1% of *S. pneumoniae*, and 92.8% of beta-hemolytic streptococci evaluated in this study. Of note, 44% of the evaluated *S. pneumoniae* and 70.4% of beta-hemolytic streptococci had agar

dilution MIC values that were 2-fold higher than broth microdilution MIC values for TR-700.

Figure 27: TR-700 Broth Microdilution vs. Agar Dilution MIC Values[a]



Source: Derived from MCR-08-0701-016 Figure 44a; MCR-12-0701-063, as described in TR701-021.0, on file with sponsor.
Abbreviations: AD=agar dilution; BMD=broth microdilution; MIC=minimum inhibitory concentration
[a]N=413; *S. aureus* (n=104), enterococci (n=105), *S. pneumoniae* (n=106), and beta-hemolytic streptococci (n=98).

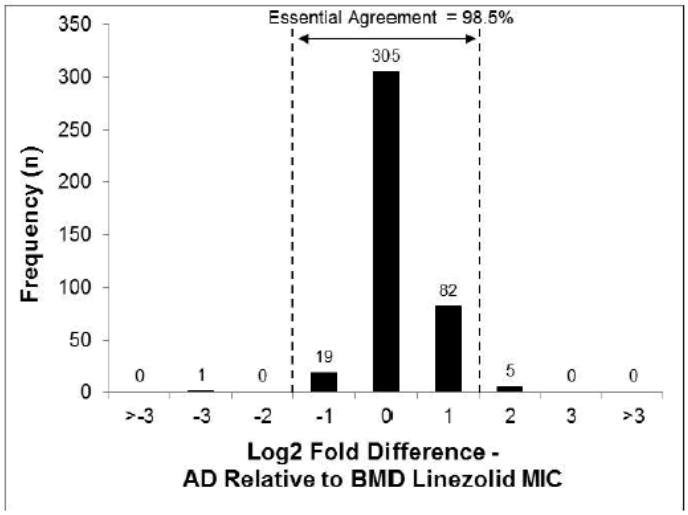
Table 49: Comparison of TR- 700 Broth Microdilution and Agar Dilution MIC Values Overall and by Organism

		Log$_2$ Difference of TR-700 AD MIC relative to BMD MIC (number of isolates [%])						
Organism	**N**	**≥-3**	**-2**	**-1**	**0**	**1**	**2**	**≥3**
Staphylococcus aureus	104	-	-	9 (8.7)	93 (89.4)	2 (1.9)	-	-
Enterococci[a]	105	-	1 (1.0)	16 (15.2)	76 (72.4)	12 (11.4)	-	-
Streptococcus pneumoniae	106	-	-	1 (0.9)	56 (52.8)	47 (44.3)	2 (1.9)	-
Streptococcus spp. (beta-hemolytic)[b]	98	1 (1.0)	-	-	22 (22.4)	69 (70.4)	6 (6.1)	-
OVERALL:	413	1 (0.2)	1 (0.2)	26 (6.3)	247 (59.8)	130 (31.5)	8 (1.9)	-

Source: Derived from MCR-08-0701-016; MCR-12-0701-063, as described in TR701-021.0, on file with sponsor.
Abbreviations: AD=agar dilution; BMD=broth microdilution; MIC=minimum inhibitory concentration
Note: Dashes represent no value
[a]Isolates include 53 *Enterococcus faecalis* and 52 *Enterococcus faecium*.
[b]Isolates include 50 *Streptococcus pyogenes*, 40 *Streptococcus agalactiae*, and 10 Group C/F/G streptococci.

The results observed with TR-700 were similar to those of linezolid (Figure 50, Table 28) where 98.5% essential agreement was observed against the same test isolates, and 74.0% of isolates had identical MIC values by agar dilution and broth microdilution.

Figure 28: Linezolid Broth Microdilution vs. Agar Dilution MIC Values



Source: Derived from MCR-08-0701-016 Figure 45a; MCR-12-0701-063, as described in TR701-021.0, on file with sponsor.
Abbreviations: AD=agar dilution; BMD=broth microdilution; MIC=minimum inhibitory concentration
[a]N=412; *S. aureus* (n=104), enterococci (n=104), *S. pneumoniae* (n=106), and beta-hemolytic streptococci (n=98).
[b]One isolate of *E. faecalis* had an undefined linezolid MIC value by BMD (>8 µg/mL) and an AD MIC value of 8 µg/mL; this isolate was removed from the linezolid analysis as the difference between AD and BMD MIC values could not be interpreted.

Table 50: Comparison of Linezolid Broth Microdilution and Agar Dilution MIC Values Overall and by Organism

Organism	N	Log$_2$ Difference of Linezolid AD MIC relative to BMD MIC (number of isolates [%])						
		≥-3	-2	-1	0	1	2	≥3
Staphylococcus aureus	104	-	-	2 (1.9)	101 (97.1)	1 (1.0)	-	-
Enterococci[a]	104[b]	-	-	11 (10.6)	92 (88.5)	1 (1.0)	-	-
Streptococcus pneumoniae	106	-	-	6 (5.7)	85 (80.2)	15 (14.2)	-	-
Streptococcus spp. (beta-hemolytic)[c]	98	1 (1.0)	-	-	27 (27.6)	65 (66.3)	5 (5.1)	-
OVERALL:	412	1 (0.2)	-	19 (4.6)	305 (74.0)	82 (19.9)	5 (1.2)	-

Source: MCR-08-0701-016; MCR-12-0701-063, as described in TR701-021.0, on file with sponsor.
Abbreviations: AD=agar dilution; BMD=broth microdilution; MIC=minimum inhibitory concentration
Note: Dashes represent no value
[a]Isolates include 53 *E. faecalis* and 52 *E. faecium*.
[b]One isolate of *E. faecalis* had an undefined linezolid MIC value by BMD (>8 µg/mL) and an AD MIC value of 8 µg/mL; this isolate was removed from the linezolid analysis as the difference between AD and BMD MIC could not be interpreted.
[c]Isolates include 50 *Streptococcus pyogenes*, 40 *Streptococcus agalactiae*, and 10 Group C/F/G streptococci.

Testing of TR-700 by Broth Microdilution Using Dry-form MIC Test Plates

The Applicant performed equivalency test between (b) (4) dried plates to the CLSI broth microdilution reference method (CLSI M7) using 200 isolates. Briefly, the susceptibility of gram-positive organisms to TR-700 using microtiter plates made with dried plate technology (b) (4) was evaluated in a study conducted (b) (4) ((b) (4) MCR-08-0701-035). The 200 isolates evaluated consisted of 70 staphylococci (40 *S. aureus*, 30 coagulase-negative staphylococci), 90 streptococci (30 *S. pneumoniae*, 20 *S. pyogenes*, 20 *S. agalactiae*, 20 viridans group streptococci [VGS]), and 40 *Enterococcus* spp. To establish the equivalency of testing TR-700 with dried plates relative to the reference method of using frozen plates, concurrent inocula of the evaluated isolates were tested simultaneously on frozen broth microtiter plates (b) (4) and on dried microtiter plates containing TR-700 and a

variety of comparator drugs. The results from the equivalency study overall and by organism are shown in Table 51. There was 100% agreement for the evaluated staphylococci and enterococci, such that TR-700 dried plate MIC values were identical or within one doubling-dilution of TR-700 frozen plate MIC values. Additionally, the Applicant sated that among streptococci, essential agreement for TR-700 during initial testing varied from 93% for *S. pneumoniae* and 95% for VGS, to 100% for beta-hemolytic streptococci (*S. pyogenes* and *S. agalactiae*). The data indicated a trend towards two-fold lower TR-700 MIC values on dried microtiter plates relative to reference frozen broth microtiter plates. However, given the effectiveness of TR-700 against streptococci, it was hypothesized that this trend is likely to have minimal to no impact on the susceptibility testing of TR-700. The Applicant also tested twenty inocula each of *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *S. pneumoniae* ATCC 49619 on both dried and on frozen microtiter plates for their susceptibility to TR-700. It was stated that all twenty inocula evaluated in this study had MIC values within the CLSI approved quality control ranges on both dried microtiter plates and reference frozen microtiter plates.

Table 51: TR- 700 MIC Values - Dried Microtiter Plates vs Reference Frozen Broth Microtiter Plates Overall and by Organism

		Log_2 Difference of TR-700 MIC on Dried Plates Relative to Frozen Plates (number of isolates [%])						
Organism	**N**	**≥-3**	**-2**	**-1**	**0**	**1**	**2**	**≥3**
Staphylococci[a]	70	-	-	-	34 (48.9)	36 (51.4)	-	-
Enterococci	40	-	-	2 (5.0)	31 (77.5)	7 (17.5)	-	-
Streptococci[b]	90	-	-	82[c] (91.1)	8 (8.9)	-	-	-
OVERALL:	200	-	-	84	73	43	-	-

Source: MCR-08-0701-035, Table derived from Tables 1A through 1G, as described in TR701-021.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration
Note: Dashes represent no value
[a]40 *S. aureus*, 30 coagulase-negative staphylococci.
[b]30 *S. pneumoniae*, 20 *S. pyogenes*, 20 *S. agalactiae*, 20 viridans group streptococci (VGS).
[c]Three isolates (2 *S. pneumoniae*, 1 VGS) initially had a Log_2 difference of -2, but on retesting in triplicate it was determined that all three had modal MIC values with a Log_2 difference of -1.

Table 52 summarizes the overall essential agreement of TR-700 and the comparators evaluated in this study on dried microtiter plates in comparison to the reference test method. These results validate the (b) (4) dried microtiter TR-700-containing plate for use in the susceptibility testing of TR-700 and comparators in accordance with the manufacturer's instructions.

Table 52: Percent Essential Agreement of TR-700 and Comparator MIC Values - Dried Microtiter Plates vs. Reference Frozen Broth Microtiter Plates

Drug	%EA Before Repeat Testing	%EA After Repeat Testing
TR-700	98.3	100
Linezolid	100	100
Clindamycin	100	100
Vancomycin	99.6	100
Daptomycin	100	100
Levofloxacin	100	100
Penicillin	98.7	100
Tigecycline	98.6	100
Oxacillin	100	100

Source: MCR-08-0701-035, Summary
Abbreviations: EA=essential agreement; MIC=minimum inhibitory concentration

EFFECT OF TESTING DYNAMICS ON TR-700 ACTIVITY:

Effect of non-standard test conditions on the in vitro activity

In another study, the effect of non-standard test conditions on the in vitro activity of TR-700 was evaluated in studies MCR-08-0701-016 and MCR-12-0701-062. In these studies, replicates of *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 (**MCR-08-0701-016**) and *S. pneumoniae* ATCC 49619 (**MCR-12-0701-062**) were tested using broth microdilution in standard and non-standard test conditions in parallel to evaluate the impact of variation in the standard testing conditions on TR-700 activity.

Conditions evaluated in this study included divalent cation concentration, media pH, inoculum size, atmosphere of incubation, the presence of lysed horse blood, and the presence of the non-ionic surfactant polysorbate-80. In a separate study (PHA-07-0701-043), the impact of testing by agar dilution in the presence of human serum was evaluated for a selection of methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE). There was no apparent impact on the activity of TR-700 against the evaluated organisms in 6 out of 7 non-standard conditions tested. MIC values under non-standard conditions were within one doubling-dilution of MIC values under standard conditions when tested by broth microdilution in non-standard concentrations of either Ca2+ or Mg2+ (Table 53), at pH higher or lower than standard pH (Table 54), when incubated in 5% to 8% CO2 rather than ambient air (Table 55), or when tested in the presence of lysed horse blood or polysorbate-80 (Table 56). The presence of human serum also had no impact on TR-700 activity when tested by agar dilution (Table 57).

Table 53: Impact of Cation Concentration on the MIC Value (mcg/mL) of TR-700

	Ca^{2+} (µg/mL):	25	20	5	25	5	10	50	25
	Mg^{2+} (µg/mL):	12	10	5	5	10	10	15	15
	Replicate	MIC (µg/mL)							
Staphylococcus aureus ATCC 29213	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	2	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	3	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	4	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	5	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Enterococcus faecalis ATCC 29212	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	2	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	3	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Streptococcus pneumoniae ATCC 49619	1	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
	2	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
	3	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12

Source: MCR-08-0701-016 Table 4; MCR-12-0701-062 Table 1
Abbreviations: ATCC=American Type Culture Collection; MIC=minimum inhibitory concentration
Note: Standard testing conditions (20-25 µg/mL Ca^{2+}; 10-12.5 µg/mL Mg^{2+}) shaded in gray.

Table 54: Impact of Media pH on the MIC Value (mcg/mL) of TR-700

		pH							
		6.0	6.5	7.0	7.2	7.3	7.4	7.6	8.0
Organism	Replicate	MIC (µg/mL)							
Staphylococcus aureus ATCC 29213	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	2	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	3	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	4	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	5	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Enterococcus faecalis ATCC 29212	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	2	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	3	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Streptococcus pneumoniae ATCC 49619	1	0.06	0.06	0.06	-	-	0.12	-	0.12
	2	0.06	0.06	0.12	-	-	0.12	-	0.12
	3	0.06	0.06	0.12	-	-	0.12	-	0.12

Source: MCR-08-0701-016 Table 5; MCR-12-0701-062 Table 2
Abbreviations: ATCC=American Type Culture Collection; MIC=minimum inhibitory concentration
Note: Standard testing conditions (pH 7.2 - 7.4) shaded in gray.

Table 55: Impact of Incubation Atmosphere on the MIC Value (mcg/mL) of TR-700

		Incubation Atmosphere	
Organism	Replicate	Ambient air	5-8% CO_2
Staphylococcus aureus ATCC 29213	1	0.5	0.5
	2	0.5	0.5
	3	0.5	0.5
	4	0.5	0.5
	5	0.5	0.5
Enterococcus faecalis ATCC 29212	1	0.5	0.5
	2	0.5	0.5
	3	0.5	0.5
	4	0.5	0.5
	5	0.5	0.5
Streptococcus pneumoniae ATCC 49619	1	0.06	0.12
	2	0.12	0.12
	3	0.12	0.12

Source: MCR-08-0701-016 Table 7; MCR-12-0701-062 Table 4
Abbreviations: ATCC=American Type Culture Collection; MIC=minimum inhibitory concentration
Note: Standard testing conditions (ambient air) shaded in gray.

Table 56: Impact of Testing in the Presence of Lysed Horse Blood or Surfactant (Polysorbate-80) on the MIC Value (mcg/mL) of TR-700

		CAMHB supplement (MIC Values µg/mL)		
Organism	**Replicate**	**None**	**3-5% LHB**	**0.2% Polysorbate-80**
Staphylococcus aureus ATCC 29213	1	0.5	0.5	0.5
	2	0.5	0.5	0.5
	3	0.5	0.5	0.5
	4	0.5	0.25	0.5
	5	0.5	0.5	0.5
Enterococcus faecalis ATCC 29212	1	0.5	0.5	0.5
	2	0.5	0.5	0.5
	3	0.5	0.5	1
	4	0.5	0.5	0.5
	5	0.5	0.5	0.5
Streptococcus pneumoniae ATCC 49619	1	0.12	0.12	0.12
	2	0.12	0.12	0.12
	3	0.12	0.12	0.12

Source: MCR-08-0701-016 Table 8 and Table 9; MCR-12-0701-062 Table 5
Abbreviations: ATCC=American Type Culture Collection; MIC=minimum inhibitory concentration; LHB=lysed horse blood; CAMHB=Cation-adjusted Mueller Hinton Broth
Note: Standard testing conditions (CAMHB, no supplement) shaded in gray.

Table 57: Impact of Human Serum on the Agar Dilution MIC Value (mcg/mL) of TR-700 and Linezolid Against MRSA and VRE

Organism	**Drug**	**Media**	**MIC range**	**MIC_{50}**	**MIC_{90}**
Staphylococcus aureus (MRSA) N=18	TR-700	MHA	0.5	0.5	0.5
		MHA w/ 20% plasma	1	1	1
	Linezolid	MHA	2	2	2
		MHA w/ 20% plasma	2 - 4	4	4
Enterococcus spp[a] (VRE) N=17	TR-700	MHA	0.25 - 0.5	0.5	0.5
		MHA w/ 20% plasma	0.5	0.5	0.5
	Linezolid	MHA	1 - 2	2	2
		MHA w/ 20% plasma	1 - 2	2	2

Source: PHA-07-0701-043 Table 1
Abbreviations: MRSA=methicillin-resistant *S. aureus*; VRE=vancomycin-resistant enterococci; MIC=minimum inhibitory concentration; MIC_{50}=minimum inhibitory concentration against 50% of the isolates; MIC_{90}=minimum inhibitory concentration against 90% of the isolates; MHA=Mueller Hinton agar
[a]Mixture of *E. faecalis* and *E. faecium*.

Effect of inoculum size on activity:

In the case of inoculum size (Table 58), MIC values were 4-fold lower at the lowest inoculum size evaluated (10^3 CFU/mL) for *S. aureus* ATCC 29213. It was noted that there was a trend towards increasing TR-700 MIC values with increasing inoculum size for all evaluated organisms. However, except for the 10^3 CFU/mL inoculum size when testing *S. aureus* ATCC 29213, observed TR-700 MIC values were within one-doubling dilution of those observed with the standard inoculum size (10^5 CFU/mL). These data illustrate that TR-700 activity as measured by broth microdilution is largely unaffected by changes that may occur during routine susceptibility testing as a result of technical error (eg, incorrect media pH, incorrect inoculum size).

Table 58: Impact of Inoculum Size on the MIC Value (mcg/mL) of TR-700

		Inoculum (CFU/mL)				
		10^3	10^4	10^5	10^6	10^7
Organism	**Replicate**	MIC (µg/mL)				
Staphylococcus aureus ATCC 29213	1	0.12	0.25	0.5	1	1
	2	0.12	0.25	0.5	1	1
	3	0.12	0.25	0.5	1	2
	4	0.12	0.25	0.5	1	1
	5	0.12	0.25	0.5	1	1
Enterococcus faecalis ATCC 29212	1	0.25	0.25	0.5	1	1
	2	0.25	0.25	0.5	0.5	1
	3	0.25	0.25	0.5	0.5	1
	4	0.25	0.25	0.5	0.5	1
	5	0.25	0.25	0.5	0.5	1
Streptococcus pneumoniae ATCC 49619	1	0.06	0.06	0.06	0.06	0.12
	2	0.06	0.06	0.06	0.12	0.12
	3	0.06	0.06	0.12	0.12	0.12

Source: MCR-08-0701-016 Table 6; MCR-12-0701-062 Table 3
Abbreviations: ATCC=American Type Culture Collection; MIC=minimum inhibitory concentration; CFU=colony forming units
Notes: Standard testing conditions (approx. 1.5×10^5 CFU/mL) shaded in gray.

DIVISION OF ANTI-INFECTIVE PRODUCTS CLINICAL MICROBIOLOGY NDA REVIEW

NDA: 205435/205436 DATE REVIEW COMPLETED: 02/24/2014
Tedizolid Phosphate (Sivextro)

Summary:

As shown in this section, TR-700 susceptibility may be determined by disk diffusion testing using a 20 mcg disk for target pathogens (staphylococci, enterococci, and streptococci). Additionally, TR-700 agar dilution MIC values also correlate with broth microdilution MIC values, indicating that agar dilution may be an acceptable method for determining the susceptibility of target pathogens to TR-700. There was minimal to no impact of non-standard testing conditions (eg, incorrect inoculum size, improper media pH/cations) on the overall activity of TR-700 against the evaluated ATCC quality control strains. The minimal impact on non-standard testing conditions may reduce the possibility of inaccurate MIC values in clinical laboratories testing TR-700 in the presence of common technical errors.

Development of Quality Control Parameters

The Applicant developed QC limits for aerobic microdilution and disk diffusion susceptibility tests of TR-700. Studies conducted to establish quality control ranges for the in vitro susceptibility testing of TR-700 were performed in accordance with guidelines established by the Clinical Laboratory Standards Institute (CLSI) as described in document M23 (CLSI M23 2001).

The acceptable quality control range for the broth microdilution testing of TR-700 against relevant ATCC quality control strains was determined in a multicenter study. The Applicant stated that testing was conducted in 8 laboratories that adhered to Tier 2 quality control guidelines as described by CLSI (MCR-08-0701-013). Participating laboratories (and principal investigators) were: (b) (4)

The Applicant stated that frozen broth microdilution trays containing TR-700 and linezolid (tested as the control agent) were commercially prepared (b) (4) and distributed to the participating laboratories for testing. Each test panel contained TR-700 in cation-adjusted Mueller- Hinton broth (CAMHB) from 3 separate manufacturers (b) (4) For the testing of *S. pneumoniae*, broth was supplemented with 3% lysed horse blood (LHB) per CLSI guidelines (CLSI M7-A6 2003). Each laboratory tested 10 independent inocula of each of the 3 relevant media lots, resulting in 30 independent MIC results for TR-700 per laboratory. In all, 240 TR-700 MIC results were reported for each QC strain. Standard QC reference strains for broth microdilution MIC testing of *Staphylococcus* spp. (*S. aureus* ATCC 29213), *Enterococcus* spp. (*E. faecalis* ATCC 29212), and *Streptococcus* spp. (*S. pneumoniae* ATCC 49619) were evaluated (Table 59).

Table 59: TR-700 Acceptable MIC Quality Control Ranges

QC Strain	TR-700 Acceptable Quality Control Ranges (MIC, µg/mL)
Staphylococcus aureus ATCC 29213	0.25 - 1
Enterococcus faecalis ATCC 29212	0.25 - 1
Streptococcus pneumoniae ATCC 49619	0.12 - 0.5

Source: CLSI M100-S22 2012, MCR-08-0701-013
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute; MIC=minimum inhibitory concentration; QC=quality control

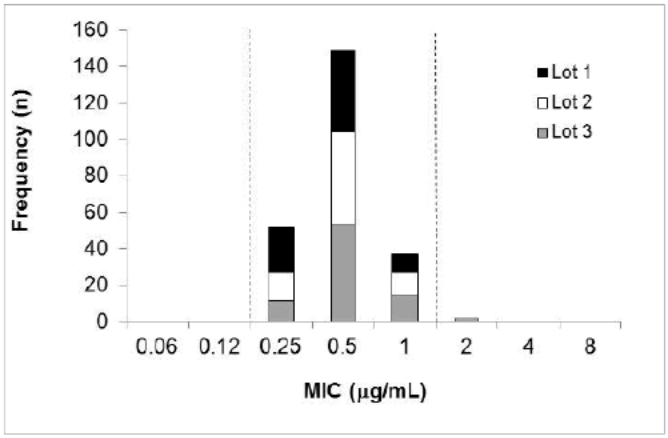
QC ranges were determined from the data based on CLSI guidelines and were confirmed by supplemental analysis of data using the Rangefinder Method of Turnidge and Bordash (Turnidge 2007). The raw data for TR-700 are shown by strain in Table 60, Table 61, and Table 62 and in Figure 47, Figure 48, and Figure 49 and are described in MCR-08-0701-013.

Table 60: *Staphylococcus aureus* ATCC 29213 TR-700 MIC Results

MIC (µg/mL)	Lot 1	Lot 2	Lot 3	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Overall
0.06	-	-	-	-	-	-	-	-	-	-	-	-
0.12	-	-	-	-	-	-	-	-	-	-	-	-
0.25	25	16	11	7	-	-	1	3	30	11	-	52
0.5	45	51	53	23	29	6	29	26	-	19	17	149
1	10	13	14	-	1	22	-	1	-	-	13	37
2	-	-	2	-	-	2	-	-	-	-	-	2
4	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-
N	80	80	80	30	30	30	30	30	30	30	30	240
GEOMEAN	0.439	0.487	0.531	0.425	0.512	0.912	0.489	0.477	0.250	0.388	0.675	0.484
MODE	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	0.25	0.5	0.5	0.5
MIN	(b) (4)											
MAX												
RANGE	3	3	4	2	2	3	2	3	1	2	2	4

Source: MCR-08-0701-013
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute; MIC=minimum inhibitory concentration, QC=quality control; N=number
Note: Shaded cells indicate MIC values within CLSI approved QC range.

Figure 29: *Staphylococcus aureus* ATCC 29213 TR-700 MIC Results



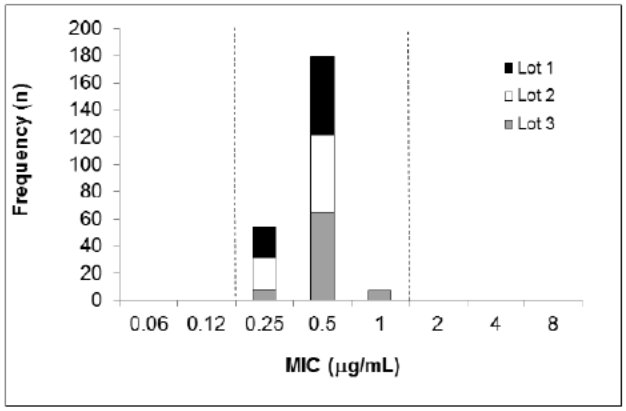
Source: MCR-08-0701-013
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute; MIC=minimum inhibitory concentration; N=number
Note: Dashed lines indicate CLSI approved quality control range.

Table 61: *Enterococcus faecalis* ATCC 29212 TR-700 MIC Results

MIC (µg/mL)	Lot 1	Lot 2	Lot 3	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Overall
0.06	-	-	-	-	-	-	-	-	-	-	-	-
0.12	-	-	-	-	-	-	-	-	-	-	-	-
0.25	23	23	8	6	23	-	-	10	4	11	-	54
0.5	57	57	65	22	7	27	29	20	26	19	29	179
1	-	-	7	2	-	3	1	-	-	-	1	7
2	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-
N	80	80	80	30	30	30	30	30	30	30	30	240
GEOMEAN	0.410	0.410	0.496	0.456	0.294	0.536	0.512	0.397	0.456	0.388	0.512	0.437
MODE	0.5	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5
[redacted]												(b) (4)
RANGE	2	2	3	3	2	2	2	2	2	2	2	3

Source: MCR-08-0701-013
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute; MIC=minimum inhibitory concentration, QC=quality control; N=number
Note: Shaded cells indicate MIC values within CLSI approved QC range.

Figure 30: *Enterococcus faecalis* ATCC 29212 TR-700 MIC Results



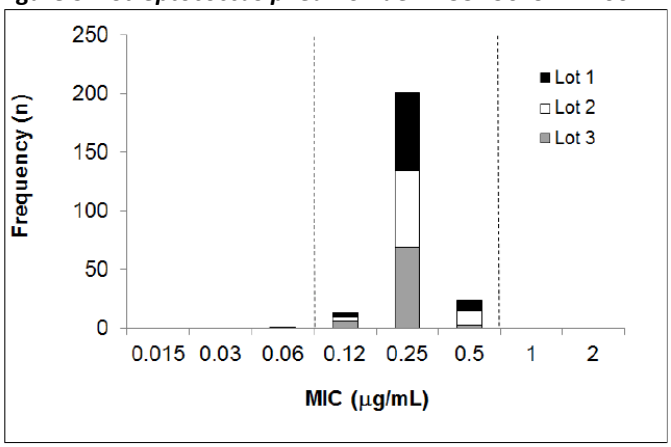
Source: MCR-08-0701-013
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute; MIC=minimum inhibitory concentration; N=number
Note: Dashed lines indicate CLSI approved quality control range.

Table 62: *Streptococcus pneumoniae* ATCC 49619 TR-700 MIC Results

MIC (µg/mL)	Lot 1	Lot 2	Lot 3	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Overall
0.015	-	-	-	-	-	-	-	-	-	-	-	-
0.03	-	-	-	-	-	-	-	-	-	-	-	-
0.06	-	-	1	-	-	-	-	1	-	-	-	1
0.12	4	3	7			3		7		1	3	14
0.25	67	65	69	30	9	25	29	22	30	29	27	201
0.5	9	12	3		21	2	1					24
1												
2												
N	80	80	80	30	30	30	30	30	30	30	30	240
GEOMEAN	0.261	0.270	0.236	0.250	0.406	0.243	0.256	0.201	0.250	0.244	0.232	0.255
MODE	0.25	0.25	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25
												(b) (4)
RANGE	3	3	4	1	2	3	2	3	1	2	2	4

Source: MCR-08-0701-013
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute; MIC=minimum inhibitory concentration, QC=quality control. N=number
Note: Shaded cells indicate MIC values within CLSI approved QC range.

Figure 31: *Streptococcus pneumoniae* ATCC 49619 TR-700 MIC Results



Source: MCR-08-0701-013
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute; MIC=minimum inhibitory concentration , N=number
Note: Dashed lines indicate CLSI approved quality control range.

The Applicant indicated that all ranges reported below were approved by the Subcommittee for Antibacterial Susceptibility Testing of CLSI. Overall, based on the data presented, there was no apparent variation across testing laboratories or media lots for TR-700 with any of the evaluated ATCC QC strains. Testing of the control agent linezolid yielded MIC values 100% within approved linezolid CLSI ranges for all evaluated ATCC QC strains. Against *S. aureus* ATCC 29213, TR-700 had MIC values of 0.25 to 2 mcg/ml and a clear mode of 0.5 mcg/mL. The approved QC range (b) (4) for *S. aureus* ATCC 29213 captured 99.2% of TR-700 test results. Against *E. faecalis* ATCC 29212, TR-700 had MIC values of 0.25 to 1 mcg/ml and a clear mode of 0.5 mcg/mL. The approved QC range (b) (4) for *E. faecalis* ATCC 29212 captured 100% of TR-700 test results. Against *S. pneumoniae* ATCC 49619, TR-700 had MIC values of 0.06 to 0.5 mcg/ml and a clear mode of 0.25 mcg/mL. The approved QC range (b) (4) for *S. pneumoniae* ATCC 49619 captured 99.6% of TR-700 test results.

Disk Diffusion Testing of Staphylococcus aureus and Streptococcus pneumoniae

In another study, the acceptable QC range for the disk diffusion testing of TR-700 against relevant ATCC QC strains was determined in a multicenter study consisting of nine laboratories that adhered to Tier 2 quality control guidelines as described by CLSI (MCR-11-0701-017). Participating laboratories (and principal investigators) were: (b) (4)

Each lab tested disks containing 20 mcg of TR-700 from two separate manufacturers (b) (4) across 3 lots of Mueller-Hinton Agar (MHA) from 3 separate manufacturers (b) (4) For the testing of *S. pneumoniae*, MHA supplemented with 5% lysed sheep blood per CLSI

guidelines was also acquired from the same 3 manufacturers. It was stated that each laboratory tested 10 independent inocula of each relevant ATCC QC strain on each lot of media for each disk lot, resulting in 60 disk zone sizes for TR-700 per lab (10 for each of 2 disk lots across 3 lots of media). In all, 540 TR-700 disk zone sizes were reported for each QC strain. Standard QC reference strains employed for the QC of disk diffusion susceptibility testing of *Staphylococcus* spp. and *Enterococcus* spp. (*S. aureus* ATCC 25923), and *Streptococcus* spp. (*S. pneumoniae* ATCC 49619) were evaluated. Quality Control ranges were determined using the Gavan statistic (CLSI M23-A2 2001). The CLSI approved quality control ranges are shown in Table 63. The raw data for TR-700 are shown by strain in Table 64 and Table 65 and in Figure 32 and Figure 33, and are described in MCR-11-0701-017.

Table 63: TR-700 Acceptable Zone Diameter Quality Control Ranges

QC Strain	TR-700 Acceptable Quality Control Ranges Zone diameter (mm)
Staphylococcus aureus ATCC 25923[a]	22 - 29
Streptococcus pneumoniae ATCC 49619	24 - 30

Source: CLSI M100-S22 2012
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute
[a]As per CLSI recommendations for linezolid, zone diameters read with transmitted light

Table 64: *Staphylococcus aureus* ATCC 25923 TR-700 Disk Diffusion Results

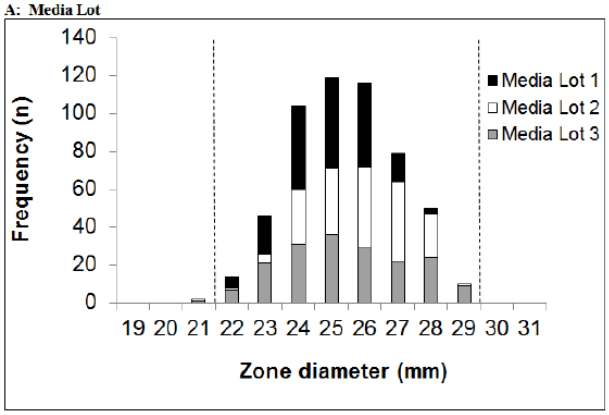
Zone diameter (mm)	Media Lot 1	Media Lot 2	Media Lot 3	Disk Lot A	Disk Lot B	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Overall
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	1	1	-	2	-	-	-	-	-	-	-	2	-	2
22	6	1	7	7	7	-	5	-	2	-	-	3	4	-	14
23	20	5	21	21	25	-	11	1	12	-	1	9	12	-	46
24	44	29	31	55	49	1	17	3	25	5	10	18	25	-	104
25	48	35	36	61	58	14	21	13	16	8	22	9	11	5	119
26	44	43	29	54	62	17	6	18	5	25	17	8	6	14	116
27	15	42	22	40	39	16	-	15	-	13	9	9	-	17	79
28	3	23	24	28	22	9	-	10	-	9	1	3	-	18	50
29	-	1	9	4	6	3	-	-	-	-	-	1	-	6	10
30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N	180	180	180	270	270	60	60	60	60	60	60	60	60	60	540
MEDIAN	25	26	25	25	25	26	24	26	24	26	25	24.5	24	27	25
MODE	25	26	25	25	26	26	25	26	24	26	25	24	24	28	25
MEAN	24.89	25.86	25.46	25.43	25.38	26.45	24.20	26.22	24.17	26.22	25.43	24.90	23.95	27.10	25.4
RANGE	7	9	9	8	9	6	5	6	5	5	6	8	6	5	9

Source: MCR-11-0701-017
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute, N=number
Note: Shaded cells indicate zone diameters within CLSI approved quality control range

Gavin statistic:	
All Lab median =	25 mm
Median of Ranges (MR) =	6
1/2 MR rounded up (R) =	3
All Lab Median +/- R =	22-28 mm
Rangefinder:	22-29 mm

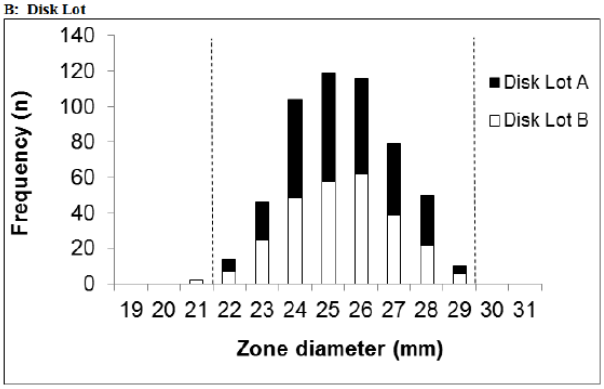
Figure 32: *Staphylococcus aureus* ATCC 25923 TR-700 Disk Diffusion Results by (A) Media Lot and (B) Disk Lot

A: Media Lot



Source: MCR-11-0701-017
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute, N=number
Note: Dashed lines indicate CLSI approved quality control range

B: Disk Lot



Source: MCR-11-0701-017
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute, N=number
Note: Dashed lines indicate CLSI approved quality control range

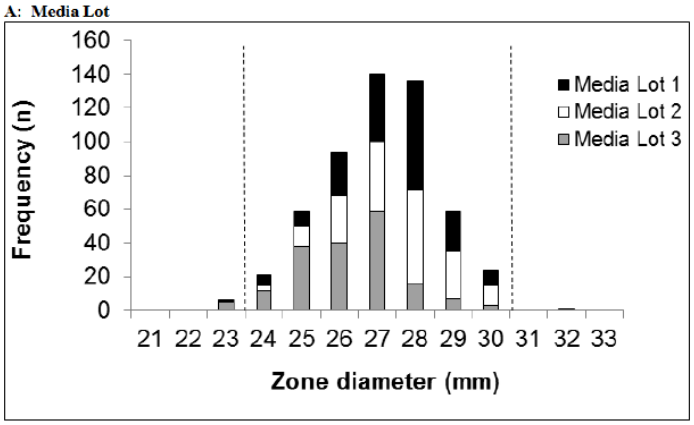
Table 65: *Streptococcus pneumoniae* ATCC 49619 TR-700 Disk Diffusion Results

Zone diameter (mm)	Media Lot 1	Media Lot 2	Media Lot 3	Disk Lot A	Disk Lot B	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Overall
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	1	-	5	2	4	-	3	1	2	-	-	-	-	-	6
24	6	3	12	10	11	1	9	2	9	-	-	-	-	-	21
25	9	12	38	25	34	8	16	6	17	5	5	-	2	-	59
26	26	28	40	46	48	17	12	7	10	17	16	7	8	-	94
27	40	41	59	65	75	20	16	14	7	12	20	12	21	18	140
28	65	55	16	74	62	9	4	11	11	14	16	20	17	34	136
29	24	28	7	34	25	5	-	10	3	8	3	13	10	7	59
30	9	12	3	14	10	-	-	8	1	4	-	8	2	1	24
31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	1	-	-	1	-	-	1	-	-	-	-	-	-	1
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N	180	180	180	270	270	60	60	60	60	60	60	60	60	60	540
MEDIAN	28	28	26	27	27	27	26	27.5	26	27	27	28	26	28	27
MODE	28	28	27	28	27	27	27	27	25	26	27	28	26	28	27
MEAN	28.00	27.51	26.26	27.19	26.93	26.72	25.68	27.52	26.02	27.25	26.93	28.05	26.03	27.85	27.06
RANGE	8	9	8	8	10	6	6	10	8	6	5	5	6	4	10

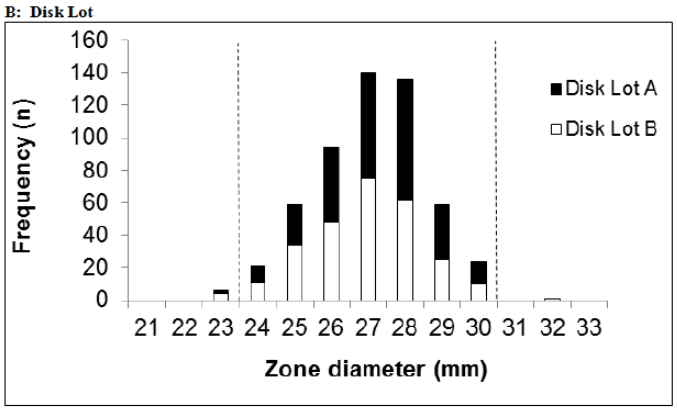
Source: MCR-11-0701-017
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute; MIC=minimum inhibitory concentration, N=number
Note: Shaded cells indicate zone diameters within CLSI approved quality control range.

Gavin statistic:	
All Lab median =	27 mm
Median of Ranges (MR) =	6
1/2 MR rounded up (R) =	3
All Lab Median +/- R =	24-30 mm
Rangefinder:	24-31 mm

Figure 33: *Streptococcus pneumoniae* ATCC 49619 TR-700 Disk Diffusion Results by (A) Media Lot and (B) Disk Lot



Source: MCR-11-0701-017
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute, N=number
Note: Dashed lines indicate CLSI approved quality control range.



Source: MCR-11-0701-017
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute, N=number
Notes: Dashed lines indicate CLSI approved quality control range

Antibacterial Interaction Studies

Antibacterial combination and synergy are important for treating pathogens in mixed infection, to enhance the killing of specific pathogens, and to prevent or delay the emergence of drug-resistant populations. The Applicant evaluated synergy of TR-700 in combination with different antibiotics using the checkerboard technique against a variety of bacterial isolates. The MIC and fractional inhibitory concentrations (FIC) and FIC indices (FICI) was used to assess drug interaction for TR-700 in combination with other antibacterials. A "synergistic interaction" is evidenced by inhibition of organism growth by combinations that are at concentrations significantly below the MIC of either compound alone, resulting in a low FICI value (≤ 0.50). The interpretation of "no interaction" results in growth inhibition at concentrations below the MICs of the individual compounds, but the effect is not significantly different from the additive effects of the two compounds, resulting in an FICI value of > 0.50 but ≤ 4.0. (The interpretation "no interaction" has previously been referred to as "additivity" or "indifference.") An "antagonistic interaction" results when the

concentrations of the compounds in combination that are required to inhibit organism growth are greater than those for the compounds individually, resulting in an FIC value of > 4.0. While there is no officially sanctioned set of FICI criteria, ≤ 0.50 was used to define synergism in this study.

The interactions of TR-700 with other agents were addressed in 3 independent studies. The first study evaluated the interaction of TR-700 with β-lactam antibiotics and colistin against methicillin-resistant *S. aureus* (MRSA), *E. coli* and *P. aeruginosa* (CPH-12-0701-052A); the second study evaluated TR-700 with various agents against methicillin-sensitive *S. aureus* (MSSA), MRSA, *E. faecalis*, *S. pyogenes*, *S. pneumoniae*, and *E. coli* (PHA-08-0701-015); and the third study evaluated the interaction of select anti-fungal agents with TR-700 against *S. aureus*, *E. faecalis*, *S. pyogenes*, and *S. pneumoniae* (PHA-08-0701-023). The MIC and FICI results for selected antibiotic combinations are detailed in Table 66. Against the evaluated MRSA strain, the activity observed with TR-700 was not altered when combined with ceftazidime, piperacillin, or colistin. Overall, FIC indices ranged from 0.63 to 2.06, indicative of no interaction for the drugs and organisms tested. Against the MRSA strain, a slight increase in activity was observed for TR-700 when combined with imipenem (FIC index of 0.63) though this increase was not sufficient to indicate synergy (FIC index ≤0.5). Against wild-type strains of *E. coli* and *P. aeruginosa*, TR-700 FIC indices were 2.06, showing no activity greater than that observed with imipenem, ceftazidime, piperacillin, or colistin alone (no interaction indicated by FIC index ranging from >0.5 to 4). Against a *P. aeruginosa* efflux deficient mutant (PA397), TR-700 had activity alone, but as with the other gram-negative bacteria evaluated in this study, the activity of imipenem, ceftazidime, piperacillin, and colistin was not affected when combined with TR-700; FIC indices ranged from 0.75 to 1.00.

Table 66: FIC Analysis of TR- 700 Combined with Imipenem, Ceftazidime , Piperacillin, and Colistin

		imipenem		ceftazidime		piperacillin		colistin	
	MIC (µg/mL) TR-700 alone	MIC (µg/mL) imipenem alone	FICI	MIC (µg/mL) ceftazidime alone	FICI	MIC (µg/mL) piperacillin alone	FICI	MIC (µg/mL) colistin alone	FICI
E. coli ATCC 25922	>32	0.25	**1.06**	0.5	**1.91**	4	**1.00**	0.5	**0.70**
P. aeruginosa PA01	>32	1	**1.06**	2	**1.12**	4	**2.06**	2	**0.97**
P. aeruginosa PA397	16	1	**1.00**	1	**1.00**	2	**0.75**	1	**1.00**
MRSA ATCC 33591	0.25	64	**0.63**	>64	**1.07**	>64	**1.07**	>64	**1.07**

Source: CPH-12-0701-052A Table 2
Abbreviations: ATCC=American Type Culture Collection; *E. coli=Escherichia coli*; FICI=fractional inhibitory concentrations index; MIC=minimum inhibitory concentration (µg/mL); MRSA=methicillin-resistant *S. aureus*; *P. aeruginosa=Pseudomonas aeruginosa;*
Note: *E. coli*, MRSA, and PA01 are wild-type. PAO397=PAO1 *ΔmexABoprM –nfxB ΔmexCD-oprJ ΔmexJKL ΔmexXY OpmH⁻ ΔopmH362 ΔmexEF-oprN*

In the second study (PHA-08-0701-015), the Applicant evaluated the activity of different antibacterial agents in combination with TR-700 (**Table 67**, Table 68, Table 69, Table 70 and Table 71). Test organisms included isolates for which TR-700 has activity (*S. aureus*, *E. faecalis*, *S. pyogenes*, and *S. pneumoniae*) and *E. coli,* against which TR-700 is inactive. Against the evaluated MRSA isolate, neither the activity of TR-700 nor the various other agents was affected when combined (FIC indices ranged between 0.52 and 1.37). It was noted that the same result was also observed for these agents in combination with TR-700 against *E. faecalis* (FIC indices ranging between 0.63 and 2.33), *S. pyogenes* (FIC indices ranging between 0.72 and 2.41), and *S. pneumoniae* (FIC indices ranging between 0.72 and 3.83). Against *E. coli*, there was no apparent antagonism or synergy for the gram-negative-active agents when combined with TR-700 (FIC indices between 0.84 and 2.14).

Table 67: FIC Analysis of TR-700 Combined with Several Comparator Agents Against MRSA

S. aureus 2053 (MRSA)				
Compound		TR-700 MIC (µg/mL)	FICI	Interpretation
Name	MIC (µg/mL)			
Aztreonam	>128	0.5	0.85	No Interaction
Ceftriaxone	>128	0.25	0.82	No Interaction
Ceftazidime	>128	0.25	1.14	No Interaction
Imipenem	128	0.25	0.85	No Interaction
Rifampin	0.004	1	1.37	No Interaction
SXT	64/1216	0.5	1.01	No Interaction
Minocycline	4	0.5	0.83	No Interaction
Clindamycin	0.25	0.25	0.92	No Interaction
Ciprofloxacin	128	0.25	0.52	No Interaction
Daptomycin	0.5	0.5	1.16	No Interaction
Vancomycin	1	0.5	1.00	No Interaction
Gentamicin	>64	0.5	0.85	No Interaction

Source: PHA-08-0701-015 Table 2
Abbreviations: FICI=fractional inhibitory concentration index; MIC=minimum inhibitory concentration; MRSA=methicillin-resistant *S. aureus*; SXT=trimethoprim/sulfamethoxazole

Table 68: FIC Analysis of TR-700 Combined with Several Comparator Agents Against *Enterococcus faecalis*

E. faecalis 795 (VSE)				
Compound		TR-700 MIC (µg/mL)	FICI	Interpretation
Name	MIC (µg/mL)			
Aztreonam	>128	0.5	1.00	No Interaction
Ceftriaxone	>128	0.5	0.78	No Interaction
Ceftazidime	>128	0.25	1.28	No Interaction
Imipenem	1	0.25	2.00	No Interaction
Rifampin	0.25	0.25	2.33	No Interaction
SXT	0.015/0.28	0.5	1.51	No Interaction
Minocycline	8	0.5	0.68	No Interaction
Clindamycin	128	0.25	1.57	No Interaction
Ciprofloxacin	128	0.25	0.63	No Interaction
Daptomycin	1	0.5	0.70	No Interaction
Vancomycin	1	0.5	1.00	No Interaction
Gentamicin	>64	0.5	1.28	No Interaction

Source: PHA-08-0701-015 Table 3
Abbreviations: FICI=fractional inhibitory concentration index; MIC=minimum inhibitory concentration; SXT=trimethoprim/sulfamethoxazole; VSE=vancomycin-susceptible enterococci

Table 69: FIC Analysis of TR-700 Combined with Several Comparator Agents Against *Streptococcus pyogenes*

S. pyogenes 717				
Compound		TR-700 MIC (µg/mL)	FICI	Interpretation
Name	MIC (µg/mL)			
Aztreonam	8	0.125	2.41	No Interaction
Ceftriaxone	0.002	0.125	1.68	No Interaction
Ceftazidime	0.015	0.125	1.12	No Interaction
Imipenem	0.015	0.125	1.39	No Interaction
Rifampin	0.06	0.25	0.94	No Interaction
SXT	0.125/2.4	0.125	1.15	No Interaction
Minocycline	0.06	0.25	0.72	No Interaction
Clindamycin	0.03	0.125	1.32	No Interaction
Ciprofloxacin	0.5	0.25	1.23	No Interaction
Daptomycin	0.06	0.125	0.75	No Interaction
Vancomycin	0.25	0.125	1.16	No Interaction
Gentamicin	8	0.125	1.45	No Interaction

Source: PHA-08-0701-015 Table 4
Abbreviations: FICI=fractional inhibitory concentration index; MIC=minimum inhibitory concentration; SXT=trimethoprim/sulfamethoxazole

Table 70: FIC Analysis of TR-700 Combined with Several Comparator Agents Against *Streptococcus pneumoniae*

S. pneumoniae 880				
Compound		TR-700 MIC (µg/mL)	FICI	Interpretation
Name	MIC (µg/mL)			
Aztreonam	>128	0.125	2.28	No Interaction
Ceftriaxone	0.125	0.03	3.17	No Interaction
Ceftazidime	4	0.03	3.83	No Interaction
Imipenem	1	0.125	1.37	No Interaction
Rifampin	0.015	0.25	1.49	No Interaction
SXT	1/19	0.125	1.31	No Interaction
Minocycline	0.06	0.125	0.86	No Interaction
Clindamycin	128	0.125	0.79	No Interaction
Ciprofloxacin	16	0.06	0.72	No Interaction
Daptomycin	0.125	0.25	0.86	No Interaction
Vancomycin	0.25	0.25	0.77	No Interaction
Gentamicin	16	0.125	0.75	No Interaction

Source: PHA-08-0701-015 Table 5
Abbreviations: FICI=fractional inhibitory concentration index; MIC=minimum inhibitory concentration; SXT=trimethoprim/sulfamethoxazole

Table 71: FIC Analysis of TR-700 Combined with Several Comparator Agents Against *Escherichia coli*

E. coli ATCC 25922				
Compound		TR-700 MIC (µg/mL)	FICI	Interpretation
Name	MIC (µg/mL)			
Aztreonam	0.03	>32	0.84	No Interaction
Ceftriaxone	0.06	>32	1.59	No Interaction
Ceftazidime	0.03	>32	1.06	No Interaction
Imipenem	0.5	>32	1.43	No Interaction
SXT	0.125/2.4	>32	1.43	No Interaction
Minocycline	0.25	>32	2.14	No Interaction
Ciprofloxacin	0.008	>32	1.78	No Interaction
Gentamicin	0.5	>32	1.99	No Interaction

Source: PHA-08-0701-015 Table 6
Abbreviations: ATCC=American Type Culture Collection; FICI=fractional inhibitory concentration index; MIC=minimum inhibitory concentration; SXT=trimethoprim/sulfamethoxazole

Another study was conducted to determine the impact of combining TR-700 with common antifungal agents on gram-positive microbial susceptibility (PHA-08-0701-023). As shown in **Table 72**, there was no interaction observed when testing TR-700 in combination with either amphotericin B, terbinafine HCl, or ketoconazole; FIC indices ranged from 1.08 to 1.19 against the evaluated MRSA, *E. faecalis*, *S. pyogenes*, and *S. pneumoniae* isolates.

Table 72: FIC Analysis of TR-700 Combined with Antifungal Agents Against Gram-positive Organisms

Compound		TR-700 MIC (µg/mL)	FICI	Interpretation
Name	MIC (µg/mL)			
Staphylococcus aureus 2053 (MRSA)				
Amphotericin B	>64	1	1.08	No Interaction
Terbinafine HCl	>64	1	1.19	No Interaction
Ketoconazole	>64	1	1.19	No Interaction
Enterococcus faecalis 0795 (VSE)				
Amphotericin B	>64	1	1.19	No Interaction
Terbinafine HCl	>64	1	1.19	No Interaction
Ketoconazole	>64	1	1.19	No Interaction
Streptococcus pyogenes 0717				
Amphotericin B	>64	0.5	1.19	No Interaction
Terbinafine HCl	>64	0.5	1.19	No Interaction
Ketoconazole	>64	0.5	1.19	No Interaction
Streptococcus pneumoniae 0880				
Amphotericin B	>64	0.5	1.19	No Interaction
Terbinafine HCl	>64	0.5	1.19	No Interaction
Ketoconazole	>64	0.5	1.19	No Interaction

Source: PHA-08-0701-023, Tables 4-7
Abbreviations: FICI=fractional inhibitory concentration index; MIC=minimum inhibitory concentration; MRSA=methicillin-resistant *S. aureus*; VSE=vancomycin-susceptible enterococci

Summary:
Studies evaluating the fractional inhibitory concentration indices of TR-700 in combination with a wide array of agents showed no apparent antagonism or synergy in vitro between TR-700 and other agents against both gram-positive and gram-negative pathogens. Furthermore, it was noted that combining TR-700 with common antifungal agents also had no impact on the in vitro antibacterial activity of the compounds for the target pathogens.

EFFECT OF MISCELLANEOUS FACTORS ON TR-700 ACTIVITY

Post Antibiotic effect (PAE)

The PAE describes the suppression of bacterial growth that occurs after short exposure to an antibiotic. The PAE is a consequence of the initial exposure to high concentrations of antibiotics rather than to persistent sub-inhibitory levels. The subinhibitory MIC effect (SME) is similar to the PAE except that the bacteria are exposed to drug at subinhibitory concentrations. The postantibiotic subinhibitory effect (PA-SME) is similar to a PAE, except that after exposure, the bacteria are further incubated in media containing subinhibitory concentrations of the agent rather than in fresh drug-free media. These effects assess the effect of the agent on bacterial growth during a typical dose interval, where drug concentrations may increase and then decrease to sub-MIC values before subsequent doses.

PAE, SME, and PA-SME are commonly evaluated by the quantitation of viable bacteria after exposure to an antibacterial agent and subsequent removal. In Study **PHA-07-0701-061**, the PAE, SME, and PA-SME of TR-700 and linezolid were evaluated against *S. aureus*, coagulase-negative staphylococci, *E. faecalis*, and *E. faecium* (Table 73). Isolates included methicillin-resistant staphylococci and vancomycin-resistant enterococci.

Table 73: PAE, SME, and PASME for TR- 700 and Linezolid

Strain	Drug	MIC	PAE (hour)	SME (hour)			PA-SME (hour)		
				0.2X MIC	0.3X MIC	0.4X MIC	0.2X MIC	0.3X MIC	0.4X MIC
MRSA 98-11-R782	TR-700	0.5	0.25	1.95	3.30	4.40	2.00	3.95	4.65
	LZD	2	0.60	1.20	2.35	3.95	1.45	2.75	2.95
MRSA M126	TR-700	0.5	0.63	3.70	6.95	9.25	3.86	8.47	14.52
	LZD	2	1.30	0.70	4.25	6.60	4.00	8.70	8.65
MSSA 96-10-2532	TR-700	0.5	0.05	0.25	0.50	1.20	0.70	0.90	1.75
	LZD	2	0.10	0.20	0.30	0.55	0.30	1.05	0.55
MRCoNS 01-8-V8	TR-700	0.25	0.30	0.60	2.08	2.74	0.92	1.74	3.28
	LZD	1	0.56	0.50	2.80	2.22	0.46	1.78	2.26
MSCoNS 01-8-P183	TR-700	0.5	0.70	2.20	2.75	4.65	2.20	2.85	5.25
	LZD	2	1.00	1.40	1.45	3.05	0.95	2.80	3.10
VR- *Enterococcus faecium* 00-5-U1211	TR-700	0.25	0.15	0.00	0.80	1.35	0.40	0.85	1.20
	LZD	1	0.65	0.25	0.45	1.20	0.15	0.55	1.40
VR-*E. faecium* 99-3-U95	TR-700	0.25	1.05	0.55	1.35	4.25	0.05	2.30	4.10
	LZD	1	1.05	1.00	1.90	2.20	1.30	2.80	3.20
Enterococcus faecalis 00-4-U518	TR-700	0.5	0.57	0.47	1.09	3.69	1.10	1.68	3.26
	LZD	2	0.77	0.87	1.93	2.16	0.90	1.52	3.80

Source: PHA-07-0701-061 Table 1
Abbreviations: LZD=linezolid; MIC=minimum inhibitory concentration in µg/mL; MRSA=methicillin-resistant *S. aureus* ; MR=methicillin-resistant; MS=methicillin-susceptible; CoNS=coagulase-negative staphylococci; PAE=post antibiotic effect; PA-SME=postantibiotic subinhibitory effect; SME=subinhibitory MIC effect; SA=*S. aureus*; VR=vancomycin-resistant

The PAE was evaluated by exposing log-phase inocula to TR-700 and linezolid at 4X the respective MIC value

for 1 hour alongside an untreated growth control. After 1 hour, drug was removed by centrifugation, and the bacteria were resuspended in drug-free media. It was stated that subsequent to removal of drug, viable bacteria were quantitated at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hours. The PAE was defined as the time after exposure and removal of the agent required to grow 1 log10 CFU/mL, minus the time required for the unexposed growth control to grow 1 log10 CFU/mL. Against the evaluated staphylococci, including methicillin-resistant isolates, TR-700 had PAE values of 0.05 to 0.7 hours compared with 0.10 to 1.30 hours observed with linezolid. Against the evaluated enterococci, including vancomycin-resistant isolates, TR-700 had PAE values of 0.15 to 1.05 hours compared with 0.65 to 1.05 hours observed with linezolid.

In another experiment, the SME was evaluated in the same fashion as PAE with the exception that log-phase inocula were exposed to TR-700 and linezolid at 0.2X, 0.3X, and 0.4X the MIC value for 1 hour before transfer to fresh drug-free media. Viable bacteria were quantitated beyond 8 hours in instances where it was apparent that the delay in regrowth was prolonged. Regrowth of the bacteria was delayed for both TR-700 and linezolid relative to PAE, as evidenced by increased SME values relative to PAE values (Table 88). SME values for both TR-700 and linezolid increased with increasing subinhibitory concentrations. Against staphylococci, SME values for TR-700 ranged from 0.25 to 3.70 hours after exposure to 0.2X the MIC value, 0.5 to 6.95 hours after exposure to 0.3X the MIC value, and 1.20 to 9.25 hours after exposure to 0.4X the MIC value. SME values against staphylococci for TR-700 at the evaluated concentrations were consistently longer than those observed with linezolid against staphylococci. Against enterococci, SME values for TR-700 ranged from 0.00 to 0.55 hours after exposure to 0.2X the MIC value, 0.80 to 1.35 hours after exposure to 0.3X the MIC value, and 1.35 to 4.25 hours after exposure to 0.4X the MIC value. At 0.4X the MIC value, TR-700 SME values against enterococci were greater than those observed with linezolid.

The PA-SME was evaluated as described above with initial exposure of log-phase bacterial cells to TR-700 and linezolid at 4X the MIC value for 1 hour before transfer into media containing the respective drugs at 0.2X, 0.3X, and 0.4X the MIC. As with the evaluation of SME, viable bacteria were quantified beyond 8 hours in instances where it was apparent that the delay in regrowth was prolonged. The observed PA-SME values for TR-700 were slightly elevated relative to the corresponding SME values. Against staphylococci, PA-SME values for TR-700 ranged from 0.70 to 3.86 hours for media containing 0.2X the MIC value, 0.90 to 8.47 hours for media containing 0.3X the MIC value, and 1.75 to 14.52 hours for media containing 0.4X the MIC value. PA-SME values for TR-700 were consistently longer than those observed with linezolid against staphylococci. Against enterococci, PA-SME values for TR-700 ranged from 0.05 to 1.10 hours for media containing 0.2X the MIC value, 0.85 to 2.30 hours for media containing 0.3X the MIC value, and 1.20 to 4.10 hours for media containing 0.4X the MIC value. At 0.4X the MIC value, the PA-SME of TR-700 was longer than linezolid for 2 of 3 evaluated strains.

To summarize, the Applicant stated that the PAE of TR-700 at suprainhibitory concentrations was shorter than that of linezolid when evaluated at 4X the MIC value against staphylococci and enterococci. Methicillin resistance among staphylococci or vancomycin resistance among enterococci didn't appear to have an impact on the PAE, SME, or PA-SME of TR-700 or linezolid.

Effect of Panton-Valentine Leukocidin Toxin on MIC

The expression of Panton-Valentine Leukocidin toxin (PVL) has been linked to community-acquired *S. aureus*, the most prevalent pathogen of acute bacterial skin and skin structure infections (ABSSSIs). The impact of this virulence factor on the activity of TR-700 and linezolid against *S. aureus* was evaluated in study PHA-08-0701-010, the results of which are summarized in **Table 74**. The susceptibility of 50 clinical isolates, which varied in the presence of genes responsible for the production of PVL, to TR-700 and linezolid, was evaluated by broth microdilution. There was no apparent difference in activity of either TR-700 or linezolid based on PVL status assessed by MIC50 or MIC90 values. PVL status did correlate with methicillin-resistance; 96% of PVL-positive strains were methicillin-resistant compared with 16% of PVL-negative strains.

Table 74: Impact of PVL on the Activity of TR-700 and Linezolid

Drug	PVL-status	MIC range	MIC_{50}	MIC_{90}
TR-700	positive (n=25)	0.25 - 1	0.25	0.25
	negative (n=25)	0.25 – 0.5	0.25	0.5
Linezolid	positive (n=25)	2 - 2	2	2
	negative (n=25)	1 - 2	2	2

Source: PHA-08-0701-010 Table 1
Abbreviations: MIC=minimum inhibitory concentration in µg/mL; MIC_{50}=minimum inhibitory concentration against 50% of isolates; MIC_{90}=minimum inhibitory concentration against 90% of isolates; PVL=Panton-Valentine Leukocidin, n=number

Effect of Phenol-Soluble Modulin (PSM) Expression

In another experiment, the impact of subinhibitory concentrations of TR-700 and clindamycin on the expression of PSMα1-4 from *S. aureus* was evaluated in PVL-positive MSSA and MRSA skin infection isolates (Yamaki 2011). Briefly, after evaluating the baseline expression of PSM, isolates were subdivided based on level of PSM expression, which varied by strain and PSM type. A subset of isolates was evaluated for PSM expression after exposure to either TR-700 or clindamycin at 0.12X, 0.25X, and 0.5X the MIC value. Quantitation of PSMα1-4 was accomplished with mass spectrometry. In addition, the transcription of the global virulence gene regulators responsible for PSM expression, RNAIII and *agr*A, was evaluated by real-time-PCR. TR-700 at 0.5X the MIC value had an overall inhibitory effect on PSM production, with the most inhibition observed with PSMα3.The data for PSM production at 0.12X and 0.25X the MIC value were more variable and frequently comparable to baseline values. However, for many of the strains, growth kinetics were negatively impacted during incubation with these agents at subinhibitory MIC values, a factor which could have influenced these results.

The Applicant noted that the expression of PSM by *S. aureus* was inhibited by TR-700 and clindamycin at 0.5X the MIC value. The inhibitory effect on PSM production is consistent with the inhibition of in vitro protein synthesis by TR-700 where TR-700 resulted in detectable inhibition of protein synthesis at concentrations 16- to 32-fold lower than the *S. aureus* MIC value. Greater than 50% inhibition of protein synthesis was observed at concentrations 4- to 8-fold below the TR-700 MIC value. However, whether the effects of concentrations lower than 0.5X the MIC value were due directly to TR-700 or clindamycin activity or to decrease rates of

growth observed with some strains is not clear. The clinical relevance of these findings has not been determined.

In vitro Activity of TR-700 Metabolites

In another experiment, the in vitro activity of TR-700 and its metabolites was evaluated against staphylococci, enterococci, streptococci, *H. influenzae*, and *E. coli*. According to the Applicant, TR-700 is excreted primarily as a sulfate conjugate (Sulfate-TR-700; detected at 10% of the dose in urine, 69% in feces), with only two other minor metabolites of TR-700 detectable in excreta (Desmethyl-TR-700 detected at 1% of the dose in urine, not detected in feces; Carboxy-TR-700 detected in 8% of the dose in urine and feces combined).

The resulting MIC values of TR-700 and TR-700 metabolites are shown in Table 75 below. The carboxy analog was inactive (MIC values >128 mcg/mL). The desmethyl metabolite exhibited a very low level of activity (MIC values 8- to 32-fold higher than TR-700). The desmethyl metabolite is said to be present in urine at very low levels; therefore, it is not anticipated to have an impact on microbial flora within the gastrointestinal tract. The sulfate sample contained 7% TR-700 parent molecule. The data suggest that TR-700 metabolites are not believed to contribute to the activity of TR-701.

Table 75: MIC Values (mcg/mL) of TR-700 and TR-700 Metabolites

Species	Strain	TR-700	Desmethyl–TR-700	Sulfate–TR-700	Carboxy–TR-700	LZD	VAN
Staphylococcus aureus	ATCC 33591	0.5	8	16	>128	2	0.5
S. aureus	ATCC 13709	0.5	8	16	>128	2	0.5
S. aureus	ATCC 13709 + serum	0.5	16	16	>128	2	0.5
S. aureus	RN4220	0.25	8	16	>128	2	0.5
Staphylococcus epidermidis	ATCC 12228	0.25	8	8	>128	1	1
Enterococcus faecalis	ATCC 29212	1	16	32	>128	4	1
Enterococcus faecium	ATCC 19434	0.5	16	16	>128	2	1
E. faecium	ATCC 700221	0.5	8	16	>128	1	>128
Streptococcus pneumoniae	ATCC 49619	0.25	4	8	>128	1	0.12
S. pneumoniae	ATCC 51916	0.25	8	16	>128	2	0.25
Streptococcus pyogenes	ATCC 19615	0.25	2	8	>128	2	0.25
Haemophilus influenzae	ATCC 49247	32	32	128	>128	16	128
Escherichia coli	ATCC 25922	>32	>128	>128	>128	>128	>128

Source: PHA-12-0701-055 Table 1
Abbreviations: ATCC=American Type Culture Collection; LZD=linezolid; MIC=minimum inhibitory concentration; VAN=vancomycin

Effect on Human Intestinal Flora

A double-blind, placebo-controlled, multiple dose, safety, tolerability and pharmacokinetic study (Module 5.3.3.3 CSR 16102 Phase 1 MAD, Safety, PK [Bayer Japan]) of IV and tablet formulation of TR-701 FA (BAY 1170438) in Japanese male subjects was conducted as part of the Phase 1 clinical program. In this study, a secondary objective was to assess the influence of TR-700 on intestinal microflora. A 200 mg once daily dose of TR-701FA was administered IV (over 60±2 min (cohort 1) with saline as placebo or orally (cohort 2) with a placebo tablet. The duration of treatment was 7 days for each cohort. Whole fecal samples were collected three times during the study period: pre-dose (Day -2 to pre-dose Day 0), during treatment (between Day 4 and Day 6), and post-dose (within 14-21 days after last dose administered).

All collected samples were held under anaerobic conditions and refrigerated, then forwarded to the laboratory for isolation and identification of the following: aerobic bacteria, anaerobic bacteria, *Enterobacteriaceae,* non-glucose fermentative gram-negative rods, *P. aeruginosa, Staphylococcus, Streptococcus, Enterococcus, Bacillus, Corynebacterium,* yeasts, *Bifidobacterium, Eubacterium, Lactobacillus, Clostridium* (lecithinase +), *Clostridium* (lecithinase-), *Clostridium difficile, Peptostreptococcus*, *Bacteroides, Fusobacterium, Veillonella,*and *Megasphaera.* Total bacteria count and the presence of *Clostridium difficile* toxin was also measured. For the determination of TR-700 concentrations in feces, a part of each fecal sample was forwarded to the bioanalysis laboratory. If the amount of collected sample was less than 5 g, the measurement of the bacterial number was prioritized. All subjects underwent the intestinal flora tests three times as planned. In all subjects in the TR-701 FA groups, fecal TR-700 was detected during the study treatment and the concentration at post-dose returned to below the lower level of quantitation (LLOQ). There was no sign indicative of microbial flora substitution after multiple IV or oral TR-701 FA doses of 200 mg once daily over 7 days in Japanese healthy male subjects.

PROPOSED INTERPRETIVE CRITERIA FOR TR-700 SUSCEPTIBILITY TESTING

In a previous submission, the Applicant proposed MIC in vitro susceptibility interpretive criteria against the target pathogens before they conducted the Phase 3 clinical trials. Tedizolid MIC and disk diffusion provisional breakpoints were proposed based upon the population distribution analysis. However, definition of final interpretive breakpoints requires the analysis of additional data collected during the TR-701 clinical program. These data include the MIC frequency distributions for clinical trial isolates of the target bacterial species, PK/PD information (including Monte Carlo simulation), and clinical and microbiological response rates based upon MIC and zone diameter values. These data are summarized in the following sections, and contribute to the proposed TR-700 interpretive breakpoints. Interpretive breakpoints for Tedizolid were determined according to CLSI guidelines available at the time the study was conducted.

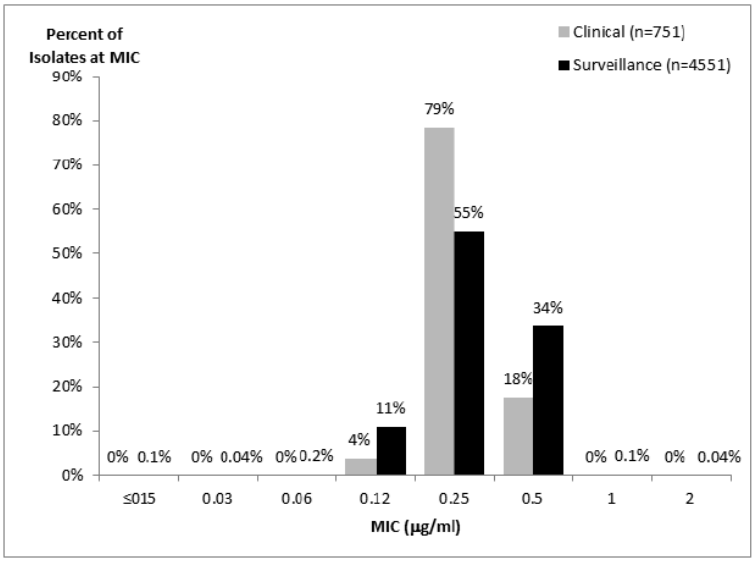
MIC Frequency Distributions:

The susceptibility of clinical trial and surveillance isolates to TR-700 for all target organisms (except *S. anginosus* Group which was not included in surveillance studies), is presented in this section as side-by-side MIC frequency distributions. The distributions, presented as histograms, are composed of TR-700 MIC values for isolates in the 3 clinical efficacy studies that used a central microbiology reference laboratory (Phase 2 Study TR701-104: 200 mg dose only; and both treatment arms of Phase 3 Study TR701-112, and Phase 3 Study TR701-113) and the combined 2011 and 2012 surveillance studies for TR-700.

Staphylococcus spp., All

The distribution of TR-700 MIC values for *Staphylococcus* spp. overall (n=5302) and for the clinical studies (n =751) was very similar to that observed for surveillance study isolates (n=4551) (Figure 34). The majority of these isolates were *S. aureus* (n=5224), with 38 *S. haemolyticus* and 40 *S. lugdunensis*. A unimodal distribution of TR-700 MIC values was evident with the most of clinical and surveillance isolates falling in the range of 0.12 to 0.5 mcg/mL. The clinical and surveillance isolates had identical MIC90 values of 0.5 mcg/mL.

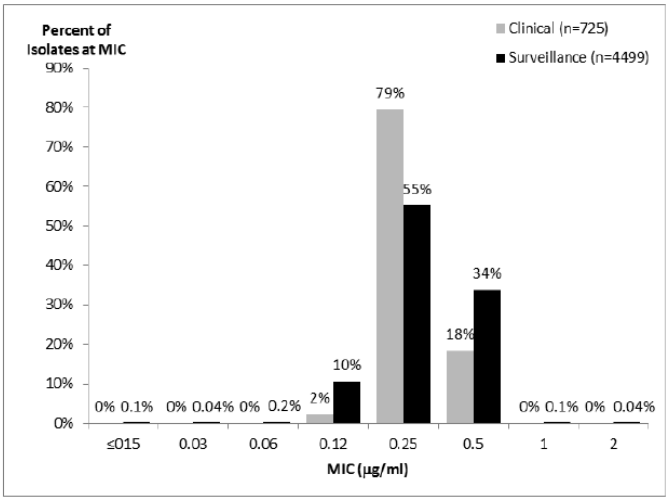
Figure 34: *Staphylococcus* spp.: MIC Population Distribution for Clinical Trial and Surveillance Isolates (n = 5302)



Source: Derived from *Scatterplot/Histogram Database v10 02JUL2013 – Bouchillon*, as described in TR701-022.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration
Note: Includes 5224 *S. aureus*, 38 *S. hemolyticus*, 40 *S. lugdunensis*

For 5224 isolates of *S. aureus* (including 3128 MSSA, 2089 MRSA, 7 LRSA), the distribution of TR-700 MIC values for the clinical trial isolates (n=725) was similar to that of the surveillance study isolates (n=4499) (Figure 35). The ratio of MSSA to MRSA in this dataset was approximately 60:40. As expected, the distribution of MIC values for *S. aureus* was nearly identical to that for *Staphylococcus* spp. since *S. aureus* accounted for ~98% of the isolates in the *Staphylococcus* spp. dataset. A unimodal distribution of TR-700 MIC values was evident with the vast majority of clinical and surveillance isolates falling in the range of 0.12 to 0.5 mcg/mL. The clinical and surveillance isolates had identical MIC90 values of 0.50 mcg/mL.

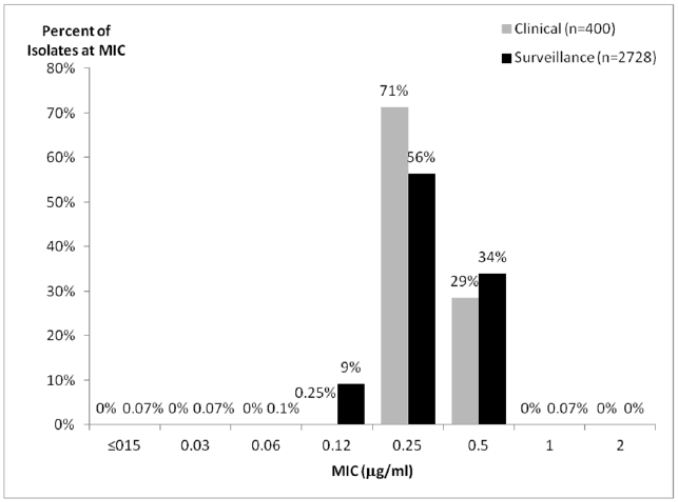
Figure 35: *Staphylococcus aureus*: MIC Population Distribution for Clinical Trial and Surveillance Isolates (n = 5224)



Source: Derived from *Scatterplot/Histogram Database v10 02JUL2013 – Bouchillon*, as described in TR701-022.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration
Note: Includes 2089 MRSA, 3128 MSSA, 7 LRSA

In another analysis, it was noted that the distribution of TR-700 MIC values for the MSSA clinical trial isolates (n=400) was similar to that of the surveillance study isolates (n=2728). A unimodal distribution of MIC values was observed with the vast majority of isolates in the range of 0.12 to 0.5 mcg/ml (MIC50/90 =0.25/0.5 mcg/mL) was observed for both clinical trial and surveillance. TR-700 MIC values for the MSSA clinical trial isolates (n=400) were similar to that of the surveillance study isolates (n=2728). A unimodal distribution of MIC values was observed with the vast majority of isolates in the range of 0.12 to 0.5 mcg/ml (MIC50/90 =0.25/0.5 mcg/mL) for both clinical trial and surveillance studies. The percentage of isolates at each concentration was similar for the clinical trial and surveillance isolates (Figure 36).

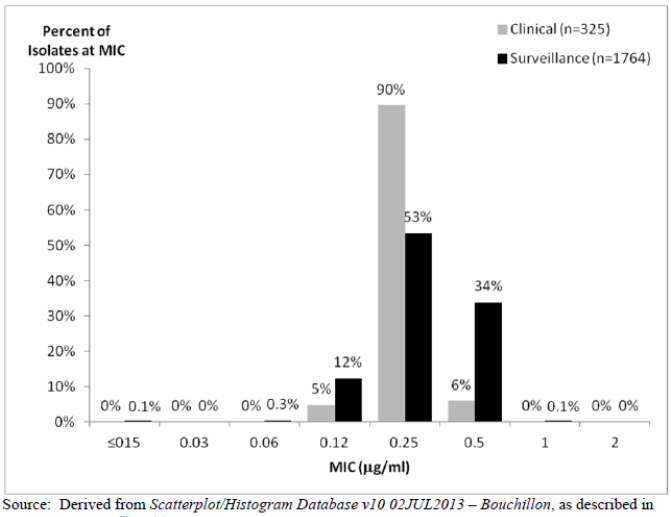
Figure 36: MSSA: MIC Population Distribution for Clinical Trial and Surveillance Isolates (n = 3128)



Source: Derived from *Scatterplot/Histogram Database v10 02JUL2013 – Bouchillon*, as described in TR701-022.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration

Against a total of 325 clinical isolates and 1764 surveillance of MRSA (Figure 37), a unimodal distribution of MIC values was observed, with the vast majority of values in the range of 0.12 to 0.5 mcg/mL. Within this relatively narrow range of MIC values, some differences in the distributions were observed. There was a higher percentage of surveillance than clinical isolates at 0.5 mcg/ml and a lower percentage at 0.25 mcg/mL. The 2-fold difference in MIC90 value is apparent in the figure at the 0.5 mcg/ml concentration (6% of clinical isolates versus 34% of surveillance isolates). The differences observed for the clinical and surveillance isolates of MRSA, while notable, all occurred within a narrow range of MIC values.

Figure 37: MRSA: MIC Population Distribution for Clinical Trial and Surveillance Isolates (n = 2089)

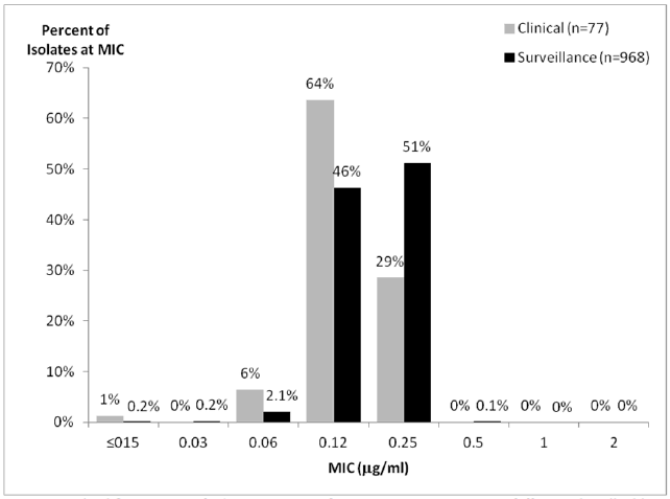


Source: Derived from *Scatterplot/Histogram Database v10 02JUL2013 – Bouchillon*, as described in TR701-022.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration

Streptococcus spp. β-hemolytic Group, All

Interpretation of MIC test values for streptococci are subject to different criteria depending upon the group of streptococci being considered (CLSI M100-S23 2013). One such grouping is "*Streptococcus spp.* β-hemolytic Group" which includes the TR-700 key target organisms *S. pyogenes* and *S. agalactiae*. The distribution of TR-700 MIC values for these organisms for the clinical trial isolates (n=77) was similar to that for the surveillance study isolates (n=968) (Figure 38). The majority of the isolates was *S. pyogenes* (44%) and *S. agalactiae* (52%), with 38 isolates of *S. dysgalactiae* (4%). The vast majority of the MIC values fell in the relatively narrow range of 0.06 to 0.25 mcg/mL. MIC50/90 values of 0.12/0.25 mcg/ml were observed for the clinical trial isolates and 0.25/0.25 mcg/ml for the surveillance isolates. Overall, the MIC population distribution for the clinical trial isolates was similar to that of the surveillance isolates.

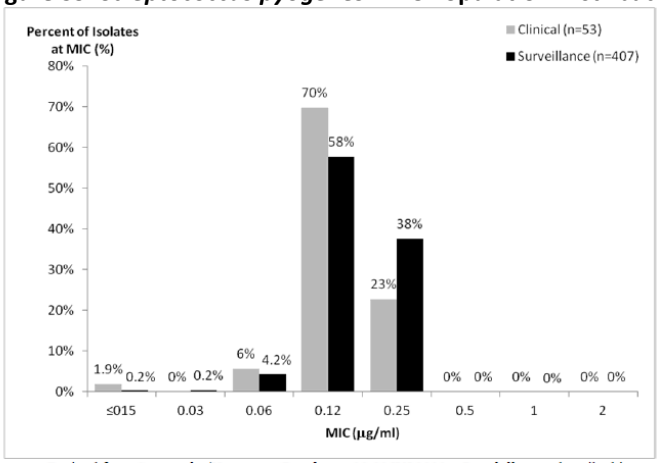
Figure 38: *Streptococcus* spp. β-hemolytic Group: MIC Population Distribution for Clinical Trial and Surveillance Isolates (n = 1045)



Source: Derived from *Scatterplot/Histogram Database v10 02JUL2013 – Bouchillon*, as described in TR701-022.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration
Note: Includes 547 *S. agalactiae*, 38 *S. dysgalactiae*, 460 *S. pyogenes*

A separate breakout of the 460 *S. pyogenes* isolates from **Figure 38** is presented in Figure 39. A unimodal distribution of MIC values was observed with the vast majority of isolates within the MIC range of 0.06 to 0.25 mcg/mL.

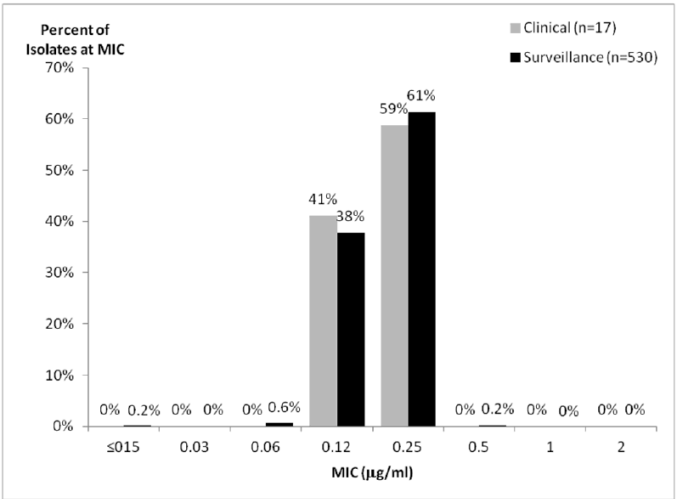
Figure 39: *Streptococcus pyogenes*: MIC Population Distribution for Clinical Trial and Surveillance Isolates (n = 460)



Source: Derived from *Scatterplot/Histogram Database v10 02JUL2013 – Bouchillon*, as described in TR701-022.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration

A separate breakout of the 547 *S. agalactiae* isolates from Figure 64 is presented in Figure 40. The distribution of TR-700 MIC values for the *S. agalactiae* clinical trial isolates (n=17) was similar to that of the surveillance study isolates (n=530). A unimodal distribution of MIC values was observed with the vast majority of isolates within the MIC range of 0.12 to 0.25 mcg/mL. The MIC population distributions for the clinical trial isolates were similar to that of the surveillance isolates.

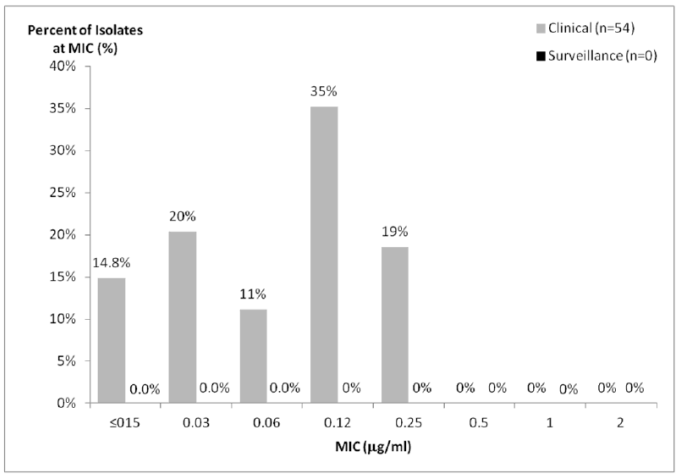
Figure 40: *Streptococcus agalactiae*: MIC Population Distribution for Clinical Trial and Surveillance Isolates (n =547)



Source: Derived from *Scatterplot/Histogram Database v10 02JUL2013 – Bouchillon*, as described in TR701-022.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration

A second interpretive grouping for streptococci is "*Streptococcus* spp. Viridans Group" (CLSI-M100-S23 2013). Included within this grouping are a number of species implicated in ABSSSI, namely the "*S. anginosus* Group". This group is composed of *S. anginosus*, *S. constellatus*, and *S. intermedius*. The MIC population distribution for 54 clinical trial isolates of *S. anginosus* Group is shown in Figure 41. Since the surveillance studies did not include this organism, only the clinical trial isolates are available. MIC values for these organisms fell within the range of ≤0.015 to 0.25 mcg/mL, with no isolates above 0.25 mcg/mL.

Figure 41: *Streptococcus anginosus* Group: MIC Population Distribution for Clinical Trial Isolates (n=54)



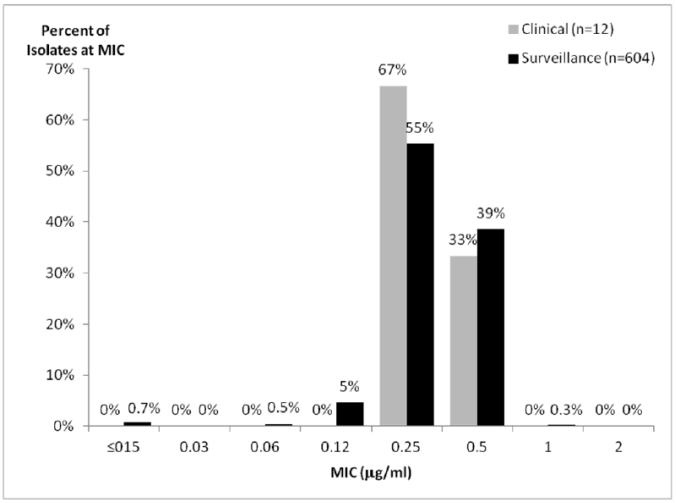
Source: Derived from *Scatterplot/Histogram Database v10 02JUL2013 – Bouchillon*, as described in TR701-022.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration

E. faecalis, Vancomycin-susceptible isolates only

TR-700 has demonstrated effective activity against both vancomycin-susceptible and -resistant enterococci in

preclinical and surveillance studies, the only species and phenotype represented in the clinical program was vancomycin-susceptible *E. faecalis* (VSE). Therefore, in this analysis, examination of enterococci is limited only to isolates of VSE (n=616). While the number of surveillance is large (n=604), the analysis is limited by the contribution of only 12 isolates from the clinical program. It was worthwhile to examine how the MIC distribution of the 12 isolates compared to that of the 604 surveillance isolates (Figure 42). The distribution of TR-700 MIC values for the clinical trial isolates was similar to that of the surveillance isolates. The vast majority of the isolates fell within the MIC range of 0.12 to 0.5 mcg/mL.

Figure 42: *E. faecalis* (vancomycin-susceptible isolates): MIC Population Distribution for Clinical Trial and Surveillance Isolates (n=616)



Source: Derived from *Scatterplot/Histogram Database v10 02JUL2013 – Bouchillon*, as described in TR701-022.0, on file with sponsor.
Abbreviations: MIC=minimum inhibitory concentration

Conclusions

The MIC population distributions demonstrate the susceptibility profile of TR-700 for the key target organisms. The distributions demonstrate that the susceptibilities of the TR-701 clinical trial isolates appear similar to those of the surveillance isolates.

HUMAN AND ANIMAL STUDIES

Animal therapeutic and Pharmacological Studies:

The Applicant has submitted data from a variety of animal models, including lethal systemic infections, skin/soft tissue infections, and pneumonia in mice; skin/soft tissue infection and pneumonia in rats; and endocarditis in rabbits. In general, infections were initiated by injecting the pathogen (in a vehicle containing 5% mucin) in sufficient numbers to kill 100% of the animals if left untreated. Animals were rendered neutropenic with cyclophosphamide treatment prior to enterococcal infections and prior to some MRSA infections. Antibiotic treatment was typically administered as a single dose of TR-701 or comparator given

orally or IV one hour postinfection. Both routes of administration were used in each study. Mortality was usually assessed over 7 days, and the dose of antibiotic required to protect 50% of the infected animals (Effective Dose 50 [ED_{50}]) was calculated. Additionally, time-kill studies were conducted in mice and summarized below.

Staphylococcal Systemic Infections in mice

The in vivo antibacterial efficacy of TR-700 was evaluated in mice systemically infected with MSSA or MRSA. Briefly, two strains of MSSA and 3 strains of MRSA were selected for this study. Four to 5 dosage groups were used for each strain, with 8 male Institute of Cancer Research mice (18-20 g) assigned to each group. Mice were given an intraperitoneal injection of S. *aureus* (2×10^7 to 1×10^8 colony forming units per mouse) suspended in 5% mucin to initiate the infection. One hour post-infection, a single dose of TR-701 or linezolid (both dissolved in distilled water) was administered intravenously (IV) or orally (PO). TR-701 was evaluated at doses ranging from 1.1 to 30 mg/kg when administered PO or IV versus all strains with the exception of MSSA Smith, which was evaluated over a dose range of 1.25 to 20 mg/kg. Linezolid was tested at dose ranges of 3.3 to 30 mg/kg (PO) and 1.1 to 30 mg/kg (IV) versus all strains with the exception of MSSA Smith, which was evaluated over a dose range of 2.5 to 20 mg/kg (PO and IV). Mice were monitored over 7 days for mortality. The total number of survivors in each dose group was used to calculate the effective dose that protected 50% of the infected mice from death (ED_{50}). The ED_{50} for TR-701 ranged from 3.2 mg/kg to 7.6 mg/kg for PO administration and from 1.5 mg/kg to 4.3 mg/kg for IV administration (Table 76). When compared to linezolid, TR-701 was up to 4 times more effective when administered orally, and as high as 8 times more effective when administered intravenously.

Table 76: Results of protection test by *Staphylococcus aureus*

Strain	Drug	Route	ED_{50} (mg/kg)	95% LIMITS
MRSA 98-12-P85 ($1.0 X10^8$ CFU/head)	DA-70218	p.o.	3.2	1.8-5.7
		i.v.	1.5	1.1-4.5
	Linezolid	p.o.	9.6	5.3-17.3
		i.v.	7.7	3.4-22.3
MRSA M126 ($1.0 X10^8$ CFU/head)	DA-70218	p.o.	3.7	2.2-6.2
		i.v.	2.8	1.6-4.8
	Linezolid	p.o.	15.6	9.1-27.6
		i.v.	12.9	7.6-22.3
MSSA 2923 ($1.0 X10^8$ CFU/head)	DA-70218	p.o.	5.5	2.9-10.3
		i.v.	4.2	2.2-7.7
	Linezolid	p.o.	16.0	7.1-93
		i.v.	20.8	-
MSSA 97-4-2022 ($4.0X10^7$ CFU/head)	DA-70218	p.o.	5.0	2.8-9.0
		i.v.	3.3	1.6-6.0
	Linezolid	p.o.	21.4	11.4-43.5
		i.v.	29.1	13.7-68.8
MSSA smith ($2.0 X 10^7$ CFU/head)	DA-70218	p.o.	7.6	5.2-11.4
		i.v.	4.3	2.9-6.3
	Linezolid	p.o.	12.4	8.1-19.8
		i.v.	16.7	10.1-29.5

Efficacy of TR-701 versus the Linezolid- and Methicillin Resistant S. aureus cfr+ Strain CM05 in Mice

In another experiment, the antibacterial activity of TR-701 was evaluated in vivo versus the linezolid-resistant MRSA strain CM05 (PHA-08-0701-002). Briefly, *S. aureus* CM05 carries the *cfr* gene encoding a 23S rRNA methyltransferase that confers resistance to linezolid as well as chloramphenicol and clindamycin through modification of a ribosomal region involved in antibiotic binding. In vivo results are summarized below MIC values against *S. aureus* CM05 were 0.5 mcg/ml for TR-700 and 8 mcg/ml for linezolid (resistance to linezolid is defined as an MIC value >4 mcg/mL) (Table 77). The activity of TR-701 was evaluated in a neutropenic mouse septicemia model. Female DBA/2 mice were rendered neutropenic with cyclophosphamide 4-days and also 1-day prior to infection, and then inoculated IP with *S. aureus* CM05 (in 5% mucin). TR-701 or linezolid were administered orally immediately following infection. The mice were divided into 8 treatment groups (4 TR-701 dose levels and 4 linezolid dose levels) and an untreated control group. The animals were monitored for 24 hours for survival; any surviving animals were euthanized at that time. Results of this study are shown in **Table 77**. Single oral-dose administration of 20 mg/kg TR-701 provided 100% protection 24 hours after dose administration. Linezolid administered at 50 mg/kg protected 9 of 10 animals after 24 hours, although the animals appeared very sick at this time. A re-evaluation of the in vitro susceptibility of the MRSA CM05 recovered from infected animals confirmed a resistance profile identical to the pre-infection strain.

Table 77: TR-701 and Linezolid Efficacy in an Experimental Systemic Infection Induced with a *cfr+* MRSA Strain in Mice

Organism (Strain)	Bacterial Challenge (CFU/mouse)	Treatment Schedule and Route	Study Drug and Dose (mg/kg)		Survival (%) 24 hours post infection		Source
					survived/group	MIC (µg/mL)[b]	
Staphylococcus aureus CM05 MRSA LZD-R (*cfr*+)	10 DBA/ 2 mice per group; 9 groups 2.2×10^7 in 5% mucin, IP		TR-701	In vitro	Prior to infection	0.5	PHA-08-0701-002 Table 3 and Table 4
			Linezolid	In vitro	Prior to infection	8	
		Cyclophosphamide[a] IP				Post-tx MIC	
		Immediately postinfection single-dose oral	TR-701	1	0/10	0.5	
				5	1/9		
				10	3/10		
				20	10/10		
		Immediately postinfection single-dose oral	Linezolid	5	0/10	8	
				10	2/10		
				20	1/10		
				50	9/10		
			Untreated	-	0/10	-	

Abbreviations: cfr=chloramphenicol-florfenicol-resistance gene encoding a 23S rRNA methyltransferase; CFU=colony-forming unit; IP=intraperitoneal; LZD-R=linezolid-resistant; MIC=minimum inhibitory concentration; MRSA=methicillin-resistant *Staphylococcus aureus*; Post-tx MIC=MIC values for MRSA strains isolated from the animals after animals received a single dose of either TR-701 or linezolid
[a]Animals were rendered neutropenic by treatment with cyclophosphamide 150 mg/kg 4 days & 100 mg/kg IP 1 day prior to infection.
[b]TR-700 or linezolid minimum inhibitory concentrations.

Coagulase-Negative Staphylococcal Systemic Infections in Mice

In this experiment, the Applicant investigated the in vivo antibacterial efficacy of TR-701 (lot no. OXA-04-001) was evaluated and compared to linezolid to assess the antibacterial activity against methicillin-resistant, coagulase-negative *Staphylococcus aureus* (MRCNS) in systemic mouse infection (Note: TR-701 is referred to as DA-7218 throughout the report). Briefly, three strains of MRCNS were selected for this study, 01-8-P1 37, 01 -8-P213, and 01-8-C172. Three or four dosage groups were used for each strain, with 8 male Institute of Cancer Research mice (18-20 g) assigned to each group. Infections were initiated by intraperitoneal injection of approximately 1 x 10^8 colony forming units of MRCNS suspended in 5% mucin. At one hour post-infection, a single dose of TR-701 (in distilled water) or linezolid (in distilled water) was administered intravenously (IV) or orally (PO). Mice infected with MRCNS 01-8-P137 were treated TR-701 and linezolid at doses ranging from 1.1 to 30 mg/kg. Mice infected with the two other strains were treated with linezolid over a dose range of 3.3 to 30 mg/kg or TR-701 over a dose range of 1.48 to 40 mg/kg. Mice were monitored over 7 days for mortality. The results of the study are shown in Table 78. The total number of survivors at each dose was used to calculate the effective dose that protected 50% of the infected mice from death (ED_{50}). The ED_{50} of TR-701 ranged from 2.01 mg/kg to 3.25 mg/kg for PO administration and from 0.46 mg/kg to 1.29 mg/kg for IV administration. When compared to linezolid, TR-701 was more effective when administered orally and intravenously (Table 78).

Table 78: Effective Dose50 Values of TR-701 and Linezolid Against Experimentally Induced Systemic Infections in Animals (MRCNS)

Organism (Strain)	Bacterial Challenge[a] (CFU/mouse)	Treatment Schedule and Route	Study Drug and Route		Protective Effect	
					ED_{50}[b] (mg/kg)	MIC[c] (µg/mL)
CoNS 01-8-P137 MR	1×10^8	1 hour postinfection single dose oral or IV	TR-701	Oral	2.56 (0.85-4.80)	0.25
				IV	0.93 (0.05-4.78)	
			Linezolid	Oral	5.77 (2.83-10.51)	1
				IV	6.56 (3.23-11.94)	
CoNS 01-8-P213 MR	1×10^8		TR-701	Oral	3.25 (1.77-5.75)	0.5
				IV	1.29 (0.55-2.66)	
			Linezolid	Oral	7.48 (3.98-13.57)	2
				IV	5.34 (2.62-10.04)	
CoNS 01-8-C172 MR	1×10^8		TR-701	Oral	2.01 (0.94-3.83)	0.25
				IV	0.46 (0.03-3.98)	
			Linezolid	Oral	2.41 (1.18-4.49)	1
				IV	1.80 (0.82-3.51)	

Systemic Enterococcal Infections in Mice

The in vivo antibacterial efficacy of TR-701 was evaluated in mice systemically infected with vancoymcin-susceptible or vancomycin-resistant enterococci (Note: TR-701 is referred to as DA-7218 throughout the report). The following strains of enterococci were selected for this study: *Enterococcus faecium* 5932, vancomycin- resistant *E. faecium* (VREF) 99-4-C159, VREF 98-7-1074, *E. faecalis* 00-4-U518, and *E. faecalis* 00-4-R417. Four or five dose groups were tested per strain, with 8 male Institute of Cancer Research mice (18-20 g) assigned to each group. Mice were rendered neutropenic by subcutaneous administration of cyclophosphamide (100 mg/kg body weight) 1 and 3 days prior to infection. Infections were initiated by intraperitoneal injection of enterococci (1.4×10^5 to 4×10^7 colony forming units per mouse) suspended in 5% mucin. One hour post-infection, a single dose of TR-701 or linezolid was administered intravenously (i.v.) or orally. Doses tested against strain VREF 99-4-C159 ranged from 1.1 to 30 mg/kg for both TR-701 and linezolid. All other strains were dosed with TR-701 at test concentrations ranging 2.5 to 40 mg/kg or linezolid at 5.0 to 40 mg/kg. Mice were monitored over 7 days for mortality. The total number of survivors at each dose was used to calculate the effective dose that protected 50% of the infected mice from death (ED_{50}). The results are summarized in the following table (Table 79).

The ED_{50} range of orally administered TR-70 I against enterococci was 4.3 to l l.3 mg/kg compared to 17.6 to 25.9 mg/kg for linezolid. When administered i.v., the ED_{50} range for TR-701 against enterococci was 2.2 to 9.1 mg/kg, compared to 11.1 to > 40 mg/kg for linezolid.

Table 79: ED_{50} of TR-701 and Linezolid Against Enterococci Systemic Infections in Neutropenic Mice

	TR-701 ED_{50} (mg/kg)		Linezolid ED_{50} (mg/kg)	
Pathogens	Oral	Intravenous	Oral	Intravenous
E. faecium 99-4-C159	11.30	8.93	25.88	> 30
E. faecium 5932	4.32	2.21	19.70	37.80
E. faecium 98-4-1074	10.88	9.10	31.62	> 40
E. faecalis 00-4-U518	6.79	4.02	17.64	21.19
E. faecalis 00-4-R417	5.33	2.77	18.09	11.12

Streptococcal Systemic Infections in Mice

In another experiment, the antibacterial efficacy of TR-701 and linezolid against penicillin-resistant streptococcal systemic infections in mice were investigated. Four pneumococcal strains were chosen for the study: *S. pneumoniae* DR9, DR10, DR11, and DR14. The methodology and results of this study are summarized in **Table 80**. TR-701 was effective against all strains tested and was generally more potent than linezolid. The ED_{50} values for the oral route ranged from 3.19 to 11.53 mg/kg for TR-701 and were 2-fold lower than linezolid for 3 of the 4 strains tested. By the IV route, TR-701 ED_{50} values ranged from 3.52 to 10.19 mg/kg, which were 3- to 8-fold lower than linezolid for all 4 strains tested.

Table 80: ED_{50} Values of TR-701 and Linezolid against Streptococcal Experimentally Induced Systemic Infections in Animals

			Oral dose[a]					Intravenous dose[e]				
PRSP[b] isolate (no. of CFU/mouse)	Antimicrobial agent	*In vitro* MIC[c] (μg/ml)	No. (%) of mice surviving after administration of dose (mg/kg/day) of: 1.48	4.44	13.33	40	ED_{50} (mg/kg/day)[d]	No. (%) of mice surviving at day 7 after administration of dose (mg/kg/day) of: 1.48	4.44	13.33	40	ED_{50} (mg/kg/day)[d]
PRSP DR9 (5×10^4)	Tedizolid phosphate	0.125	0 (0.0)	2 (25.0)	8 (100)	8 (100)	5.70 (3.45–9.38)	1 (12.5)	3 (37.5)	7 (87.5)	8 (100)	4.89 (2.95–8.04)
	Linezolid	0.5	0 (0.0)	2 (25.0)	3 (37.5)	8 (100)	11.06 (6.73–18.22)	0 (0.0)	0 (0.0)	1 (12.5)	5 (62.5)	31.84 (18.16–59.08)
PRSP DR10 (7×10^3)	Tedizolid phosphate	0.125	1 (12.5)	6 (75.0)	7 (87.5)	8 (100)	3.19 (1.63–5.94)	2 (25.0)	3 (37.5)	8 (100)	8 (100)	3.52 (1.82–6.50)
	Linezolid	0.5	1 (12.5)	2 (25.0)	6 (75.0)	8 (100)	6.38 (3.48–11.55)	0 (0.0)	2 (25.0)	2 (25.0)	6 (75.0)	17.62 (9.51–34.24)
PRSP DR11 (2×10^4)	Tedizolid phosphate	0.125	0 (0.0)	1 (12.5)	7 (87.5)	8 (100)	7.63 (4.81–12.09)	0 (0.0)	0 (0.0)	6 (75.0)	8 (100)	10.19 (6.38–16.14)
	Linezolid	0.5	0 (0.0)	1 (12.5)	3 (37.5)	7 (87.5)	14.85 (6.38–16.14)	0 (0.0)	0 (0.0)	1 (12.5)	5 (62.5)	30.83 (18.93–52.29)
PRSP DR14 (2.5×10^4)	Tedizolid phosphate	0.125	0 (0.0)	0 (0.0)	5 (62.5)	8 (100)	11.53 (3.90–32.77)	0 (0.0)	1 (12.5)	5 (62.5)	8 (100)	10.01 (3.56–28.53)
	Linezolid	0.5	0 (0.0)	1 (12.5)	3 (37.5)	8 (100)	12.98 (4.65–37.90)	0 (0.0)	1 (12.5)	1 (12.5)	2 (25)	39.53 (9.98–233.33)

[a] $n = 8$ mice per dose group. Survival data are for day 7 after infection.
[b] Penicillin resistance was determined on the basis of the oral penicillin resistance MIC breakpoint for nonmeningitis pneumococcal isolates (>2 μg/ml).
[c] The MICs reported are for tedizolid, which is formed *in vivo* after administration of its prodrug, tedizolid phosphate.
[d] Data in parentheses are 95% CIs.

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Effect of TR-701 against MRSA in mouse skin and soft tissue infection

The in vivo efficacy of TR-70 1 (lot no. Oxa-04-001) against methicillin-resistant *Staphylococcus aureus* (MRSA) M126 was assessed in 2 skin and soft tissue mouse infection models (Note: TR-701 is referred to as DA-7218 throughout the report). Male Institute for Cancer Research (ICR) mice (18 to 20 g) were rendered neutropenic by intraperitoneal injection of cyclophosphamide. Approximately 4.5 x 10^4 colony forming units (CFU) of MRSA M126 in a 0.1 mL saline suspension were introduced into the right thigh of each mouse. Two hours post-infection, TR-701 in distilled water or linezolid in distilled water (for comparison) was administered orally once- or twice-daily to groups containing 5 animals each at doses of 5, 10, or 20 mg/kg for 2 days. Viable bacterial counts in thigh homogenates were determined 24 and 48 hours post-administration and compared to untreated controls (Table 81). TR-701 treatment reduced CFU counts to levels ranging from 0.12 to 1 log below the starting inoculum with the exception of once-daily dosing at 5 mg/kg, where CFU counts were slightly increased above the initial inoculum. In contrast, linezolid-treated animals showed increased CFU counts ranging from 0.4 to 1.9 logs above the starting inoculum.

Table 81: Bacterial Counts 48 Hours Postinfection in Mice Infected with Methicillin-Resistant *Staphylococcus aureus* Strain M126

Dose Regimen	Dose (mg/kg)	Colony Forming Units log_{10}/mL (SD)			
		TR-701		Linezolid	
		Thigh Model	Air Pouch Model	Thigh Model	Air Pouch Model
-	Control	7.52 (0.11)	9.07 (0.21)	7.52 (0.11)	9.07 (0.21)
Once Daily	5	5.21 (1.36)	3.40 (0.25)	6.07 (0.51)	8.49 (0.35)
	10	4.60 (0.68)	2.44 (0.12)	5.62 (1.36)	7.12 (1.16)
	20	3.16 (0.14)	2.33 (1.08)	5.47 (0.47)	5.40 (1.29)
Twice Daily	5	5.42 (1.03)	5.00 (0.16)	7.44 (0.35)	8.41 (0.11)
	10	3.51 (0.17)	3.25 (0.23)	6.04 (1.51)	8.29 (0.11)
	20	3.17 (0.33)	2.84 (0.67)	4.69 (1.06)	3.60 (0.95)

Source: PHA-07-0701-057 Table 1 and Table 2
Abbreviations: SD=standard deviation

Briefly, air pouches were induced in male ICR mice by injecting 5 mL of sterile filtered air into loose connective dorsal tissue. Bacterial suspensions containing approximately 1 x 10^6CFU/mL of MRSA were introduced into the pouches 24 hours post-induction. Immediately post-infection, TR-701 or linezolid were administered orally once-or twice-daily at doses of 5, 10, or 20 mg/kg for 2 days. Viable bacterial counts in air pouch fluid were determined 24 and 48 hours post-administration and compared to untreated controls. In the untreated control group, viable bacterial counts after 24 hours at all doses, while TR-701 treatment reduced CFU. The data indicate that TR-701 is effective in the treatment of MRSA skin and soft tissue infections, when compared with linezolid at equivalent mg/kg doses.

In Vivo Pharmacodynamics of TR-701 Against Methicillin Susceptible and Methicillin-Resistant S. aureus Strains in a Mouse Thigh Infection Model

The in vivo pharmacodynamics of TR-701 in a neutropenic mouse thigh model of MRSA (ATCC 33591) infection in comparison with linezolid treatment was determined. The Applicant stated that the overall goal of the experiments was to identify the dose and schedule of TR-701 administration associated with optimal antibacterial effect. Forty-eight-hour dose ranging and 24-hour dose fractionation studies were conducted in association with single-dose PK determinations. A further dose-range experiment compared the efficacies of TR-701 and linezolid against an MSSA strain (ATCC 29213) and a community-associated MRSA strain (CA-MRSA) 6-8548A. TR-700 and linezolid MIC values were determined for these MSSA and MRSA strains using the broth macrodilution method (CLSI M7-A7 2006).

For the study, female Swiss Webster mice were rendered neutropenic and in support of PK/PD analyses, PK profiles were determined for TR-700 in neutropenic mice administered TR-701 (prodrug) doses associated with 10, 20, 40, or 80 mg/kg doses of the active moiety, TR-700 (referred to here as adjusted TR-701 doses). The mice were infected with MRSA ATCC 33591 (10^5 CFU in each posterior thigh muscle) 2 hours prior to IP administration of TR-701. Blood samples were collected for plasma concentration analysis at 0.5, 1, 2, 3, 4, 6, 8, and 10 hours after drug administration (n=2-3/time point/dose). Plasma AUC, Cmax, and time above the MIC value were calculated for each dose examined. The TR-700 MIC value was 0.5 mcg/ml for all strains tested; in the presence of 80% mouse serum and also tested in 80% human serum, the MIC values increased

2- to 4-fold to 1 and 2 mcg/mL, respectively. The linezolid MIC value was 2 mcg/ml for all strains tested; in the presence of mouse and human serum, the MIC value increased to 4 mcg/mL.

The dose-ranging experiment was performed to model the dose-effect relationship based on the dose at which 50% maximal kill was achieved and the microbial density associated with maximal microbial reduction. Neutropenic mice (n=10/group) were inoculated with 10^5 CFU/thigh of MRSA ATCC 33591; TR-701 was administered at adjusted IP doses ranging from 10 to 240 mg/kg/day as divided doses twice per day for 2 days. For comparison, linezolid was administered IP at 120 mg/kg/day in 2 divided daily doses for 2 days. Bacterial counts in thigh tissue were performed at 24 and 48 hours after the start of drug treatment. Adjusted TR-701 doses ≥80 mg/kg/day resulted in a 1.5 $\log_{10}$ CFU/g decrease in bacterial load between the 24 and 48 hour time points. Stasis and a 1-$\log_{10}$ CFU/g reductions in bacterial concentration in thigh tissue were associated with doses of 38.5 and 82.6 mg/kg/day, respectively, for the 24-hour time point. For the 48-hour time point, stasis and a 1-$\log_{10}$ CFU/g reduction were associated with doses of 33.6 and 44.4 mg/kg/day, respectively. These dosages correspond to *f*AUC/MIC ratios of 37.6 for stasis and 80.4 for a 1-$\log_{10}$ reduction (24 hour), and 32.8 and 43.3 (48 hour), respectively. Adjusted for protein binding the AUC/MIC ratios would be increased by a factor of 5 to 188 for stasis and 400 for a 1-$\log_{10}$ reduction (24 hour) and 164 and 216.5 (48 hour) for stasis and a 1-log10 reduction, respectively.

The Applicant stated that administration of linezolid at 120 mg/kg/day did not result in stasis (*f*AUC/MIC = 74.6). For the dose-fractionation study, adjusted IP doses of TR-701 identified from the flat portion of the initial dose-response curve (10 to 72 mg/kg) were administered to MRSA-infected neutropenic mice (10 per group; 9 treatment groups) either as a single daily dose or as divided doses 2 times or 4 times daily. Thigh tissue homogenates were prepared 24 hours after treatment to determine CFU counts, and the relationship between exposure and antibacterial activity was determined.

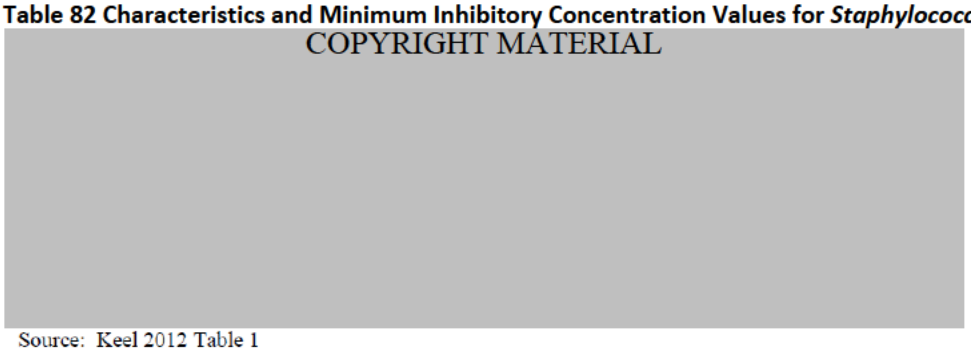
The results demonstrated that the area under the concentration-time curve over 24 hours divided by the MIC (AUC/MIC ratio) was the PD index for TR-701/TR-700 that was linked with efficacy. The target total and free drug AUC/MIC ratios were calculated as 250 and approximately 50, respectively. In the comparative dose ranging experiments with TR-701 and linezolid, neutropenic mice were infected with either 10^5 CFU of MSSA or CA-MRSA 6-8548A; one hour after bacterial challenge, mice were treated IP with TR-701 (adjusted) or linezolid doses of up to 150 mg/kg once daily for 2 days. Bacterial counts in thigh homogenates were determined at 24 or 48 hours after treatment. TR-701 was found to be highly active against MSSA and CA-MRSA, and the doses required to achieve 1 $\log_{10}$ and 2 $\log_{10}$ CFU/mL reductions were similar for the 2 strains. For the CA-MRSA strain, stasis was associated with adjusted TR-701 doses of 36.2 mg/kg/day and 39.8 mg/kg/day at 24 and 48 hours, respectively. For the MSSA strain, stasis was associated with adjusted TR-701 doses of 40.6 and 39.9 mg/kg/day at 24 and 48 hours, respectively. The Applicant stated that linezolid treatment did not result in stasis at either time point tested.

NDA: 205435/205436 DATE REVIEW COMPLETED: 02/24/2014
Tedizolid Phosphate (Sivextro)

Comparative Efficacy of Human Simulated Exposures of TR-701 and Linezolid for the Treatment of S. aureus in the Murine Thigh Model

A study was conducted to evaluate the efficacy of TR-701 and linezolid against MSSA and MRSA pathogens in a mouse thigh infection model. The principal goal of the study was to establish the efficacy profile of the two drugs at plasma exposures simulating those associated with TR-700 and linezolid administration in humans. Four MRSA strains (3 hospital-associated MRSA [HA-MRSA] including one vancomycin-resistant [VRSA], one CA-MRSA) and one MSSA strain were studied. MIC values ranged from 0.25 to 0.5 mcg/ml for TR-700 and from 2 to 4 mcg/ml for linezolid (**Table 82**).

Table 82 Characteristics and Minimum Inhibitory Concentration Values for *Staphylococcus aureus* Isolates Used for In Vivo Testing



Source: Keel 2012 Table 1
Abbreviations: CA-MRSA=community-acquired methicillin-resistant *Staphylococcus aureus*; HA-MRSA=hospital-acquired methicillin-resistant *S. aureus*; MSSA=methicillin-susceptible *S. aureus*; VRSA=vancomycin-resistant *S. aureus*

For PK determinations, single doses of TR-701 or linezolid were administered to immunocompetent, female, specific-pathogen-free (SPF) ICR mice (groups of 6 mice at 3 or 4 time points) to simulate human steady-state exposures following a regimen of 200 mg orally once daily for TR-701 or linezolid 600 mg IV every 12 hours. The experiments targeted free (unbound) 24-hour exposures ($fAUC_{0\text{-}24}$) of 3 mcg·hr/mL and 137 mcg·hr/mL for TR-700 and linezolid, respectively. Free concentrations were estimated based on assumption of protein binding values of 85% for TR-700 and 30% for linezolid. Blood samples were collected via cardiac puncture at 3 to 4 time points over 12 to 24 hours.

For PD assessments, mice (n=3/group/time point) were inoculated in the thigh muscle with 0.1 mL of 10^8 CFU/mL isolates; 2 hours later, the human simulated regimen of TR-701 (IP) or linezolid (SC) was administered. The drugs were administered to simulate a human steady-state 24-hour area under the free concentration-time curve of 200 mg every 24 hours (once daily) for TR-701 or 600 mg every 12 hours for linezolid over a 3-day treatment period. Treated mice were sacrificed at 4, 8, 12, 24, 36, 48 and 72 hours after initiation of treatment. Thigh tissue was homogenized, and bacterial counts were determined. Efficacy was determined by the change in bacterial density in tissue compared to the density in the untreated controls.

The mean bacterial density for control mice prior to initiating administration was 6.89 log_{10} CFU/mL, and the mean bacterial density increased to 7.08 log_{10} CFU/mL after 72 hours. The human simulated regimen for TR-701 and for linezolid produced reductions against all staphylococcal isolates as shown in Table 83. Both agents were bacteriostatic at 24 hours and bactericidal on the third day of treatment. Any statistical difference between TR-701 and linezolid were transient and did not persist throughout the 72-hour period.

Table 83: Mean Change in MRSA Bacterial Density after Treatment with TR-701 or Linezolid in an Experimental Mouse Thigh Infection Model

Source: Keel 2012 Figures 1A through 1E
Abbreviations: CFU=colony-forming unit; *f*AUC_{0-24}=human steady-state 24-hour area under the free concentration-time curve; MRSA=methicillin-resistant *S. aureus; S. aureus=Staphylococcus aureus*
[a]Mean change in MRSA density after TR-701 or linezolid treatment at human simulated doses compared to bacterial density in untreated controls.
[b]TR-701 was administered to simulate a *f*AUC_{0-24} curve of 200 mg every 24 h (once daily) over a 3-day treatment period. *f*AUC_{0-24} was 2.99 µg·h/mL for TR-701.
[c]Linezolid was administered to simulate a human *f*AUC_{0-24} curve of 600 mg every 12 h over a 3-day treatment period. *f*AUC_{0-24} was 144 µg·h/mL for linezolid.
*Bacteriostatic
**Bactericidal=>3 log_{10} reduction in bacterial density compared to untreated controls

The Applicant stated that the human simulated regimen of TR-700 resulted in a mean total drug AUC_{0-24}/MIC of 79.7 and 39.8 for isolates with MIC values of 0.25 and 0.5 mcg/mL, respectively. Overall, the 2 regimens produced similar antibacterial effects over the 72-hour treatment period regardless of the genotype or phenotype of the staphylococcal strain used to initiate the infection. This study of MRSA and MSSA thigh infections established the efficacy profiles of TR-701 and linezolid at target plasma exposures simulating those associated with human administration of the two drugs. The dose regimens employed produced similar antibacterial effects for TR-701 and linezolid over the 72-hr treatment period for all isolates tested.

The Applicant stated that in a post hoc analysis of the exposures attained for each drug revealed that the actual human target AUC exposures in mice were not achieved. For TR-701, the actual *f*AUC_{0-24} was 2.99 mcg·hr/mL (under human target of 5.2 mcg·hr/mL) and for linezolid the actual *f*AUC_{0-24} was 144 mcg·hr/mL (above human target of 96.6 mcg·hr/mL. Therefore, actual drug exposure was approximately 42% lower than target for TR-701 and approximately 33% higher than target for linezolid. This could explain the comparable efficacy results observed for Tedizolid and linezolid in this study, rather than improved efficacy for TR-701 as might be expected.

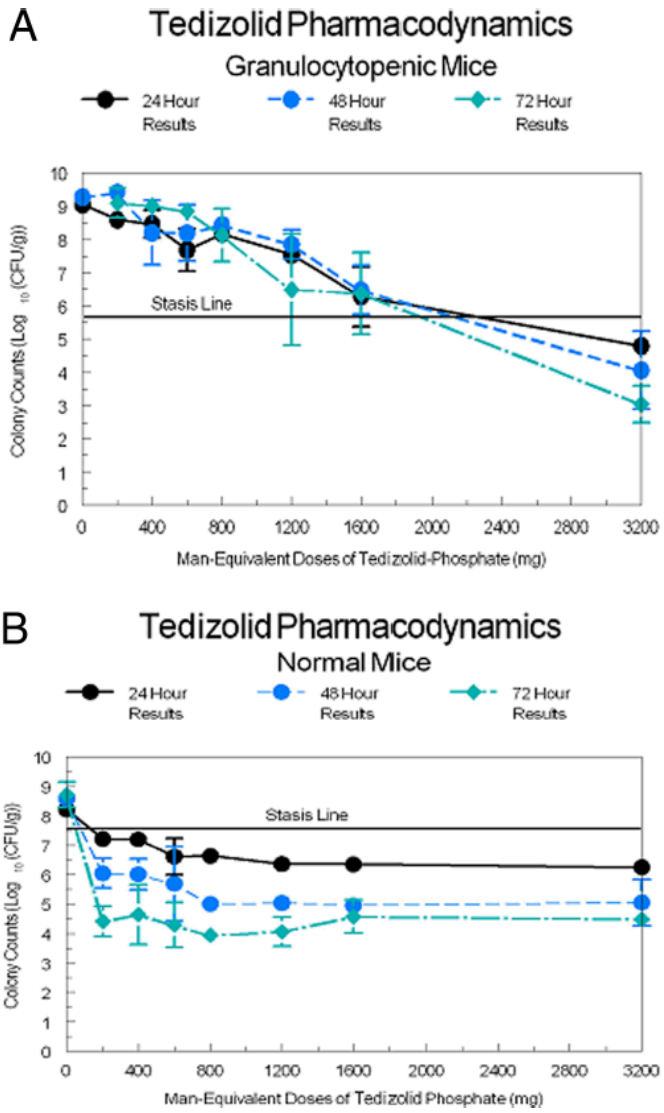
Impact of Granulocytes on the Antibacterial Effect of TR-701 in a Mouse Thigh Infection Model

The above thigh infection model used dose range and dose fractionation studies for TR-701 in a neutropenic mouse; and demonstrated that the area under the concentration-time curve over 24 hours in the steady state divided by the MIC (AUC/MIC) is the pharmacodynamic index most closely linked to the bacterial cell killing

rate. TR-701 significantly outperformed the comparator linezolid. Given this finding, it was hypothesized that treatment with TR-701 would result in a significant increase in activity in the presence of granulocytes [(polymorphonuclear leukocytes (PMN)] relative to the period when the animals were neutropenic. In studies described above in the section entitled "Intracellular Antibacterial Concentration Assessment" it was indicated that TR-701 penetrated into macrophages and white cells. This penetration was hypothesized to boost the microbiological activity of the TR-701.

In this experiment, the accumulation of TR-701 in white blood cells in relation to potential microbiological activity was explored. In an in vitro study, TR-700 accumulated in human macrophages to a significantly greater extent than linezolid, an effect that was associated with a higher rate of *S. aureus* killing. Granulocytes play an important role in the physiological response to bacterial skin and skin structure infections. In 2011, Drusano et al. evaluated the in vivo interaction between TR-701 and granulocytes on staphylococcal killing in mice[13]. Briefly, Female Swiss Webster mice (4/group) were rendered granulocytopenic by IP administration of cyclophosphamide at Day -4 (150 mg/kg) and Day -1 (100 mg/kg) prior to bacterial challenge. This treatment resulted in a neutrophil count of less than 100 cells/mL for at least 5 days. Other mice were not treated with cyclophosphamide, remaining granulocyte-replete. MRSA (strain ATCC 33591) was inoculated into the posterior thigh muscle of nongranulocytopenic and granulocytopenic animals at concentrations of 10^7 CFU/thigh and 5×10^5 CFU/thigh, respectively. The TR-700 MIC value for the challenge strain was 0.5 mcg/mL. Treated mice were administered a single IP dose of TR-701 at doses intended to produce AUC_{0-24} TR-700 exposures in plasma equivalent (after conversion) to human doses of 200 (the recommended human dose), 400, 600, 800, 1200, 1600, or 3200 mg. The doses were scaled to reflect the finding that a dose of 8.42 mg/kg IP in mice approximated the human-equivalent AUC_{0-24} for a 200-mg oral dose. Thigh muscle was obtained at 24, 48, and 72 hour after treatment initiation to determine MRSA viable counts. A dose-dependent response to TR-701 treatment was observed in both granulocytopenic and nongranulocytopenic mice as shown in Figure 43. Bacterial growth/kill curves for the granulocytopenic animals are displayed in Figure 43A and for the normal mice in Figure 43B.

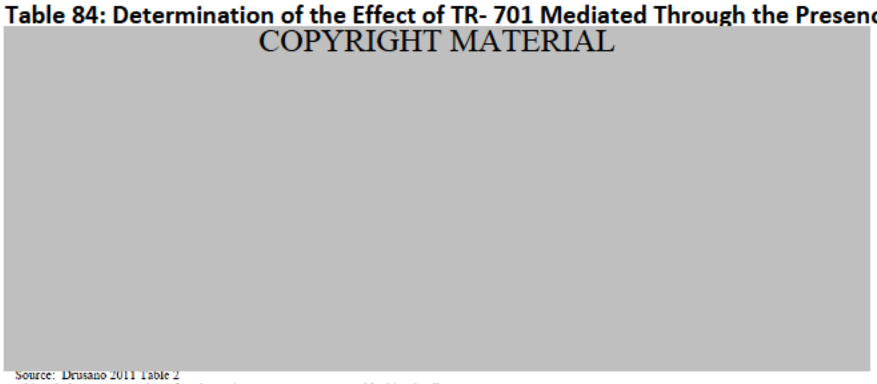
Figure 43: (A) Colony counts over 72 h of *S. aureus* in the thighs of granulocytopenic mice. (B) Colony counts over 72 h of *S. aureus* in the thighs of granulocyte-replete mice.



Stasis was achieved in granulocytopenic mice at slightly less than the human-equivalent doses of 2300 mg/day at 24 hours, 2100 mg/day at 48 hours, and 2000 mg/day at 72 hours. In immunocompetent mice (bottom panel), stasis was achieved at human-equivalent dose of slightly greater than 100 mg/day at 24 hours and less than 100 mg/day at 48 and 72 hours. The data suggest that the majority of bacterial killing was attributable to effects of TR-700 mediated through granulocytes. The maximal killing rate in normal animals was achieved at 72 hours, with the lowest dose providing an effect similar to that seen with the highest dose. Stasis was reached on Day 1 in immunocompetent animals for both the lowest and highest dose groups. However, in granulocytopenic animals, doses higher than the recommended human dose were required to achieve stasis. The cumulative effects of the presence of granulocytes plus the direct drug effect and the drug effect mediated by granulocytes can be seen in the nongranulocytopenic mice receiving drug therapy at the lowest

dose (200-mg/day equivalent). The bacterial levels in this immunocompetent treatment group were reduced by 3.12, 5.12, and 6.43 log_{10} CFU/g at 24, 48, and 72 hours, respectively, compared with the neutropenic TR-701- treated cohort. Data were analyzed to estimate the relative contributions of these factors to the large difference in efficacy between granulocytopenic and nongranulocytopenic animals as summarized in Table 84.

Table 84: Determination of the Effect of TR- 701 Mediated Through the Presence of Granulocytes



Source: Drusano 2011 Table 2
Abbreviations: CFU=colony-forming unit; Log=Log10; WBC=white blood cell
[a]The actual direct drug effect cannot be calculated for the 72-hour time point, because all the animals in the granulocytopenic no-treatment control cohort had expired before that time. As the actual value for 48 hours was a negative value, the direct effect was fixed to zero for this time point.
[b]A fair appraisal cannot be made of direct granulocyte effect to examine amplification in the granulocytopenic no-treatment control cohort versus the normal no-treatment control cohort, because of the different starting inocula, but the upper boundary is fixed in both instances because of the stationary-phase growth status. The direct effect was estimated by assuming that the granulocytopenic no-treatment control cohort amplification started at 7.54 log CFU/g, which is the same as the value seen with the normal no-treatment control cohort, and that the direct granulocyte effect would be the difference in levels of organism amplification between the granulocytopenic and normal no-treatment control cohorts. Again, for the 72-hour time point, the value from 48 hours was used because all animals in the granulocytopenic cohort had expired.

Stasis was achieved in immunocompetent animals at human-equivalent exposures of slightly more than 100 mg/day at the 24 hour time-point and less than 100 mg/day at the 48 hour and 72 hour time-points. The difference in the response between groups was related to the increased exposure as a function of the presence of granulocytes. These in vivo results suggest that the increased activity TR-701 observed in a *S. aureus* non-neutropenic infection model may be due to improved granulocyte penetration of TR-700 and also to improved killing of the staphylococcal strain within granulocytes.

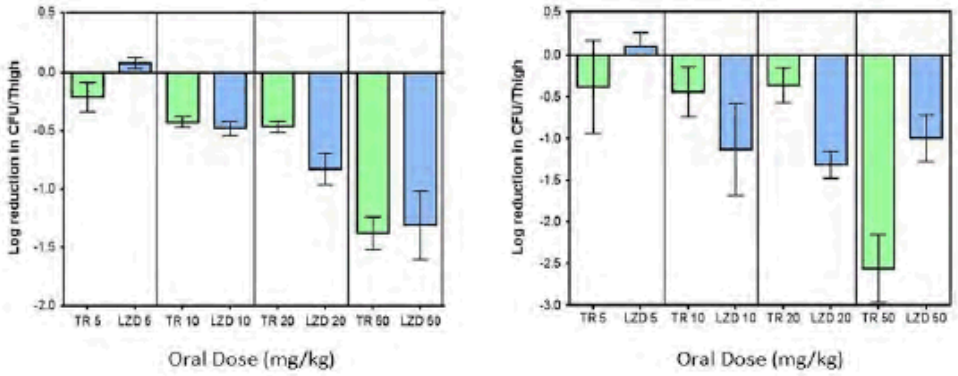
Summary:

Treatment with TR-701 resulted in a significant increase in activity in the presence of granulocytes relative to the period when the animals were neutropenic. A close examination of both normal and granulocytopenic animals indicate an improvement in the exposure response as a function of the presence of granulocytes. For the granulocytopenic animals, stasis was achieved with a human equivalent dose exposure of approximately 2000, 2100 and 2300 mg administered daily for the 72-, 48-, and 12-hours endpoints, respectively. However, in normal animals stasis was achieved at human-equivalent exposure doses of approximately 100 mg/day at the 24-hour endpoint and less than 100 mg/day at the 48-hour and 72-hour endpoints. The maximal killing rate was achieved at 72 h, with the lowest dose (200 mg/day) providing an effect similar to that seen with the highest dose (3,200 mg/day). Those regimens were associated with bacterial burdens in thigh muscles of ~4 log10 CFU/g and ~4.5 log10 CFU/g, respectively, which represent bacterial killing levels that were about 3 log10 CFU/g greater than the stasis response. Thus, after 72 h of therapy with TR-701, the lowest and the highest doses appear to produce equivalent microbiological effects. Although the mechanism behind this finding is unclear, it is possible that the generation of reactive oxygen species (ROS) during the phagocytosis response maybe contributing to the enhanced activity of TR-700.

Efficacy of TR-701 against MRSA in a skin and soft tissue infection rat model

In another experiment, the efficacy of TR-701 against MRSA in a skin and soft tissue infection rat model was investigated. Male Sprague-Dawley rats (6/group) were rendered neutropenic prior to infection by IP injection of cyclophosphamide on Day -4 (150 mg/kg) and Day -1 (100 mg/kg) relative to inoculation. Rats were inoculated (approximately 1×10^7 CFU/thigh) by intramuscular (IM) injection with MRSA strain ATCC 33591. Treatment drugs (TR-701 or linezolid) were administered 2 hours after inoculation at doses of 5, 10, 20, or 50 mg/kg orally. In addition, a control group received a single dose of vehicle (water for injection) 2 hours after inoculation. One additional group of infected rats was left untreated. The untreated group was sacrificed immediately after inoculation, and groups treated with drug or vehicle were sacrificed 24 hours after infection. Tissue from the right thigh was removed, homogenized, and cultured for bacterial counts. Both TR-701 and linezolid produced a clear antibacterial response relative to untreated controls at the 50 mg/kg dose. TR-701 was slightly more effective at this dose in both experiments, providing a 1.4 to 2.56 $\log_{10}$ CFU reduction in bacterial count, whereas linezolid produced a 1 to 1.3 $\log_{10}$ CFU reduction as shown in Figure 44. TR-701 and linezolid generally produced more modest efficacy at other doses tested.

Figure 44: Mean Bacterial Count Reduction in Thigh Tissue of Rats Infected with MRSA 24 Hours After a Single Oral Dose of TR-701 or Linezolid



The relationship between the TR-700 exposure, as measured by the pharmacodynamic indices *f*AUC/MIC ratio, *f*Cmax/MIC ratio, and *f*Time>MIC, and microbiological effect was also assessed. The pharmacodynamic index that best correlated with treatment effect was the *f*AUC/MIC ratio. The *f*AUC/MIC ratio versus the $\log_{10}$ (CFU/g) for TR-701 administration intervals of 6, 12, or 24 hours indicated that once-daily administration provided the same efficacy as giving the same total daily dose as multiple, equally divided doses each day.

Lung infections epithelial lining fluid (ELF):

The Applicant evaluated the in vivo antibacterial activity and epithelial lining fluid (ELF) exposures of TR-700, linezolid, and vancomycin. Female BALB/c mice (6/group) were inoculated with 3 strains (internally designated) of MRSA via oral instillation (aspiration) into the lungs (0.05 mL of 109 CFU/mL). Characteristics and MIC values for each strain are presented in **Table 85.**

Table 85: Characteristics and Minimum Inhibitory Concentration Values for Methicillin-resistant *S. aureus* Used for In Vivo Testing

Source: Tessier 2012 Table 1
Abbreviations: CA-MRSA=community-acquired methicillin-resistant *S. aureus*; HA-MRSA=hospital-acquired methicillin-resistant *S. aureus*
[a]Internal designation

Three hours after inoculation, infected mice were administered antibiotic regimens chosen to produce AUC_{24} exposures in ELF comparable to human ELF exposures associated with a TR-701 FA dose of 200 mg once in 24 hours, a linezolid dose of 600 mg twice over 24 hours, or a vancomycin dose of 1 g twice over 24 hours. The mice were administered either 20 mg/kg TR-701 by IP injection once in 24 hours, 120 mg/kg linezolid by SC injection twice over 24 hours (every 12 hours) or 25 mg/kg vancomycin by SC injection twice over 24 hours. Bronchoalveolar lavage (BAL) fluid and blood were collected over the 12 to 24 hour dose intervals and analyzed for urea concentrations. The drug concentrations in ELF were calculated. TR-701 diminished CFU loads by 0.76 to 1.4 log10 CFU/lung at 24 hours, whereas linezolid lowered bacterial density by 1.2 to 2.0 log10 CFU/lung. Vancomycin was less effective. Vancomycin was also less protective than either TR-701 or linezolid (Figure 45).

Figure 45: Changes in Bacterial Density After 24 Hours for TR-701-, Linezolid-, and Vancomycin- Treated Groups

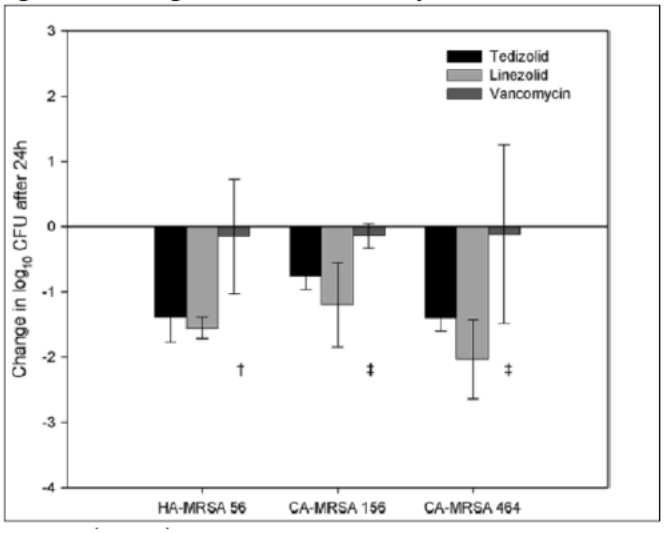
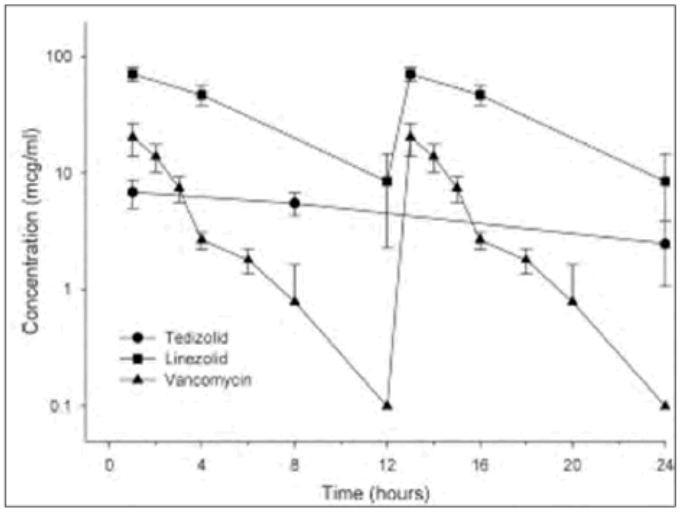


Figure 46 shows the concentration-time profiles for TR-700, linezolid, and vancomycin. The study shows that when human exposures were simulated in this MRSA pneumonia model, antibacterial efficacy was similar for TR-701 and linezolid. Vancomycin was less effective than either of the oxazolidinones in terms of efficacy and reduction in mortality at the human exposures simulated in this model.

Figure 46: Lung ELF Concentration Time-Course for TR-700, Linezolid, and Vancomycin in Mice



In another experiment, the in vivo antibacterial characteristics of TR-700 and linezolid against 4 strains of MSSA and 7 strains of MRSA in a neutropenic mouse pneumonia model. In this study, the PD target value (AUC/MIC) was identified by PK/PD modeling for each compound. Minimum inhibitory concentration values for TR-700 and linezolid (Table 86) were determined

Table 86: Characteristics and Minimum Inhibitory Concentration Values for *Staphylococcus aureus* Strains

Pathogen *S. aureus* Strain	Resistance Designation	Minimum Inhibitory Concentration (µg/mL)		
		TR-700	Linezolid	Methicillin
ATCC 25923	MSSA	0.06	2	0.25
ATCC 29213	MSSA	0.25	2	0.125
ATCC 6538P	MSSA	0.125	NR	0.5
ATCC Smith	MSSA	0.5	NR	0.125
04-045	CA-MRSA	0.125	2	>16
04-154	CA-MRSA	0.25	2	>16
MW2	CA-MRSA;USA400	0.5	2	>16
307109[a]	CA-MRSA	0.25	NR	>16
ATCC 33591	HA-MRSA;USA200	0.25	NR	>16
R2527	CA-MRSA;USA300	0.125	NR	>16
05-051	CA-MRSA	0.25	NR	>16

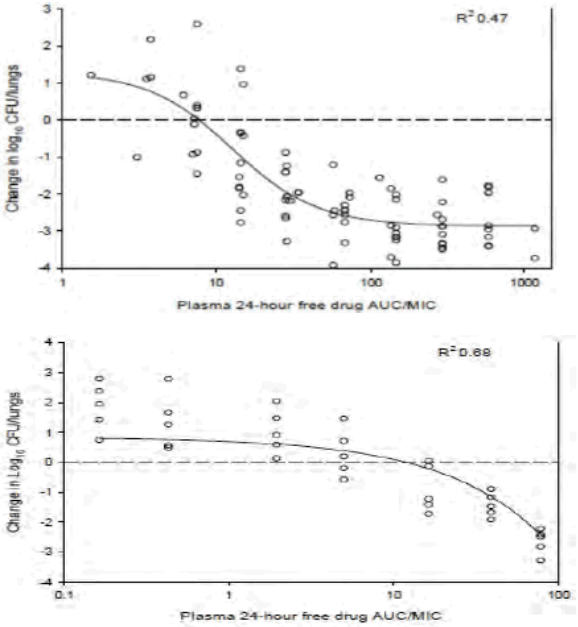
Pharmacodynamics of TR-700 and Linezolid Neutropenic Mouse *S. aureus* Pneumonia Model:

In this study, neutropenic female ICR/Swiss mice were inoculated with 10^6-10^7 CFU/ml of bacteria into the nares and inhaled into the lung. Drug treatment began 2 hours after infection and was administered orally every 12 hours over dose ranges of 0.625 to 160 mg/kg for TR-701 and 1.25 to 80 mg/kg for linezolid. After 24 hours, drug-treated mice were sacrificed, and bacterial counts in lung tissue were performed. Untreated control mice were sacrificed and assessed at the time treatment began (2-hours postinfection) for drug-treated mice. For PK evaluation, single-dose PK data were collected from parallel groups (n=3) of infected mice administered PO doses of TR-701 (0.625, 2.5, 10, and 40 mg/kg) or linezolid (0.625, 2.5, 10, and 40 mg/kg).

Blood samples were obtained 0.25, 0.5, 1, 3, 6, 9, 12, and 24 hours after treatment and analyzed. The dose needed to achieve net stasis and 1 $\log_{10}$ kill was determined, and total drug AUC/MIC and free drug AUC/MIC (*f*AUC/MIC) were calculated. The values used for protein binding were 85% for TR-700 and 30% for linezolid.

The bacterial count in lung tissue was approximately 6.24±0.40 $\log_{10}$ CFU/lungs at the time of treatment initiation. After 24 hours of treatment, the maximal reductions in mean bacterial burden in mice treated with TR-701 and linezolid were 3.93 (±0.24) log10 CFU/lung and 3.27 (±0.56) log10 CFU/lung, respectively, compared to levels of 7.92 (±1.02) log10 CFU/lungs in untreated animals after 24 hours. The mean 24 hour total drug AUC/MIC values associated with a static endpoint were 133 and 27.2 for TR-700 and linezolid, respectively. When protein binding was considered, the mean *f*AUC/MIC values for TR-700 and linezolid were similar at 20 and 19, respectively. The mean 24 hour *f*AUC/MIC values associated with 1-log kill reduction were roughly 2-fold higher than that needed for stasis (34.6 for TR-700 and 46.1 for linezolid). The plasma 24 hour *f*AUC/MIC values for TR-700 associated with net stasis and 1-log kill were, however, not significantly different among the 4 MSSA strains and 7 MRSA strains tested. The analysis of exposure-response relationships for TR-700 and linezolid are presented in **Figure 47**.

Figure 47: Relationship Between TR-700 (Upper Panel) and Linezolid (Lower Panel) Free Drug AUC/MIC Values and In Vivo Efficacy



Efficacy of TR-701 in a Murine *Streptococcus pneumoniae* Pneumonia Model

This study evaluated the effects of TR-701 and linezolid against a penicillin-susceptible *S. pneumoniae* (PSSP) mouse pneumonia model of infection. Mice were intranasally inoculated with a suspension containing 2.5×107 CFU of the PSSP Type III strain. Infected mice were treated orally with TR-701 (2.5, 5, 10 or 20 mg/kg once daily) or linezolid (2.5, 5, 10, or 20 mg/kg twice daily) for 2 days, with the first administration occurring 4 hours postinfection. A control group of infected mice was left untreated.

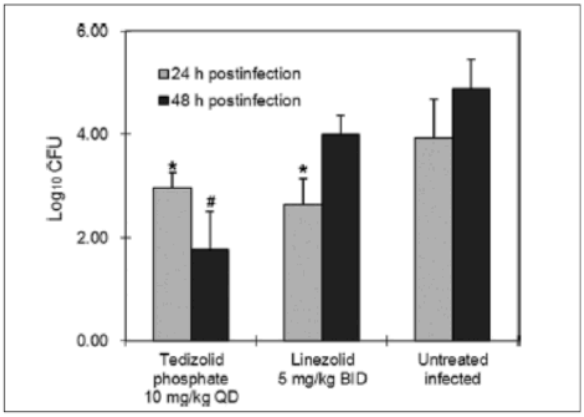
The data in Table 100 show ED50 values. Both TR-701 (2.80 mg/kg/day [confidence interval {CI} 1.41 to 4.44]) and linezolid (8.09 mg/kg/day [CI 4.74 to 11.91]) were effective against PSSP infection (**Table 87**). The cumulative survival of vehicle-treated infected mice was 10%.

Table 87: In Vivo Efficacy of TR-701 and Linezolid Against *S. pneumoniae* (PSSP) in A Murine Pneumonia Model

Compound	Dose (mg/kg/d)	Regimen	Survival rate (%)	MSD (day)[a]	ED_{50} (mg/kg/d)[b]
TR-701	2.5	once daily	50	9.7	2.80 (1.41-4.44)
	5		80	12.2	
	10		100	14.0	
	20		100	14.0	
Linezolid	5	twice daily	30	7.0	8.09 (4.74-11.91)
	10		70	11.1	
	20		90	13.0	
	40		100	14.0	
Untreated Infected Control	-	-	10	5.7	-

Compared to untreated infected controls, is was reported that lung tissue from mice in a subgroup analysis treated for 2 days with 10 mg/kg/day TR-701 or 10 mg/kg/day linezolid displayed significantly fewer bacteria (by 1 log10 /lung) by Day 1 postinfection as shown in **Figure 48**. On Day 2 postinfection, only mice treated with TR-701 displayed a significantly reduced bacterial count relative to controls (3.1 log10 CFU/lung).

Figure 48: Pneumococcal Clearance from Lungs of *Streptococcus pneumoniae*-infected Mice by TR-701 and Linezolid



Heart Valve Infections:

Comparing TR-701 at Different Doses for Treatment of Experimental *S. aureus* Endocarditis in Rabbit

In this study, the efficacy of TR-701 versus vancomycin and daptomycin in an experimental staphylococcal aortic valve endocarditis in rabbits was determined. A catheter was positioned across the aortic valve of each New Zealand White male rabbit (2.5 to 3.0 kg) 48 hours prior to the IV introduction of 3.7×107 CFU of *S. aureus* COL (an MRSA strain). Untreated controls were sacrificed 18 hours later, and the remaining rabbits were treated with either TR-701 (15 mg/kg IV twice daily), vancomycin (30 mg/kg IV twice daily), or daptomycin (18 mg/kg IV once daily) for 4 days. Blood was obtained 1 hour and 9 hours (TR-701 only) after IV

injection to determine serum levels. Rabbits were sacrificed the following day (Day 5), and the hearts, spleens, and kidneys were quantitatively cultured. Mean drug plasma concentrations (mg/L) after 1 hour were 9.7±1.8 for TR-700, 34±10 for vancomycin, and 93±13 for daptomycin. At 9 hours, the TR-700 mean plasma concentration was 5.8±2.2. Results of the mean change from baseline in MRSA cell count in the organs after 4 days of antibiotic treatment are shown in **Table 88**.

Table 88: Mean MRSA Cell Counts from Endocarditis in Rabbits After Treatment with TR-701, Vancomycin, or Daptomycin

Tissues	MRSA Cell Count±SD (log_{10} CFU/g tissue)			
Treatment groups	Untreated Control (n=9)	TR-701 (n=14)	Vancomycin (n=16)	Daptomycin (n=16)
Vegetations	7.7 ±1.2	6.0 ±2.0	5.5 ±2.6	3.5 ±2.6
Spleen	4.6 ±0.7	3.0 ±1.4	3.2 ±1.5	2.3 ±1.5
Kidney	3.5 ±1.3	2.5 ±1.1	2.6 ±1.2	2.2 ±1.0
Minimum inhibitory concentration				
MIC (µg/mL)	--	0.125	1	1

The efficacy of TR-701 was comparable to that of vancomycin, each producing about a 2 log10 CFU/g kill, and slightly less than that of daptomycin in the rabbit aortic endocarditis model.

Time-Kill Studies

In Vivo Time-Kill Effects of TR-701 in Mice

The in vivo activity of TR-701 was determined in a mouse infection with *S. aureus* (ATCC 13709; Smith strain). The MIC values previously determined for TR-700, TR-701, and linezolid were 0.5, >32, and 2 mcg/mL, respectively. The in vivo bactericidal time course was evaluated in female BALB/c mice (10/time point) inoculated by intraperitoneal (IP) injection with approximately $5x10^3$ CFU *S. aureus* in 5% mucin. Surviving mice were euthanized at 0.5, 1, 2, 4, 6, 8, 18, and 24 hours after inoculation, and bacterial counts were conducted for both kidneys. Bactericidal activity was defined as a >3 log unit reduction in CFU/gram tissue by the 24 hour time point. Based on mouse survival and CFU/g results in kidney tissue, an 8 hour time course was chosen for the time-kill assessment. TR-701 (1.5, 3, 6, and 12 mg/kg) or linezolid (3, 6, 12, and 25 mg/kg) was administered as a single oral dose within 15 minutes of inoculation with $3.5x10^3$ CFU *S. aureus* in 5% mucin (n=10/group). Mice were euthanized 8 hours after inoculation, and bacterial counts were conducted on tissue from both kidneys.

The results of the in vivo time-kill assay are presented in Table 89. Both TR-701 and linezolid were bactericidal in vivo. In the untreated control animals, mean staphylococcal counts reached 6.3 log_{10} CFU/g of kidney tissue by 8 hours after inoculation. Treatment with TR-701 produced approximately 1-log greater activity at the dose of 3 mg/kg compared to the reduction in CFU/g with linezolid. Both drugs demonstrated bactericidal activity at higher doses, although TR-701 was more effective at lower concentrations (3 mg/kg) than linezolid.

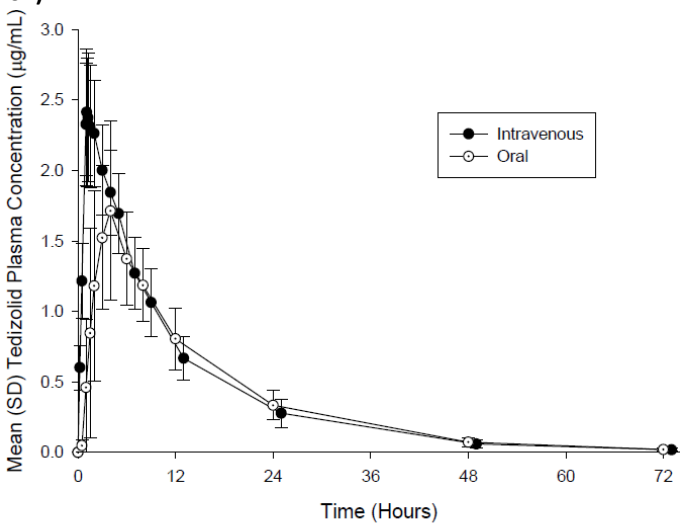
Table 89: Bactericidal Effect After Administration of TR-701 and Linezolid in a *S. aureus* Mouse Kidney Infection Model

Dose (mg/kg)	Log_{10} CFU/g (±SEM)	
	TR-701	Linezolid
0	6.3 (±0.12)	6.3 (±0.12)
1.5	-2.12 (±0.22)	NT
3	-2.68 (±0.17)	-1.61 (±0.21)
6	-2.86 (±0.13)	-2.98 (±0.11)
12	-3.70 (±0.09)	-3.32 (±0.11)
25	Not Tested	-4.05 (±0.07)

Human Pharmacokinetics

Based on the Applicant's data, TR-701 FA has been shown to be rapidly converted by phosphatases to the microbiologically active moiety TR-700. The absolute bioavailability of TR-700 following oral administration of TR-701 FA is said to be high (>80%); thus, the same therapeutic dosage can be used by either oral or IV delivery as shown in the Phase 1 Study TR701-108. Steady-state concentrations is said to be achieved within 3 days and indicate modest drug accumulation of approximately 30% following multiple once-daily oral or IV administration (Studies TR701-101 and Study TR701-107). Following oral administration, peak plasma TR-700 concentrations are achieved within approximately 3 hours after administration (Figure 49 and Table 90). Pharmacokinetic studies have demonstrated that TR-700 rapidly distributes into tissues and penetrates into the interstitial space fluid of adipose and skeletal muscle tissue, resulting in TR-700 exposures in these compartments that were similar to free drug exposure in plasma (Study TR701-102). Protein binding was observed with TR-700 in human plasma (70% to 90%, primarily to albumin), and binding appeared to be independent of concentration. The majority of elimination occurs via the liver, with 81.5% of the product eliminated in feces and 18.0% in urine (Study TR701-106).

Figure 49: Mean TR-700 Plasma Concentrations Following Single Oral and IV Administration of 200 mg TR-701 FA (Study TR701-107)



Source: Module 5.3.1.1 TR701-107 CSR Report Body Post-text Table 14.2.1-1c
Abbreviations: SD=Standard deviation

Table 90: Single and Multiple Administration of 200 mg TR-701 FA Pharmacokinetic Parameters (Mean [Standard Deviation])

	Oral		IV	
	Single Administration	Steady State	Single Administration	Steady State
AUC[a] (µg·h/mL)	23.8 (6.8)	25.6 (8.4)	26.6 (5.2)	29.2 (6.2)
C_{max} (µg/mL)	2.0 (0.66)	2.2 (0.64)	2.3 (0.64)	3.0 (0.66)
C_{min} (µg/mL)	NA	0.44 (0.19)	NA	0.36 (0.09)
t_{max} (h)[b]	2.5 (1 – 8)	3.5 (1 – 6)	1.1 (0.9 – 1.5)	1.2 (0.9 – 1.5)
CL or CL/F (L/h)	6.9 (1.7)	8.4 (2.1)	6.4 (1.2)	5.87 (1.4)

Source: TR701-124 for oral single administration, TR701-114 for oral multiple administration, and TR701-107 Part B for IV administration.
Abbreviations: AUC=area under the concentration-time curve; $AUC_{0-\infty}$=AUC from Time 0 to infinity; AUC_{0-24}=AUC from Time 0 to 24 hours; CL=clearance; CL/F=apparent oral clearance; C_{max}=maximum concentration; C_{min}=minimum concentration; IV=intravenous; t_{max}=time to maximum concentration; NA=not applicable
[a]AUC is $AUC_{0-\infty}$ for single administration and AUC_{0-24} for multiple administration.
[b]Median (Minimum, Maximum).

PROVISIONAL INTERPRETIVE BREAKPOINTS

In a previous submission, the Applicant has proposed MIC in vitro susceptibility interpretive criteria against the target pathogens before they conducted the Phase 3 clinical trials. Provisional MIC and disk diffusion zone diameter interpretive breakpoints were proposed for TR-700 based on a review of in vitro test results across a broad range of bacterial pathogens representing multiple species and phenotypic groups (MCR-08-0701-016). The provisional breakpoints proposed were based solely on MIC population distributions and MIC: Zone diameter scatter plots, and are shown in **Table 91**.

Table 91: Tedizolid Provisional Interpretive Criteria Based Upon MIC Population Distributions and MIC:Zone Diameter Scatterplots Only

(b) (4)

HUMAN CLINICAL TRIALS

TR-701 FA has been studied in a complete Phase 1 through Phase 3 clinical trial program for acute bacterial skin and skin structure infections (ABSSSI). The TR-701 FA 200 mg QD dose proposed for marketing was studied in two pivotal Phase 3 trials, while the 200 mg QD disodium salt form of TR-701 was studied in one Phase 2 study. During the Phase 2 and 3 clinical trials, these quality control ranges were employed by the central laboratory to validate the testing results for clinical trial isolates. One or more quality control organisms was included in each assay that evaluated clinical trial isolates; if the results for the quality control

organism(s) were not within the recommended ranges for TR-700 and the comparator agents, the assay of the clinical isolates was repeated. The quality control ranges are shown in Table 92.

Table 92: CLSI Approved Quality Control Ranges for TR-700

QC Strain	Range	
	MIC (µg/mL)	Zone Diameter (mm)
Staphylococcus aureus ATCC 29213	0.25 - 1	--
Staphylococcus aureus ATCC 25923	--	22 - 29
Enterococcus faecalis ATCC 29212	0.25 - 1	--
Streptococcus pneumoniae ATCC 49619	0.12 - 0.5	24 - 30

Source: CLSI M100-S23 2013
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute; MIC=minimum inhibitory concentration; QC=quality control

In the clinical trial the MIC observed for the quality control strains validated the testing of the clinical isolates. For *S. aureus* ATCC 29213, 100% of results (248/248) were within the quality control range (b) (4) For *S. pneumoniae* ATCC 49619, 97.0% of results (130/134) were within the quality control range (b) (4) The 4 out-of-range results for *S. pneumoniae* of 0.06 mcg/ml (Study TR701-113) were only one 2-fold dilution below the lower bound of the quality control range.

For disk diffusion, all of the 30 TR-700 zone diameter values generated for *S. aureus* ATCC 25923 in this trial (Table 93) fell within the approved zone diameter quality control range (b) (4) Of the 11 TR-700 zone diameter values generated for *S. pneumoniae* ATCC 49619 in this study, 9 were within the approved zone diameter quality control range (b) (4) (81.8%). Two values fell below the lower boundary of the quality control range. Overall, the disk diffusion data for 2 CLSI quality control organisms validated the testing results for the clinical isolates from the Phase 2 Study TR701-104. However, even though the number of assays was few, the out-of-range results observed for *S. pneumoniae* were notably high, and the performance of the TR-700 disk and QC ranges for this organism will warrant continued monitoring.

Table 93: Zone Diameter Quality Control Values for *S. aureus* ATCC 25923 and *S. pneumoniae* ATCC 49619

	Zone Diameter (mm)[a]	
Strain	*S. aureus* ATCC 25923	*S. pneumoniae* ATCC 49619
Study	TR701-104	
Total No. Determinations	30	11
Range	23 - 29	21 - 29
CLSI Range	22 - 29	24 - 30
Percent Within CLSI Range	100	81.8
Study	TR701-112	
Total No. Determinations	65	39
Range	19-35	21-31
CLSI Range	22-29	24-30
Percent Within CLSI Range	92.3	84.6
Study	TR701-113	
Total No. Determinations	125	81
Range	19-35	6-31
CLSI Range	22-29	24-30
Percent Within CLSI Range	92.8	86.4

Source: Appendix 12 Supplemental Quality Control Results –Tables A12-7 to A12-12
Abbreviations: ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute; *S. aureus=Staphylococcus aureus*; *S. pneumoniae=Streptococcus pneumonia;* TZD=tedizolid/TR-700
[a]TZD, tedizolid/TR-700, 20 µg disk.

NDA: 205435/205436 DATE REVIEW COMPLETED: 02/24/2014
Tedizolid Phosphate (Sivextro)

Overall, the zone diameter results observed for the quality control strains generally validated the testing of the clinical trial isolates. For *S. aureus* ATCC 25923, 93.6% of test results (206/220) were within the quality control range (b) (4) For *S. pneumoniae* ATCC 49619, 85.5% of results (112/131) were within the quality control range (b) (4)

Phase 2 and 3 Clinical Studies Description

Microbiological data were analyzed from the following TR-701/FA clinical trials:

- **TR701-104**: A Phase 2, multicenter, randomized, double-blind, non-controlled study of safety, tolerance and efficacy of oral TR-701 200 mg daily (QD) versus TR-701 300 mg (QD) versus TR-701 400 mg (QD) in patients with cSSSI in the US. Only data for the 200 mg treatment arm are included here.

- **TR701-112**: A multinational Phase 3 randomized, double-blind, multicenter study comparing the efficacy and safety of 6-day oral TR-701 FA and 10-day oral linezolid for the treatment of ABSSSI.

- **TR701-113**: A multinational Phase 3 randomized, double-blind, multicenter study comparing the efficacy and safety of IV to oral 6-day TR-701 FA once daily for 6 days and IV to oral linezolid 600 mg BID for 10 days for the treatment of ABSSSI .

An overview of these studies is provided in Table 94.

Table 94: Overview of Clinical Efficacy and Safety Studies Included in the Microbiological Analysis

Study ID	Study Objectives	Design	Indication	Key Inclusion/Exclusion Criteria	Treatment Regimen	No. Enrolled
Phase 2 (Uncontrolled Study)						
TR701-104	Safety, clinical, and micro response, PK	Randomized, multicenter, no control	cSSSI	• Cellulitis, abscess and wound infection not defined per current FDA guidance; • Systemic sign of infection not required if longest dimension >5cm • 24 hours of antibiotics in the 96 hours prior to first dose of study drug allowed	TR-701 capsule, QD oral 200, 300, or 400 mg (5 to 7 days)	64, 64, and 64 [Total 192]
Phase 3 (Controlled Studies)						
TR701-112[a]	Efficacy, safety, PK	Randomized, multicenter, active controlled, non-inferiority	ABSSSI	• Cellulitis/erysipelas, major cutaneous abscess, wound infection defined per current FDA guidance including size (≥75 cm^2) requirement • At least one regional or systemic sign of infection • Antibiotics within 96 hours of first dose of study drug not allowed	TR-701 FA tablet; oral 200 mg QD x 6; placebo QD x 4	332
					LZD oral 600 mg BID x 10 days	335
TR701-113[b]	Efficacy, safety, PK	Randomized, multicenter, active controlled, non-inferiority	ABSSSI	• Cellulitis/erysipelas, major cutaneous abscess, wound infection defined per current FDA guidance including size (≥75 cm^2) requirement • At least one regional or systemic sign of infection • Antibiotics within 96 hours of first dose of study drug not allowed;	TR-701 FA IV with possible switch to oral 200 mg QD x 6; placebo QD x 4	332
					LZD IV with possible switch to oral 600 mg BID x 10 days	334

Source: Module 5 Clinical Study Reports Section 2; TR701-104, TR701-112, TR701-113
Abbreviations: ABSSSI=acute bacterial skin and skin structure infection; BID=twice daily; cSSSI=complicated skin and skin structure infection (aligned with former FDA/EMA guidelines with clinical response determined by the Investigator); FA=free acid; IV=intravenous; PK=pharmacokinetic; QD=once daily; LZD=linezolid; No.=number
[a]82 sites in 11 countries: North America (US, Canada), Europe including the European Union (Czech Republic, Germany, Hungary, Latvia, Slovakia, and Ukraine), and other countries (Argentina, Brazil and, Peru).
[b]95 sites (58 sites enrolled patients) in 10 countries: North America (US), Europe (Germany, Poland, Spain, Russia), South Africa, Australia/New Zealand, and Latin America (Argentina, Mexico).

The Phase 3 studies TR701-112 (conducted August 2010 to September 2011) and TR701-113 (conducted

September 2011 to January 2013) were randomized, non- inferiority (NI) clinical trials comparing the efficacy of TR-701 FA (200 mg daily for 6 days) with linezolid (600 mg every 12 hours for 10 days) in adult patients (and adolescents in TR701-113) with ABSSSI. TR-701 FA was administered orally in Study TR701-112 once daily and, in Study TR701-113 was initially administered intravenously (IV) with the option of later switching to the oral route after at least 2 IV infusions (1 active TR-701 FA infusion plus placebo infusion or 2 linezolid infusions). Switching TR-701 FA or linezolid to IV infusion required that 2 of these 4 criteria were met: cessation of spread of the primary ABSSSI lesion, absence of fever, no local signs or symptoms of the primary ABSSSI site worsening, and/or improvement of at least 1 local sign or symptom of the primary ABSSSI site. Patients could remain on IV therapy for the entire treatment duration based on the investigator's discretion. These 2 protocols were designed to address both the FDA and European Medicines Agency (EMA) regulatory requirements. While the EMA supports the traditional assessment of clinical response by Investigators at post-therapy evaluation (PTE) as a primary endpoint, the FDA focuses on an earlier primary endpoint (ie, at least a 20% reduction in lesion area at 48-72 hours) (see Module 5.3.5.3 *Integrated Summary of Efficacy [ISE] Section 2.1* for discussion regarding the Special Protocol Assessment [SPA] letters of agreement and ISE endpoint). Secondary outcome measures of programmatic determination of clinical response and the Investigator's assessment of clinical response at the end-of-treatment (EOT) visits were defined. The Investigator-assessed clinical outcome at the PTE Visit (formerly the Test of Cure evaluation in Study TR701-104) was used as the primary outcome for the EMA (EMA Workshop on Antibacterials Report, March 2011). The differences in the key efficacy endpoints in Studies TR701-112 and TR701-113 are summarized in Table 95.

Table 95: Efficacy Endpoints in Studies TR701-112 and TR701-113

Endpoint	TR701-112	TR701-113
Primary endpoint (at the 48 to 72 Hour Visit)		
Temperature component	Temperature was ≤37.6° and at next measurement taken within 24 hours of 48 to 72 Hour Visit is also ≤37.6°	None
Lesion component	No increase in lesion area (length × width) compared with baseline	≥20% reduction in area of erythema, edema, and/or induration (length × width) of the primary ABSSSI lesion compared with baseline
Secondary endpoint: programmatic determination of clinical response at EOT		
Clinical response at EOT	Presence/absence of pain included	Presence/absence of pain excluded

Source: Module 5.3.5.3 ISE Table 1
Abbreviations: ABSSSI=Acute bacterial skin and skin structure infection; EOT=End of Therapy

Patients with an ABSSSI caused by suspected or documented gram-positive pathogens(s) at baseline were randomized 1:1 to study treatment. Randomization was stratified by the presence/absence of fever at baseline (TR701-112 only), geographic region, and clinical syndrome (cellulitis/erysipelas, major cutaneous abscess [maximum of 30% of the total study population], and wound infection).

Adjunctive antibacterial therapy was prohibited in patients with cellulitis/erysipelas or major cutaneous abscess. Patients with wound infections could have been treated with adjunctive aztreonam and/or metronidazole if a gram-negative pathogen was suspected (eg, Gram stain) or confirmed by culture. A patient with a wound infection found to have a gram-negative pathogen after randomization, but no gram-positive pathogen, was to discontinue study drug. After randomization, all temperature measurements were to be

considered, and the highest value recorded. Phase 2 Study TR701-104 was a randomized, multicenter trial in the United States comparing TR-701 administered at 3 dose levels once daily for 5 to 7 days in 192 adults with cSSSIs. Results from this study indicated that the lowest efficacious dose regimen was 200 mg once daily. Because TR701-104 was conducted from September 2008 to February 2009, definitions for cellulitis, abscess, and wound infection in this study differed from the current FDA guidance, e.g., a smaller criterion for acceptable lesion size (5 cm^2) than the current standard of ≥75 cm^2 was used.

Microbiological Evaluations

The study design, inclusion/exclusion criteria, and procedures for microbiological evaluations performed during the clinical trials are summarized in this section. The study visits as defined in the protocol for each study are summarized in Table 96.

Table 96: Study Visits for Studies TR701-104, TR701-112, and TR701-113

Study Visit	Study Day[a]	Notes
Baseline	Day -1 or Day 1	Last measurement prior to the first dose of study drug.
48-72 Hour	Day 2 or Day 3	TR701-104: Not applicable
EOT (End of Therapy)	Last dose of study drug	TR701-104: could occur on Day 5, 6, or 7 or within 24 hours of designation of a patient as a clinical failure TR701-112, TR701-113: Day 11 (+2 days after the last dose) or within 2 days of last dose and before receiving rescue therapy in patients considered a clinical failure or who had early discontinuation of study drug
PTE (Post-therapy Evaluation)	7-14 days after last dose of study drug/EOT Visit	This visit is termed Test of Cure (TOC) in protocol TR701-104
LFU (late follow-up visit)	18-28 days after last dose of study drug	TR701-104:21-28 days after the last dose of study drug TR701-112, TR701-113: 18-25 days after the last dose of study drug

Source: Module 5.3.5.3 ISE Table 43
[a]Study Day is calculated relative to the first dose of study drug (Day 1); there is no Day 0 – the day prior to the first dose of study drug is Day -1.

Inclusion and exclusion criteria for the 3 Phase 2 and 3 studies are summarized in the individual clinical study reports (CSRs) (Module 5.3.5.2, TR701-104; Module 5.3.5.1, TR701-112, and TR701-113). The schedule for performing microbiologically-related study assessments during the clinical trial is summarized in Table 10 for the Phase 3 controlled studies.

Microbiological Sampling

In Studies TR701-112 and TR701-113, appropriate specimens (aspirates, biopsy, deep swabs) of the primary ABSSSI site and blood were collected at timepoints indicated in the Schedule of Study Procedures (Table 97). Superficial swabs of the lesion site were not acceptable. Specimens were required for abscesses and wounds at Screening; cellulitis specimens were collected according to standard practice at the study site. Two sets of aerobic/anaerobic blood cultures (obtained at the different anatomic collection sites) were also required at Screening. Similar specimens and sampling methods were employed in Study TR701-104 (Module 5.3.5.2).

Table 97 Schedule of Microbiology -Related Study Procedures for Phase 3 Studies TR701- 112 and TR701- 113

Study Procedures	Screening (≤24 h of 1st dose)	Study Visits						
		Day					PTE	LFU
		1	2	48-72 hour	7	EOT[a] 11+2 d	7-14 d after EOT	18-25 d after EOT
Verify that patient meets inclusion and exclusion criteria	X	X						
Exam, measurement, signs & symptoms of ABSSSI	X	X[b]	X	X	X	X	X[c]	
Obtain ABSSSI site specimen[d]	X	X[b]		X[e]	X[e]	X[e]	X[e]	X[c,f]
Blood for culture (2 aerobic, 2 anaerobic)	X	X[b]	X[g]	X	X	X	X	X[c,f]
Perform Investigator's assessment of clin response				X	X	X	X[c]	
Assess clinical relapse (if cure at PTE)								X
Record prior and concomitant medications	X	X	X	X	X	X	X	X

Source: Module 5.3.5.1 Protocol TR701-112 Appendix J and Protocol TR701-113 Appendix K
Abbreviations: ABSSSI=acute bacterial skin and skin structure infection; d=day(s); EOT=end of treatment; h=hour(s); PTE=post treatment; LFU=late follow-up
[a]If patient discontinues study treatment before Day 10, perform the EOT assessments within 2 days after the last dose.
[b]Omit if Screening procedures were performed on the same day that Dose 1 is administered and omit a blood sample if the sample obtained at Screening was adequate.
[c]Perform only for patients not categorized as a Failure at prior visit.
[d]Appropriate ABSSSI site specimens (superficial swab not acceptable) at baseline was evaluated with Gram stain and by culture and susceptibility testing. After baseline, ABSSSI site specimens were only required if no improvement or deterioration of lesion, and if easily accessible. Obtain a specimen on Day 1 only if the baseline sample was inadequate or not available for testing. Patients with cellulitis/erysiplelas or major cutaneous abscess and a culture or Gram stain that indicates or suggests the presence of a gram-negative pathogen causing the ABSSSI were to be excluded from enrollment. Patients with cellulitis/erysipelas or major cutaneous abscess randomized before culture results are available and found to have a gram-negative pathogen that requires an antibiotic with specific gram-negative coverage discontinued study drug and completed assessments for safety. A patient with a wound infection found to have a gram-negative pathogen after randomization, but no gram-positive pathogen, discontinued study drug and completed assessments for safety.
[e]If clinically indicated.
[f]Perform at on-site visit only if clinically indicated.
[g]Repeat after baseline only if previously positive or as clinically indicated.

Specimens from the primary ABSSSI site were collected and sent to the study site's local laboratory for Gram stain and culture. Accreditation for local laboratories is available and located in the Clinical Trial Master File. Identification of pathogens from the ABSSSI specimens and blood cultures was performed according to the local study site standard procedures. For some study sites in South America, South Africa, and Russia, a regional laboratory was designated for identification and susceptibility testing.

In addition, the following regional laboratories were used for some sites in Phase 3 studies where local laboratories were not available: (b) (4) (Studies TR701-112 and TR701-113), (b) (4) (Study TR701-112), (b) (4) (Study TR701-113), (b) (4) (Study TR701-113), (b) (4) (Study TR701-112), (b) (4). All unique organisms from the ABSSSI site and/or blood samples were to be stored and sent to the Trius-designated central laboratory for confirmation of identification and susceptibility testing, unless indication in the protocol as "never a pathogen". Isolates from Study TR701-112 and -113 were all transferred to and analyzed (b) (4) Instructions for processing and transporting isolates to the central laboratory were provided in the Microbiology Laboratory Manual. A summary of the relevant microbiological study procedures for the uncontrolled trial are summarized in Table 98.2.

Table 98: Schedule of microbiology related study procedures for phase 2 study TR701-104.

Study Procedures	Screening (Day -1 to 0)	Day 1[a]	Day 2	Day 3	Day 4	Day 5	Day 6[b]	EOT[c]	TOC 7-14 d post-treatment	LFU[d] 21-28 d post-treatment
Examination, measurements, and signs and symptoms of cSSSI	X	X	X	X		X		X	X	
Record cSSSI procedures	X	X	X	X		X		X	X	X
Microbiology testing	X[e]	X		X	X	X	X	X	X	X
Blood for culture	X[f]			X[g]	X[g]	X[g]	X[g]	X[g]	X[h]	X[g]
Assessment of clinical outcome								X	X	
Assessment of clinical relapse										X
Prior or concomitant medications	X	X	X	X	X	X	X	X	X	X[h]

Source: Module 5.3.5.2 TR701-104 CSR Body Report Table 9-2
Abbreviations: cSSSI=complicated skin and skin structure infection; d=day(s); EOT=End of Therapy visit; LFU=Late Follow Up visit; TOC=Test of Cure visit (called Post-therapy evaluation in the Phase 3 studies)
[a]If Day 1 is the same day as the Screening visit, procedures do not need to be repeated.
[b]If therapy is continued after 5 days.
[c]EOT was the last day of study drug administration.
[d]LFU visit be conducted by telephone.
[e]An appropriate cSSSI site specimen is to be obtained at baseline and Gram stain and susceptibility tests performed. After baseline, cSSSI site specimens are to be obtained when medically indicated and a focus of infection is present. If the patient's culture is positive for a gram-negative pathogen at baseline and requires additional antibiotic therapy, the patient is not eligible to participate in the study. If the patient is enrolled before culture results are available and the culture is positive for a gram-negative pathogen and requires additional antibiotic therapy, the patient must be discontinued from the study drug.
[f]Two separate blood samples are to be collected for anaerobic and aerobic evaluations for a total of 4 samples.
[g]After baseline, blood cultures should only be repeated if previously positive or medically indicated.
[h]All concomitant medications are to be collected through LFU.

Pathogen Determination

Pathogen determination was based on the genus and species identification from the central laboratory. Any discrepancies between identification by the central laboratory and the local laboratory were resolved by requesting a back-up isolate from the study site and retesting by the central laboratory. The result from the central laboratory was recorded in the database. If for some reason the central laboratory never received a specimen, the local laboratory determination was used. In general, the following organisms were always considered a pathogen in studies TR701-112, TR701-113, and TR701-104:

- Monomicrobial infections caused by
  - *S. aureus*
  - *S. haemolyticus*
  - *S. lugdunensis*
  - *S. pyogenes*
  - *S. agalactiae,*
  - *S. dysgalactiae,*
  - *S. anginosus* Group (eg, *S. anginosus, S. intermedius, and S. constellatus*)
  - *E. faecalis*
  - *E. faecium*
  - Gram-positive anaerobes
- Polymicrobial infections and all other organisms were reviewed on a case-by-case basis via manual review by Trius prior to each study's database lock and a determination was made of whether the organism was a pathogen.

The controlled studies protocols state that the following organisms were never considered to be a pathogen: *Staphylococcus saprophyticus*, *Corynebacterium* spp., *S. epidermidis*, *Bacillus* spp., diphtheroids, micrococci,

and *Candida* spp, *Aspergillus* spp, or other fungi. Any isolates not falling into either of these 2 categories, any organisms isolated from a blood culture, and all gram-negative organisms were reviewed on a case-by-case basis by Trius prior to database lock and a determination was made as to whether the organism was a pathogen.

Definition of Analysis Populations

The analysis populations defined for the 2 controlled studies and the uncontrolled study in the clinical program are summarized in the following 2 tables. Definitions for analysis sets differed between the Controlled Studies and the Uncontrolled Study. The definition for each analysis set and the number of patients in each analysis dataset for Studies TR701-112 and TR701-113 are shown in **Table 99**. The analysis sets for all studies overall and by geographic region are shown in Table 100. Note that patients from Study TR701-126 are not included in the microbiological analysis and only patients in Study TR701-104 who received the 200-mg daily dosage of TR-701 are included in the microbiological analysis.

Table 99: Analysis Data Sets for Controlled Studies

Abbrev.	Data Set	Description	TR701-112 and TR701-113 Integrated Data	
			TR-701 FA N	Linezolid N
ITT	Intent to Treat	All randomized patients	664	669
MITT	Microbiological Intent to Treat	Patients with a baseline gram-positive pathogen from a blood culture or from the primary ABSSSI site	406	412
CE-EOT	Clinically Evaluable at EOT	Patients who completed 48-72 Hour and EOT assessments without protocol violations impacting efficacy	597	598
CE-PTE	Clinically Evaluable at PTE	Patients who completed EOT and PTE assessments without protocol violations impacting efficacy unless assessed as a clinical failure	569	560
ME	Microbiologically Evaluable	Patients in both the MITT and CE-EOT data sets	358	366
		Patients in both the MITT and CE-PTE data sets	342	340

Source: Module 5.3.5.3 ISE Table 48 and Figure 3 and Section 8 Appendix Post-text Table 1
Abbreviations: ABSSSI=acute bacterial skin and skin structure infection; CE=clinically evaluable; EOT=end of therapy; ITT=Intent to Treat; ME=microbiologically evaluable; MITT=Microbiological Intent to Treat; N=number of patients; PTE=post treatment evaluation

Table 100: Analysis Data Sets for the 200-mg, Daily Dosage Groups in the Uncontrolled Study — Study TR701-104

Abbrev.	Data Set	Description	TR701-104
			N[a]
ITT	Intent to Treat	All randomized patients	64
MITT	Modified ITT	ITT patients with at least 1 dose of study drug	63
cMITT	Clinical MITT	MITT Patients with a clinical diagnosis of cSSSI	63
mMITT	Microbiological Modified Intent to Treat	cMITT patients with a baseline gram-positive cSSSI pathogen	49
CE[b]	Clinically Evaluable (Test of Cure Visit)	cMITT patients without protocol violations impacting efficacy assessments	56
ME[b]	Microbiologically Evaluable (Test of Cure Visit)	Patients in both the mMITT and CE data sets	43

Source: Module 5.3.5.2 TR701-104 CSR Body Report Table 11-1 and Figure 2
Abbreviations: CE=clinically evaluable; cMITT=clinically Modified Intent to Treat; ITT=Intent to Treat; ME=microbiologically evaluable; MITT=Modified Intent to Treat; mMITT=microbiological Modified Intent to Treat; N=number of patients.
[a]Total patients in data set.
[b]Sample size determined at Test of Cure Visit.

The **Microbiological Intent-to-Treat (MITT)** analysis set consists of all patients who were randomized and who have at least one gram-positive bacterial pathogen identified from a blood culture or from a culture of a microbiological sample obtained from the primary cSSSI (called mMITT in TR701-104) or ABSSSI (TR701-112 and TR701-113) site at baseline using a valid sampling technique. To be considered **Clinically Evaluable**, patients had to meet the conditions in the protocol inclusion criterion describing cSSSI (TR701-104 only) or ABSSSI (ie, clinical syndromes including cellulitits/erysipelas, major cutaneous abscess, and wound infection starting within 7 days of the Screening Visit) that required antibiotic therapy. Inclusion criteria specified details of the size and appearance of the local infection and the systemic signs of infection. Two CE analysis sets were defined: CE-EOT and CE-PTE. In addition, depending on the efficacy outcome analyzed, the definition of the CE analysis sets will differ slightly as indicated below. Criteria for inclusion in the CE analysis sets follow the definitions provided in the SAP for each individual study. Differences between the criteria for the controlled and uncontrolled study are summarized below:

- Diagnosis of cSSSI (TR701-104) or ABSSSI (TR701-112 and TR701-113)
- Prior systemic antibiotic therapy – In study TR701-104, prior antibiotic therapy (<24 hours in the previous 96 hours) was allowed per the inclusion criteria. In studies TR701-112 and TR701-113, prior systemic or topical antibiotic therapy was not allowed, and thus, patients who received any systemic or topical antibiotic (on the primary lesion) within 96 hours prior to first dose of study drug were excluded from the CE-EOT and CE-PTE analysis sets.
- Concomitant antibiotic therapy – Systemic concomitant antibiotic therapy was prohibited from the first dose of study drug through the EOT or PTE visits, ie, potentially effective therapy against the baseline pathogen (other than adjunctive aztreonam and/or metronidazole in patients with infected wounds with gram-negative infections [studies TR701-112 and TR701-113]). These patients were excluded from the CE-EOT and CE-PTE analysis sets for the analysis of Investigator's assessment of clinical response.
- Only Studies TR701-112 and TR701-113 were included in the analysis of the programmatic determination of clinical response at EOT. For this outcome measure, patients who received any systemic concomitant antibiotic therapy from the first dose of study drug through the EOT visit that is potentially effective against the baseline pathogen (other than adjunctive aztreonam and/or metronidazole in patients with gram-negative infections) are considered **clinical failures**. Thus, these patients are included in the CE-EOT analysis set for the analysis of this secondary outcome measure.

- Concomitant surgical procedures – Patients who received an unplanned major surgical procedure for the treatment of the primary ABSSSI are defined as a **clinical failure** for both the Investigator's assessment of clinical response and the programmatic determination of clinical response at EOT and thus, are included in the CE analysis sets. Patients with a surgical procedure other than for treatment of the primary ABSSSI that potentially confounds the outcome assessment are excluded from the CE analysis sets.

- Study Drug Therapy

    - Received at least one dose of study drug and the correct study drug based on the randomization assignment
    - For studies TR701-112 and TR701-113, study personnel involved in the assessment of efficacy remained blinded to study treatment, unless a treatment limiting adverse event occurred which required emergency unblinding.
    - **Evaluable failure**: The patient received the first 2 doses of active study drug (TR-701 FA group) or first 4 doses of active study drug (linezolid group) and the Investigator classified the patient as a clinical failure unless the patient had a treatment limiting adverse event. For the analysis of the programmatic determination of clinical response at EOT, only studies TR701-112 and TR701-113 were used.
    - **Evaluable success**: The patient received at least 5 doses of active study drug (TR-701 FA group) or at least 10 doses of active study drug (linezolid group) and the Investigator classifies the patient as a clinical success at the EOT and PTE Visits.

- Clinical Outcome Assessment - Patients must meet the following to be included in the CE analysis sets:
  For the CE-EOT analysis set:
    - Completed the clinical response outcome assessment at the EOT Visit (not an indeterminate response)
    - The EOT Visit occurred in the protocol specified window (unless deemed a failure prior to the scheduled visit).

- For the CE-PTE analysis set:
    - Completed the Investigator's assessment of clinical response at the PTE Visit (not an indeterminate response), unless defined as a clinical failure based on Investigator's assessment at the EOT Visit.
    - The PTE Visit occurred in the protocol specified window (unless deemed a failure prior to the scheduled visit).
- Baseline or Inter-current medical events – Patients were excluded from the CE analysis set if they met a protocol-defined exclusion criteria at baseline (ie, prior to randomization) that could have affected the efficacy assessment.

The **Microbiological Evaluable** (ME) analysis set consists of all patients in both the MITT and CE-EOT analysis set. The ME-PTE analysis set consists of all patients in both the MITT and CE-PTE analysis sets.

Primary Efficacy Clinical Outcome Measures

Briefly, the primary outcome measure was early clinical response at 48 to 72 hours after the first dose of study

drug in the ITT analysis set. Only studies TR701-112, TR701-113, and TR701-126 (not included in the microbiology analysis), were included in the primary efficacy outcome, since lesion measurement data were not collected within the 48-72 hour timeframe in the TR701-104 study. The primary outcome measure is the early clinical response at 48 to 72 hours after the first dose of study drug in the ITT Analysis Set. For the EMA, early clinical response is an additional efficacy outcome measure. Patients were programmatically defined as a responder or a nonresponder to therapy (Table 101).

Table 101: Clinical Response at 48 to 72 Hours after First Dose of Study Drug

Clinical Response	Definition

Responder	Patients were programmatically defined as a **responder** if:
	The patient had at least a 20% decrease from baseline in lesion area 48 to 72 hours after the first dose of study drug.[a]
	• Lesion area was defined as:
	  ○ longest head-to-toe length of the erythema x widest perpendicular width of the erythema (TR701-112)
	  ○ longest head-to-toe length of the largest of erythema, induration and/or edema x widest perpendicular width of the largest of erythema, induration and/or edema (TR701-113).

Nonresponder	Patients were programmatically defined as a **nonresponder** if any of the criteria outlined below were met: • At the 48 to 72 Hour Visit, less than a 20% decrease from baseline in lesion area, compared with baseline. • Through 72 hours after the first dose of study drug, receipt of any systemic concomitant antibiotic therapy that is potentially effective against the baseline pathogen with the exception of adjunctive aztreonam and/or metronidazole in patients with wound infections. If a patient did not have a pathogen isolated at baseline and the systemic concomitant antibiotic received has gram-positive activity, the patient was defined as a failure. • Through 72 hours after the first dose of study drug, death (all-cause mortality).
Indeterminate	Patients were considered to have an **indeterminate** response if they had missing data such that a response could not be determined. Patients with an indeterminate response are included in the denominator for the determination of the response rate, therefore these patients are essentially defined as nonresponders.

Source: Module 5.3.5.3 ISE Statistical Analysis Plan V2.0 Section 3.4.1
[a]Lesion measurement data were not collected within the 48-72 hour timeframe in the TR701-104 study.

If no lesion measurements taken prior to the first dose of study drug were available, lesion measurements taken within 6 hours after the first dose of study drug could be used as the baseline measurement. The 48-72 hour visit occurred no later than 73 hours (ie, 72 hours and 59 minutes) after the first dose of study drug.

Microbiological Outcomes

Microbiological response definitions for the evaluations performed at the EOT and PTE Visits are presented in Table 102. Note that post-baseline microbiological samples were collected only in patients with no improvement or with deterioration of lesions and if easily accessible.

Table 102: Microbiological Response Definitions at the EOT and PTE Visits

Term	Definition
Eradication	Absence of original baseline pathogen(s)
Presumed Eradication	No source specimen to culture in a patient assessed as a clinical success: • Based on the programmatic determination of clinical response for the EOT Visit • Based on the Investigator's assessment of clinical response for the PTE Visit
Persistence	Continued presence of the original baseline pathogen(s)
Presumed Persistence	No source specimen to culture in a patient assessed with a clinical failure: • Based on the programmatic determination of clinical response for the EOT Visit • Based on the Investigator's assessment of clinical response for the PTE Visit
Indeterminate	The patient's clinical response was indeterminate or other circumstance that precluded a microbiological evaluation

Source: Module 5.3.5.3 ISE Statistical Analysis Plan V2.0 Table 8.
Abbreviations: EOT=end of treatment; PTE=post therapy evaluation

For patients with multiple microbiological samples taken either while the patient was on study drug (for determination of superinfection) or post-treatment (for determination of new infection) all cultures were used in the analysis. Microbiological outcome categories at the EOT and PTE Visits are eradication, presumed eradication, persistence, presumed persistence, and indeterminate. **Favorable microbiological outcomes** are eradication or presumed eradication. **Unfavorable microbiological outcomes** are persistence or presumed persistence. Microbiological response was derived using electronic microbiology data from the central laboratory (or the local laboratory if the central laboratory never received a specimen) and from pathogen determination provided by Trius for each baseline isolate. Per-patient microbiological responses were based on per-pathogen outcomes.

Microbiological Efficacy Analyses

The clinical response assessment by key target pathogen was determined as the proportion of patients with a clinical success at the EOT Visit and the PTE Visit, for each key target pathogen isolated at baseline for the blood culture and separately for the culture of the ABSSSI site. The number and percentage of patients in each treatment group with a clinical success was tabulated per key target pathogen for the MITT and ME analysis sets. The Investigator's assessment of clinical response at the PTE Visit in the MITT and ME analysis sets was also provided by mono-microbial and poly-microbial infections (gram-positive only and mixed infections [gram-positive and gram-negative]) from the ABSSSI site.

Per-pathogen microbiological response and Investigators assessment of clinical response at EOT (MITT analysis set) and PTE (MITT and ME analysis set) were summarized by MIC of TR-700 or linezolid and for the disk diffusion zone diameters of TR-700 for the key target pathogens from the primary infection site or blood. Pathogens from the primary infection site and blood culture were combined for summary of per pathogen outcomes. Microbiological categories for pathogens identified after baseline assessment are Superinfection and New Infection. The number and percentage of patients with a Superinfection or New Infection after baseline was presented by treatment group for the MITT and ME analysis sets.

The following microbiological analyses were completed for the subgroups:

- Baseline pathogens from the primary infection site and from the blood culture in the MITT analysis set

- Baseline MIC of TR-700 and linezolid to key target pathogens from the primary infection site or blood culture in the MITT analysis set by Geographic Region
- MIC summary statistics of TR-700 and linezolid to key target pathogens from the primary infection site or blood culture in the MITT analysis set by Geographic Region
- Per-pathogen microbiologic response at EOT (MITT analysis set) and PTE (MITT and ME analysis sets) by MIC of TR-700 or linezolid for key target pathogens from the primary infection site or blood culture by Geographic Region
- Per-pathogen microbiologic response at EOT (MITT analysis set) and PTE (MITT and ME analysis sets) by disk diffusion zone diameter of TR-700 for key target pathogens from the primary infection site or blood culture by Geographic Region
- Per-pathogen Investigators assessment of clinical response at EOT (MITT analysis set) and PTE (MITT and ME analysis sets) by MIC of TR-700 or linezolid for key target pathogens from the primary infection site or blood culture by Geographic Region
- Per-pathogen Investigators assessment of clinical response at EOT (MITT analysis set) and PTE (MITT and ME analysis sets) by disk diffusion zone diameter of TR-700 or linezolid for key target pathogens from the primary infection site or blood culture by Geographic Region
- Per-pathogen programmatic determination of clinical response at EOT (MITT analysis set) by MIC of TR-700 or linezolid for key target pathogens from the primary infection site or blood culture by Geographic Region

Baseline Isolates in Clinical Trials

Microbiological samples were required only at baseline and in patients with no improvement or with deterioration of lesion, and, for cellulitis, if easily accessible. The distribution of species and the in vitro susceptibility of baseline pathogens in the MITT and ME populations are summarized in the following sections. Summary data presented include the distribution of baseline pathogenic organisms by primary infection site or blood culture and MIC and zone diameter values for TR-700 and linezolid. The data are further analyzed by geographic region, clinical syndrome, and the IV drug abuser subpopulations. Early clinical studies used the disodium salt of TR-701 (TR-701), while Phase 3 studies used the free acid of TR-701 (TR-701 FA). TR-701/FA is used in this section when reference is made to both the disodium salt and the free acid of TR-701.

The tables and figures will present the data for the MITT and/or ME populations as indicated in **Table 103**. The ME population (at PTE) as a percentage of the MITT population was 87.7% and 84.2%, respectively, for the TR-701/FA treatment group of the uncontrolled and controlled studies. For the combined studies, the ME population was 84.6% of the MITT population. The ME population as a percentage of the MITT population was 82.5% for linezolid.

Table 103: Integrated Summary of Microbiology Data from Central Laboratory

Population	Geographic Region	Uncontrolled Study (104)	Controlled Studies (112/113)		Combined Uncontrolled and Controlled Studies Data (104/112/113)
		TR-701 200 mg	TR-701 FA 200 mg	Linezolid 1200 mg	TR-701/FA 200 mg
MITT, No. Patients	All	49	406	412	455
	US/Canada	49	297	303	346
	Europe	0	77	84	77
	EU[a]	0	15	13	15
	Other	0	32	25	32
ME, No. Patients at PTE	All	43	342	340	385
	US/Canada	43	243	247	286
	Europe	0	72	72	72
	EU	0	11	12	11
	Other	0	27	21	27
ME at PTE as Percentage of MITT (All Regions)		87.7	84.2	82.5	84.6

Source: Appendix 9 Summary Tables – Table 1.1, Table 1.1.1, Table 1.1.2, and Table 8.2, Table 8.2.1, and Table 8.2.2
Abbreviations: MITT=Microbiological intent to treat; ME=Microbiologically evaluable; PTE=post-therapy evaluation
[a]EU, European Union as a subset of Europe.

Microbiological Response

Overall, high rates of per-pathogen and per-patient microbiological responses were seen in the TR-701/FA and linezolid groups at the EOT and PTE Visits across analysis sets, and rates were generally consistent across geographic regions and clinical syndromes. Almost all favorable responses were based on clinical response and not on confirmed pathogen eradication.

Microbiological outcome categories at the EOT and PTE Visits were eradication, presumed eradication, persistence, presumed persistence, and indeterminate. Favorable microbiological outcomes were eradication or presumed eradication. Unfavorable microbiological outcomes were persistence or presumed persistence. Microbiological response was derived using data from the central laboratory and from pathogen determination provided by the Sponsor for each baseline isolate.

Per-patient microbiological responses were based on per-pathogen outcomes. To have an overall per-patient favorable microbiologic response, the outcome for each baseline pathogen must have been favorable (eradicated or presumed eradicated). If the outcome for any pathogen was unfavorable (persistence or presumed persistence), the patient was considered to have an unfavorable per-patient microbiologic response. If the same pathogen was isolated from both the blood and the ABSSSI site culture, the worst outcome was used to determine per-patient microbiologic response. Superinfections were not considered in the microbiological response.

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Tedizolid Phosphate (Sivextro)

Per-Pathogen Microbiological Response (ME population)

In the TR-701 FA and linezolid groups, 95.6% and 98.0% of patients, respectively, had a favorable microbiological response for *Staphylococcus aureus* at the EOT Visit. The favorable response rates for MRSA were 92.6% for the TR-701/FA group and 96.8% for the linezolid group, and for MSSA were 97.7% and 98.9%, respectively. Per-pathogen microbiological responses for key target pathogens from the primary infection site or blood cultures at the EOT Visit were similar in both treatment groups in the ME Analysis Set. The few exceptions, where the difference in response rate was greater than 10% between the TR-701 FA and linezolid groups, were seen with pathogens that involved smaller numbers of patients. The per-pathogen microbiological response for key target pathogens from the primary infection site or blood cultures at the EOT Visit in the ME Analysis Set is presented in Table 104.

Table 104: Per-pathogen Microbiological Response for Key Target Pathogens from the Primary Infection Site or Blood Cultures at the EOT Visit (ME Analysis Set)

Per-pathogen Microbiological Response at the EOT Visit	Controlled Studies (112/113)	
	TR-701/FA 200 mg (N=358) n (%)	Linezolid 1200 mg (N=366) n (%)
Staphylococcus aureus,N1	293	306
Favorable	280 (95.6)	300 (98.0)
Eradication	0	2 (0.7)
Presumed Eradication	280 (95.6)	298 (97.4)
Unfavorable or Indeterminate	13 (4.5)	6 (2.0)
Persistence	4 (1.4)	1 (0.3)
Presumed Persistence	8 (2.7)	3 (1.0)
Indeterminate	1 (0.3)	2 (0.7)
MRSA,N1	121	125
Favorable	112 (92.6)	121 (96.8)
Eradication	0	0
Presumed Eradication	112 (92.6)	120 (96.0)
Unfavorable or Indeterminate	9 (7.4)	4 (3.2)
Persistence	1 (0.8)	1 (0.8)
Presumed Persistence	7 (5.8)	2 (1.6)
Indeterminate	1 (0.8)	1 (0.8)
MSSA,N1	172	183
Favorable	168 (97.7)	181 (98.9)
Eradication	0	2 (1.1)
Presumed Eradication	168 (97.7)	178 (97.3)
Unfavorable or Indeterminate	4 (2.3)	2 (1.1)
Persistence	3 (1.7)	0
Presumed Persistence	1 (0.6)	1 (0.5)
Indeterminate	0	1 (0.5)
Streptococcus pyogenes,N1	31	17
Favorable	31 (100.0)	17 (100.0)
Eradication	1 (3.2)	1 (5.9)
Presumed Eradication	30 (96.8)	16 (94.1)
Unfavorable or Indeterminate	0	0
Persistence	0	0
Presumed Persistence	0	0
Indeterminate	0	0
Streptococcus anginosus group,N1	22	26
Favorable	21 (95.5)	25 (96.2)
Eradication	0	0
Presumed Eradication	21 (95.5)	25 (96.2)
Unfavorable or Indeterminate	1 (4.5)	1 (3.8)
Persistence	0	0
Presumed Persistence	1 (4.5)	1 (3.8)
Indeterminate	0	0
Peptostreptococcus spp.,N1	3	5
Favorable	3 (100.0)	5 (100.0)
Eradication	0	0
Presumed Eradication	3 (100.0)	5 (100.0)
Unfavorable or Indeterminate	0	0
Persistence	0	0
Presumed Persistence	0	0
Indeterminate	0	0
Enterococcus faecalis,N1	8	4
Favorable	6 (75.0)	4 (100.0)
Eradication	0	0
Presumed Eradication	6 (75.0)	4 (100.0)
Unfavorable or Indeterminate	2 (25.0)	0
Persistence	0	0
Presumed Persistence	2 (25.0)	0
Indeterminate	0	0
Staphylococcus haemolyticus,N1	5	8
Favorable	5 (100.0)	8 (100.0)
Eradication	0	1 (12.5)
Presumed Eradication	5 (100.0)	7 (87.5)
Unfavorable or Indeterminate	0	0
Persistence	0	0
Presumed Persistence	0	0
Indeterminate	0	0
Staphylococcus lugdunensis,N1	3	5
Favorable	3 (100.0)	5 (100.0)
Eradication	0	0
Presumed Eradication	3 (100.0)	5 (100.0)
Unfavorable or Indeterminate	0	0
Persistence	0	0
Presumed Persistence	0	0
Indeterminate	0	0
Streptococcus agalactiae,N1	9	9
Favorable	8 (88.9)	8 (88.9)
Eradication	0	0
Presumed Eradication	8 (88.9)	8 (88.9)
Unfavorable or Indeterminate	1 (11.1)	1 (11.1)
Persistence	0	0
Presumed Persistence	1 (11.1)	0
Indeterminate	0	1 (11.1)

Source: Post-text Table 35.2

Abbreviations: ABSSSI=acute bacterial skin and skin structure infection; EOT=end of therapy; ME=microbiologically evaluable; MRSA=methicillin-resistant *Staphylococcus aureus*; MSSA=methicillin-susceptible *Staphylococcus aureus*

Notes: N=number of patients in the ME analysis set; n=number of patients in the specific category. N1=number of patients with the specific pathogen isolated from the ABSSSI. Percentages are calculated as 100 × (n/N1). Presumed eradication and presumed persistence is based on the programmatic determination of clinical response at the EOT.

Favorable per-pathogen microbiological response for key target pathogens from the primary infection site or blood cultures at the PTE Visit was similar in both treatment groups in Controlled Studies in the ME Analysis Set. The few exceptions, where the difference in response rate was greater than 10% between TR-701 FA and linezolid groups, was seen with pathogens that involved smaller numbers of patients. The TR-701/FA response rate was similar in the Controlled Studies and the Combined Uncontrolled and Controlled Studies. The per-pathogen microbiological response for key target pathogens from the primary infection site or blood culture at the PTE Visit in the ME Analysis Set is presented in Table 105.

Table 105 Per-Pathogen Microbiological Response for Key Target Pathogens from the Primary Infection Site or Blood Culture at the PTE Visit (ME Analysis Set)

Per-Pathogen Microbiological Response at the PTE Visit	Controlled Studies (112/113)		Combined Uncontrolled and Controlled Studies (104/112/113)
	TR-701/FA 200 mg (N=342) n (%)	Linezolid 1200 mg (N=340) n (%)	TR-701/FA 200 mg (N=385) n (%)
Staphylococcus aureus, N1	285	284	324
Favorable	271 (95.1)	279 (98.2)	310 (95.7)
Eradication	0	2 (0.7)	0
Presumed Eradication	271 (95.1)	277 (97.5)	310 (95.7)
Unfavorable or Indeterminate	14 (4.9)	5 (1.8)	14 (4.3)
Persistence	3 (1.1)	0	3 (0.9)
Presumed Persistence	11 (3.9)	5 (1.8)	11 (3.4)
Indeterminate	0	0	0
MRSA, N1	122	116	154
Favorable	112 (91.8)	113 (97.4)	144 (93.5)
Eradication	0	0	0
Presumed Eradication	112 (91.8)	112 (96.6)	144 (93.5)
Unfavorable or Indeterminate	10 (8.2)	3 (2.6)	10 (6.5)
Persistence	1 (0.8)	0	1 (0.6)
Presumed Persistence	9 (7.4)	3 (2.6)	9 (5.8)
Indeterminate	0	0	0
MSSA, N1	163	170	170
Favorable	159 (97.5)	168 (98.8)	166 (97.6)
Eradication	0	2 (1.2)	0
Presumed Eradication	159 (97.5)	165 (97.1)	166 (97.6)
Unfavorable or Indeterminate	4 (2.5)	2 (1.2)	4 (2.4)
Persistence	2 (1.2)	0	2 (1.2)
Presumed Persistence	2 (1.2)	2 (1.2)	2 (1.2)
Indeterminate	0	0	0
Streptococcus pyogenes, N1	30	18	30
Favorable	28 (93.3)	18 (100.0)	28 (93.3)
Eradication	0	1 (5.6)	0
Presumed Eradication	28 (93.3)	17 (94.4)	28 (93.3)
Unfavorable or Indeterminate	2 (6.7)	0	2 (6.7)
Persistence	0	0	0
Presumed Persistence	2 (6.7)	0	2 (6.7)
Indeterminate	0	0	0
Streptococcus anginosus-milleri group, N1	20	24	20
Favorable	19 (95.0)	23 (95.8)	19 (95.0)
Eradication	0	0	0
Presumed Eradication	19 (95.0)	23 (95.8)	19 (95.0)
Unfavorable or Indeterminate	1 (5.0)	1 (4.2)	1 (5.0)
Persistence	0	0	0
Presumed Persistence	1 (5.0)	1 (4.2)	1 (5.0)
Indeterminate	0	0	0
Peptostreptococcus spp.,N1	2	3	4
Favorable	2 (100.0)	2 (66.7)	4 (100.0)
Eradication	0	0	0
Presumed Eradication	2 (100.0)	2 (66.7)	4 (100.0)
Unfavorable or Indeterminate	0	1 (33.3)	0
Persistence	0	0	0
Presumed Persistence	0	1 (33.3)	0
Indeterminate	0	0	0
Enterococcus faecalis,N1	9	4	9
Favorable	7 (77.8)	4 (100.0)	7 (77.8)
Eradication	0	0	0
Presumed Eradication	7 (77.8)	4 (100.0)	7 (77.8)
Unfavorable or Indeterminate	2 (22.2)	0	2 (22.2)
Persistence	0	0	0
Presumed Persistence	2 (22.2)	0	2 (22.2)
Indeterminate	0	0	0
Staphylococcus haemolyticus,N1	5	6	5
Favorable	5 (100.0)	6 (100.0)	5 (100.0)
Eradication	0	0	0
Presumed Eradication	5 (100.0)	6 (100.0)	5 (100.0)
Unfavorable or Indeterminate	0	0	0
Staphylococcus lugdunensis,N1	4	6	6
Favorable	4 (100.0)	6 (100.0)	6 (100.0)

Per-Pathogen Microbiological Response at the PTE Visit	Controlled Studies (112/113)		Combined Uncontrolled and Controlled Studies (104/112/113)
	TR-701/FA 200 mg (N=342) n (%)	Linezolid 1200 mg (N=340) n (%)	TR-701/FA 200 mg (N=385) n (%)
Eradication	0	0	0
Presumed Eradication	4 (100.0)	6 (100.0)	6 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus agalactiae,N1	8	8	9
Favorable	7 (87.5)	8 (100.0)	8 (88.9)
Eradication	0	0	0
Presumed Eradication	7 (87.5)	8 (100.0)	8 (88.9)
Unfavorable or Indeterminate	1 (12.5)	0	1 (11.1)
Persistence	0	0	0
Presumed Persistence	1 (12.5)	0	1 (11.1)
Indeterminate	0	0	0
Streptococcus dysgalactiae,N1	1	0	1
Favorable	1 (100.0)	0	1 (100.0)
Eradication	0	0	0
Presumed Eradication	1 (100.0)	0	1 (100.0)
Unfavorable or Indeterminate	0	0	0

Source: Post-text Table 36.2
Abbreviations: ABSSSI=acute bacterial skin and skin structure infection; ME=microbiologically evaluable; MRSA=methicillin-resistant Staphylococcus aureus; MSSA=methicillin-susceptible Staphylococcus aureus; PTE=Post-Therapy Evaluation; spp.=species
Notes: N=number of patients in the ME analysis set; N1=Number of patients with the specific pathogen isolated from the ABSSSI; n=number of patients in the specific category. Percentages are calculated as 100 × (n/N). Presumed eradication and presumed persistence is based on the Investigator assessment of clinical response at the PTE Visit.

Per-Pathogen Microbiological Response by Subgroup at the EOT and PTE Visits

Favorable per-pathogen microbiological responses for key target pathogens from the primary infection site or blood cultures at the EOT Visit were similar in both treatment groups in Controlled Studies in the ME Analysis Set by geographic region. For the most commonly isolated pathogen, *Staphylococcus aureus*, positive microbiological responses were seen more frequently in Europe than in the United States/Canada, based primarily on the higher rate of presumed response in Europe. MRSA pathogens had a slightly lower rate of microbiological response compared to MSSA pathogens across all geographic regions. Results were similar for TR-701/FA patients in the Combined Uncontrolled and Controlled Studies. Per-pathogen microbiological responses for key target pathogens from the primary infection site or blood cultures at the EOT Visit by geographic region in the ME Analysis Set are presented in Table 106.

Table 106: Per-Pathogen Microbiological Response for Key Target Pathogens from the Primary Infection Site or Blood Cultures at the EOT Visit by Geographic Region (ME Analysis Set)

Per-Pathogen Microbiological Response at the EOT Visit	Controlled Studies (112/113)		Combined Uncontrolled and Controlled Studies (104/112/113)
	TR-701/FA 200 mg (N=358) n (%)	Linezolid 1200 mg (N=366) n (%)	TR-701/FA 200 mg (N=403) n (%)
United States/Canada			
Staphylococcus aureus, N1	216	222	257
Favorable	204 (94.4)	216 (97.3)	245 (95.3)
Unfavorable or Indeterminate	12 (5.6)	6 (2.7)	12 (4.7)
MRSA, N1	118	121	152
Favorable	109 (92.4)	117 (96.7)	143 (94.1)
Unfavorable or Indeterminate	9 (7.6)	4 (3.3)	9 (5.9)
MSSA, N1	98	103	105
Favorable	95 (96.9)	101 (98.1)	102 (97.1)
Unfavorable or Indeterminate	3 (3.1)	2 (1.9)	3 (2.9)
Streptococcus pyogenes, N1	6	4	6
Favorable	6 (100.0)	4 (100.0)	6 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus anginosus-milleri group, N1	22	24	22
Favorable	21 (95.5)	23 (95.8)	21 (95.5)
Unfavorable or Indeterminate	1 (4.5)	1 (4.2)	1 (4.5)
Peptostreptococcus spp.,N1	2	3	4
Favorable	2 (100.0)	3 (100.0)	4 (100.0)
Unfavorable or Indeterminate	0	0	0
Enterococcus faecalis,N1	3	1	3
Favorable	2 (66.7)	1 (100.0)	2 (66.7)
Unfavorable or Indeterminate	1 (33.3)	0	1 (33.3)
Staphylococcus haemolyticus,N1	1	1	1
Favorable	1 (100.0)	1 (100.0)	1 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus lugdunensis,N1	2	4	4
Favorable	2 (100.0)	4 (100.0)	4 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus agalactiae,N1	7	8	8
Favorable	6 (85.7)	7 (87.5)	7 (87.5)
Unfavorable or Indeterminate	1 (14.3)	1 (12.5)	1 (12.5)
Streptococcus dysgalactiae,N1	1	0	1
Favorable	1 (100.0)	0	1 (100.0)
Unfavorable or Indeterminate	0	0	0
Europe			
Staphylococcus aureus, N1	55	68	55
Favorable	54 (98.2)	68 (100.0)	54 (98.2)
Unfavorable or Indeterminate	1 (1.8)	0	1 (1.8)
MRSA, N1	0	2	0
Favorable	0	2 (100.0)	0
Unfavorable or Indeterminate	0	0	0
MSSA, N1	55	66	55
Favorable	54 (98.2)	66 (100.0)	54 (98.2)
Unfavorable or Indeterminate	1 (1.8)	0	1 (1.8)
Streptococcus pyogenes, N1	20	12	20
Favorable	20 (100.0)	12 (100.0)	20 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus anginosus-milleri group, N1	0	2	0
Favorable	0	2 (100.0)	0
Unfavorable or Indeterminate	0	0	0
Peptostreptococcus spp.,N1	1	1	1
Favorable	1 (100.0)	1 (100.0)	1 (100.0)
Unfavorable or Indeterminate	0	0	0
Enterococcus faecalis,N1	2	2	2
Favorable	2 (100.0)	2 (100.0)	2 (100.0)
Unfavorable or Indeterminate	0	0	0
Staphylococcus haemolyticus,N1	4	3	4
Favorable	4 (100.0)	3 (100.0)	4 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus agalactiae,N1	2	1	2
Favorable	2 (100.0)	1 (100.0)	2 (100.0)
Unfavorable or Indeterminate	0	0	0
Other Region			
Staphylococcus aureus, N1	22	16	22
Favorable	22 (100.0)	16 (100.0)	22 (100.0)
Unfavorable or Indeterminate	0	0	0
MRSA, N1	3	2	3
Favorable	3 (100.0)	2 (100.0)	3 (100.0)
Unfavorable or Indeterminate	0	0	0
MSSA, N1	19	14	19
Favorable	19 (100.0)	14 (100.0)	19 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus pyogenes, N1	5	1	5
Favorable	5 (100.0)	1 (100.0)	5 (100.0)
Unfavorable or Indeterminate	0	0	0
Peptostreptococcus spp.,N1	0	1	0
Favorable	0	1 (100.0)	0
Unfavorable or Indeterminate	0	0	0
Enterococcus faecalis,N1	3	1	3
Favorable	2 (66.7)	1 (100.0)	2 (66.7)
Unfavorable or Indeterminate	1 (33.3)	0	1 (33.3)
Staphylococcus haemolyticus,N1	0	4	0
Favorable	0	4 (100.0)	0
Unfavorable or Indeterminate	0	0	0
Streptococcus lugdunensis,N1	1	1	1
Favorable	1 (100.0)	1 (100.0)	1 (100.0)
Unfavorable or Indeterminate	0	0	0

Source: Post-text Table 42.1
Abbreviations: ABSSSI=acute bacterial skin and skin structure infection; EOT=end of therapy; ME=microbiologically evaluable; MRSA=methicillin-resistant *Staphylococcus aureus*; MSSA=methicillin-susceptible *Staphylococcus aureus*
Notes: N=number of patients in the ITT analysis set; N1=number of patients in the specific with the specific pathogen isolated from the ABSSSI; n=number of patients in the specific category. Percentages are calculated as 100 × (n/N1). Presumed eradication and presumed persistence is based on the programmatic determination of clinical response at the EOT Visit.

Favorable per-pathogen microbiological responses for key target pathogens from the primary infection site or blood cultures at the EOT Visit were similar in both treatment groups in Controlled Studies in the ME Analysis Set by clinical syndrome. MRSA pathogens had a slightly lower rate of microbiological response compared to MSSA pathogens across all clinical syndromes. Results were similar for TR-701/FA patients in the Combined Uncontrolled and Controlled Studies. Per-pathogen microbiological responses for key target pathogens from the primary infection site or blood cultures at the EOT Visit by clinical syndrome in the ME Analysis Set are presented in Table 107.

Table 107: Per-Pathogen Microbiological Response for Key Target Pathogens from the Primary Infection Site or Blood Cultures at the EOT Visit by Clinical Syndrome (ME Analysis Set)

Per-Pathogen Microbiological Response at the EOT Visit	Controlled Studies (112/113)		Combined Uncontrolled and Controlled Studies (104/112/113)
	TR-701/FA 200 mg (N=358) n (%)	Linezolid 1200 mg (N=366) n (%)	TR-701/FA 200 mg (N=403) n (%)
Cellulitis/erysipelas			
Staphylococcus aureus, N1	75	91	80
Favorable	71 (94.7)	89 (97.8)	76 (95.0)
Unfavorable or Indeterminate	4 (5.3)	2 (2.2)	4 (5.0)
MRSA, N1	25	31	29
Favorable	22 (88.0)	29 (93.5)	26 (89.7)
Unfavorable or Indeterminate	3 (12.0)	2 (6.5)	3 (10.3)
MSSA, N1	50	60	51
Favorable	49 (98.0)	60 (100.0)	50 (98.0)
Unfavorable or Indeterminate	1 (2.0)	0	1 (2.0)
Streptococcus pyogenes, N1	16	7	16
Favorable	16 (100.0)	7 (100.0)	16 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus anginosus-milleri group, N1	2	5	2
Favorable	2 (100.0)	4 (80.0)	2 (100.0)
Unfavorable or Indeterminate	0	1 (20.0)	0
Peptostreptococcus spp.,N1	0	1	0
Favorable	0	1 (100.0)	0
Unfavorable or Indeterminate	0	0	0
Enterococcus faecalis,N1	3	3	3
Favorable	1 (33.3)	3 (100.0)	1 (33.3)
Unfavorable or Indeterminate	2 (66.7)	0	2 (66.7)
Staphylococcus haemolyticus,N1	4	1	4
Favorable	4 (100.0)	1 (100.0)	4 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus agalactiae,N1	2	3	2
Favorable	1 (50.0)	3 (100.0)	1 (50.0)
Unfavorable or Indeterminate	1 (50.0)	0	1 (50.0)
Infected wound			
Staphylococcus aureus, N1	113	123	113
Favorable	110 (97.3)	120 (97.6)	110 (97.3)
Unfavorable or Indeterminate	3 (2.7)	3 (2.4)	3 (2.7)

Per-Pathogen Microbiological Response at the EOT Visit	Controlled Studies (112/113)		Combined Uncontrolled and Controlled Studies (104/112/113)
	TR-701/FA 200 mg (N=358) n (%)	Linezolid 1200 mg (N=366) n (%)	TR-701/FA 200 mg (N=403) n (%)
MRSA, N1	42	38	42
Favorable	41 (97.6)	37 (97.4)	41 (97.6)
Unfavorable or Indeterminate	1 (2.4)	1 (2.6)	1 (2.4)
MSSA, N1	71	86	71
Favorable	69 (97.2)	84 (97.7)	69 (97.2)
Unfavorable or Indeterminate	2 (2.8)	2 (2.3)	2 (2.8)
Streptococcus pyogenes, N1	11	8	11
Favorable	11 (100.0)	8 (100.0)	11 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus anginosus-milleri group, N1	12	12	12
Favorable	12 (100.0)	12 (100.0)	12 (100.0)
Unfavorable or Indeterminate	0	0	0
Peptostreptococcus spp.,N1	1	2	1
Favorable	1 (100.0)	2 (100.0)	1 (100.0)
Unfavorable or Indeterminate	0	0	0
Enterococcus faecalis,N1	2	1	2
Favorable	2 (100.0)	1 (100.0)	2 (100.0)
Unfavorable or Indeterminate	0	0	0
Staphylococcus haemolyticus,N1	1	4	1
Favorable	1 (100.0)	4 (100.0)	1 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus lugdunensis,N1	2	0	2
Favorable	2 (100.0)	0	2 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus agalactiae,N1	3	4	3
Favorable	3 (100.0)	4 (100.0)	3 (100.0)
Unfavorable or Indeterminate	0	0	0
Major cutaneous abscess			
Staphylococcus aureus, N1	105	92	141
Favorable	99 (94.3)	91 (98.9)	135 (95.7)
Unfavorable or Indeterminate	6 (5.7)	1 (1.1)	6 (4.3)
MRSA, N1	54	56	84
Favorable	49 (90.7)	55 (98.2)	79 (94.0)
Unfavorable or Indeterminate	5 (9.3)	1 (1.8)	5 (6.0)

Per-Pathogen Microbiological Response at the EOT Visit	Controlled Studies (112/113)		Combined Uncontrolled and Controlled Studies (104/112/113)
	TR-701/FA 200 mg (N=358) n (%)	Linezolid 1200 mg (N=366) n (%)	TR-701/FA 200 mg (N=403) n (%)
MSSA, N1	51	37	57
Favorable	50 (98.0)	37 (100.0)	56 (98.2)
Unfavorable or Indeterminate	1 (2.0)	0	1 (1.8)
Streptococcus pyogenes, N1	4	2	4
Favorable	4 (100.0)	2 (100.0)	4 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus anginosus-milleri group, N1	8	9	8
Favorable	7 (87.5)	9 (100.0)	7 (87.5)
Unfavorable or Indeterminate	1 (12.5)	0	1 (12.5)
Peptostreptococcus spp.,N1	2	2	4
Favorable	2 (100.0)	2 (100.0)	4 (100.0)
Unfavorable or Indeterminate	0	0	0
Enterococcus faecalis,N1	3	0	3
Favorable	3 (100.0)	0	3 (100.0)
Unfavorable or Indeterminate	0	0	0
Staphylococcus haemolyticus,N1	0	3	0
Favorable	0	3 (100.0)	0
Unfavorable or Indeterminate	0	0	0
Streptococcus lugdunensis,N1	1	5	3
Favorable	1 (100.0)	5 (100.0)	3 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus agalactiae,N1	4	2	5
Favorable	4 (100.0)	1 (50.0)	5 (100.0)
Unfavorable or Indeterminate	0	1 (50.0)	0
Streptococcus dysgalactiae,N1	1	0	1
Favorable	1 (100.0)	0	1 (100.0)
Unfavorable or Indeterminate	0	0	0

Source: Post-text Table 42.2
Abbreviations: ABSSSI=acute bacterial skin and skin structure infection; EOT=end of therapy; ME=microbiologically evaluable; MRSA=methicillin-resistant *Staphylococcus aureus*; MSSA=methicillin-susceptible *Staphylococcus aureus*
Notes: N=number of patients in the ITT analysis set; N1=number of patients in the specific category with the specific pathogen isolated from the ABSSSI; n=number of patients in the specific category. Percentages are calculated as 100 × (n/N1). Presumed eradication and presumed persistence is based on the programmatic determination of clinical response at the EOT Visit.

Favorable per-pathogen microbiological responses for key target pathogens from the primary infection site or blood culture at the PTE Visit were similar in both treatment groups in Controlled Studies in the ME Analysis Set by geographic region. For the most commonly isolated pathogen, *Staphylococcus aureus*, positive microbiological responses were seen more frequently in Europe than in the United States/Canada, primarily based on the higher rate of presumed response in Europe. MRSA pathogens had a slightly lower rate of microbiological response compared to MSSA pathogens across all geographic regions. Results were similar for TR-701/FA patients in the Combined Uncontrolled and Controlled Studies. Per-pathogen microbiological responses for key target pathogens from the primary infection site or blood cultures at the PTE Visit by geographic region in the ME Analysis Set are presented in Table 108.

Table 108 Per-Pathogen Microbiological Response for Key Target Pathogens from the Primary Infection Site or Blood Cultures at the PTE Visit by Geographic Region (ME Analysis Set)

Per-Pathogen Microbiological Response at the PTE Visit	Controlled Studies (112/113)		Combined Uncontrolled and Controlled Studies (104/112/113)
	TR-701/FA 200 mg (N=342) n (%)	Linezolid 1200 mg (N=340) n (%)	TR-701/FA 200 mg (N=385) n (%)
United States/Canada			
Staphylococcus aureus, N1	209	206	248
Favorable	198 (94.7)	201 (97.6)	237 (95.6)
Unfavorable or Indeterminate	11 (5.3)	5 (2.4)	11 (4.4)
MRSA, N1	119	112	151
Favorable	110 (92.4)	109 (97.3)	142 (94.0)
Unfavorable or Indeterminate	9 (7.6)	3 (2.7)	9 (6.0)
MSSA, N1	90	96	97
Favorable	88 (97.8)	94 (97.9)	95 (97.9)
Unfavorable or Indeterminate	2 (2.2)	2 (2.1)	2 (2.1)
Streptococcus pyogenes, N1	7	4	7
Favorable	6 (85.7)	4 (100.0)	6 (85.7)
Unfavorable or Indeterminate	1 (14.3)	0	1 (14.3)
Streptococcus anginosus-milleri group, N1	20	23	20
Favorable	19 (95.0)	22 (95.7)	19 (95.0)
Unfavorable or Indeterminate	1 (5.0)	1 (4.3)	1 (5.0)
Peptostreptococcus spp.,N1	1	2	3
Favorable	1 (100.0)	1 (50.0)	3 (100.0)
Unfavorable or Indeterminate	0	1 (50.0)	0
Enterococcus faecalis,N1	4	1	4
Favorable	3 (75.0)	1 (100.0)	3 (75.0)
Unfavorable or Indeterminate	1 (25.0)	0	1 (25.0)
Staphylococcus haemolyticus,N1	1	1	1
Favorable	1 (100.0)	1 (100.0)	1 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus lugdunensis,N1	3	5	5
Favorable	3 (100.0)	5 (100.0)	5 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus agalactiae,N1	7	7	8
Favorable	6 (85.7)	7 (100.0)	7 (87.5)
Unfavorable or Indeterminate	1 (14.3)	0	1 (12.5)
Streptococcus dysgalactiae,N1	1	0	1
Favorable	1 (100.0)	0	1 (100.0)
Unfavorable or Indeterminate	0	0	0
Europe			
Staphylococcus aureus, N1	54	62	54
Favorable	53 (98.1)	62 (100.0)	53 (98.1)
Unfavorable or Indeterminate	1 (1.9)	0	1 (1.9)
MRSA, N1	0	2	0
Favorable	0	2 (100.0)	0
Unfavorable or Indeterminate	0	0	0
MSSA, N1	54	60	54
Favorable	53 (98.1)	60 (100.0)	53 (98.1)
Unfavorable or Indeterminate	1 (1.9)	0	1 (1.9)
Streptococcus pyogenes, N1	18	12	18
Favorable	18 (100.0)	12 (100.0)	18 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus anginosus-milleri group, N1	0	1	0
Favorable	0	1 (100.0)	0
Unfavorable or Indeterminate	0	0	0
Peptostreptococcus spp.,N1	1	0	1
Favorable	1 (100.0)	0	1 (100.0)
Unfavorable or Indeterminate	0	0	0
Enterococcus faecalis,N1	2	2	2
Favorable	2 (100.0)	2 (100.0)	2 (100.0)
Unfavorable or Indeterminate	0	0	0
Staphylococcus haemolyticus,N1	4	3	4
Favorable	4 (100.0)	3 (100.0)	4 (100.0)

Per-Pathogen Microbiological Response at the PTE Visit	Controlled Studies (112/113)		Combined Uncontrolled and Controlled Studies (104/112/113)
	TR-701/FA 200 mg (N=342) n (%)	Linezolid 1200 mg (N=340) n (%)	TR-701/FA 200 mg (N=385) n (%)
Unfavorable or Indeterminate	0	0	0
Streptococcus agalactiae,N1	1	1	1
Favorable	1 (100.0)	1 (100.0)	1 (100.0)
Unfavorable or Indeterminate	0	0	0
Other Region			
Staphylococcus aureus, N1	22	16	22
Favorable	20 (90.9)	16 (100.0)	20 (90.9)
Unfavorable or Indeterminate	2 (9.1)	0	2 (9.1)
MRSA, N1	3	2	3
Favorable	2 (66.7)	2 (100.0)	2 (66.7)
Unfavorable or Indeterminate	1 (33.3)	0	1 (33.3)
MSSA, N1	19	14	19
Favorable	18 (94.7)	14 (100.0)	18 (94.7)
Unfavorable or Indeterminate	1 (5.3)	0	1 (5.3)
Streptococcus pyogenes, N1	5	2	5
Favorable	4 (80.0)	2 (100.0)	4 (80.0)
Unfavorable or Indeterminate	1 (20.0)	0	1 (20.0)
Peptostreptococcus spp.,N1	0	1	0
Favorable	0	1 (100.0)	0
Unfavorable or Indeterminate	0	0	0
Enterococcus faecalis,N1	3	1	3
Favorable	2 (66.7)	1 (100.0)	2 (66.7)
Unfavorable or Indeterminate	1 (33.3)	0	1 (33.3)
Staphylococcus haemolyticus,N1	0	2	0
Favorable	0	2 (100.0)	0
Unfavorable or Indeterminate	0	0	0
Streptococcus lugdunensis,N1	1	1	1
Favorable	1 (100.0)	1 (100.0)	1 (100.0)
Unfavorable or Indeterminate	0	0	0

Source: Post-text Table 42.4
Abbreviations: ABSSSI=acute bacterial skin and skin structure infection; ME=microbiologically evaluable; MRSA=methicillin-resistant *Staphylococcus aureus*; MSSA=methicillin-susceptible *Staphylococcus aureus*; PTE=post-therapy evaluation
Notes: N=number of patients in the ITT analysis set; N1=number of patients in the specific category with the specific pathogen isolated from the ABSSSI; n=number of patients in the specific category. Percentages are calculated as 100 × (n/N1). Presumed eradication and presumed persistence is based on the programmatic determination of clinical response at the EOT Visit.

Favorable per-pathogen microbiological responses for key target pathogens from the primary infection site or blood cultures at the PTE Visit were similar in both treatment groups in Controlled Studies in the ME Analysis Set by clinical syndrome. MRSA pathogens had a slightly lower rate of microbiological response compared to MSSA pathogens across all clinical syndromes. Results were similar for TR-701/FA patients in the Combined Uncontrolled and Controlled Studies. Per-pathogen microbiological responses for key target pathogens from the primary infection site or blood cultures at the PTE Visit by clinical syndrome in the ME Analysis Set are presented in Table 109.

Table 109 Per-Pathogen Microbiological Response for Key Target Pathogens from the Primary Infection Site or Blood Cultures at the PTE Visit by Clinical Syndrome (ME Analysis Set)

Per-Pathogen Microbiological Response at the EOT Visit	Controlled Studies (112/113)		Combined Uncontrolled and Controlled Studies (104/112/113)
	TR-701/FA 200 mg (N=342) n (%)	Linezolid 1200 mg (N=340) n (%)	TR-701/FA 200 mg (N=385) n (%)
Cellulitis/erysipelas			
Staphylococcus aureus, N1	75	84	81
Favorable	70 (93.3)	82 (97.6)	76 (93.8)
Unfavorable or Indeterminate	5 (6.7)	2 (2.4)	5 (6.2)
MRSA, N1	27	29	32
Favorable	24 (88.9)	27 (93.1)	29 (90.6)
Unfavorable or Indeterminate	3 (11.1)	2 (6.9)	3 (9.4)
MSSA, N1	48	55	49
Favorable	46 (95.8)	55 (100.0)	47 (95.9)
Unfavorable or Indeterminate	2 (4.2)	0	2 (4.1)
Streptococcus pyogenes, N1	17	8	17
Favorable	16 (94.1)	8 (100.0)	16 (94.1)
Unfavorable or Indeterminate	1 (5.9)	0	1 (5.9)
Streptococcus anginosus-milleri group, N1	3	3	3
Favorable	3 (100.0)	2 (66.7)	3 (100.0)
Unfavorable or Indeterminate	0	1 (33.3)	0
Enterococcus faecalis,N1	3	3	3
Favorable	1 (33.3)	3 (100.0)	1 (33.3)
Unfavorable or Indeterminate	2 (66.7)	0	2 (66.7)
Staphylococcus haemolyticus,N1	4	1	4
Favorable	4 (100.0)	1 (100.0)	4 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus agalactiae,N1	2	3	2
Favorable	1 (50.0)	3 (100.0)	1 (50.0)
Unfavorable or Indeterminate	1 (50.0)	0	1 (50.0)
Infected wound			
Staphylococcus aureus, N1	107	112	107
Favorable	103 (96.3)	110 (98.2)	103 (96.3)
Unfavorable or Indeterminate	4 (3.7)	2 (1.8)	4 (3.7)
MRSA, N1	43	35	43
Favorable	41 (95.3)	34 (97.1)	41 (95.3)
Unfavorable or Indeterminate	2 (4.7)	1 (2.9)	2 (4.7)
MSSA, N1	64	78	64
Favorable	62 (96.9)	77 (98.7)	62 (96.9)
Unfavorable or Indeterminate	2 (3.1)	1 (1.3)	2 (3.1)
Streptococcus pyogenes, N1	9	8	9
Favorable	8 (88.9)	8 (100.0)	8 (88.9)
Unfavorable or Indeterminate	1 (11.1)	0	1 (11.1)
Streptococcus anginosus-milleri group, N1	11	12	11
Favorable	11 (100.0)	12 (100.0)	11 (100.0)
Unfavorable or Indeterminate	0	0	0
Peptostreptococcus spp.,N1	1	2	1
Favorable	1 (100.0)	2 (100.0)	1 (100.0)
Unfavorable or Indeterminate	0	0	0
Enterococcus faecalis,N1	2	1	2
Favorable	2 (100.0)	1 (100.0)	2 (100.0)
Unfavorable or Indeterminate	0	0	0
Staphylococcus haemolyticus,N1	1	3	1
Favorable	1 (100.0)	3 (100.0)	1 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus lugdunensis,N1	2	0	2
Favorable	2 (100.0)	0	2 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus agalactiae,N1	2	4	2
Favorable	2 (100.0)	4 (100.0)	2 (100.0)
Unfavorable or Indeterminate	0	0	0
Major cutaneous abscess			
Staphylococcus aureus, N1	103	88	136
Favorable	98 (95.1)	87 (98.9)	131 (96.3)
Unfavorable or Indeterminate	5 (4.9)	1 (1.1)	5 (3.7)

Per-Pathogen Microbiological Response at the EOT Visit	Controlled Studies (112/113)		Combined Uncontrolled and Controlled Studies (104/112/113)
	TR-701/FA 200 mg (N=342) n (%)	Linezolid 1200 mg (N=340) n (%)	TR-701/FA 200 mg (N=385) n (%)
MRSA, N1	52	52	79
Favorable	47 (90.4)	52 (100.0)	74 (93.7)
Unfavorable or Indeterminate	5 (9.6)	0	5 (6.3)
MSSA, N1	51	37	57
Favorable	51 (100.0)	36 (97.3)	57 (100.0)
Unfavorable or Indeterminate	0	1 (2.7)	0
Streptococcus pyogenes, N1	4	2	4
Favorable	4 (100.0)	2 (100.0)	4 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus anginosus-milleri group, N1	6	9	6
Favorable	5 (83.3)	9 (100.0)	5 (83.3)
Unfavorable or Indeterminate	1 (16.7)	0	1 (16.7)
Peptostreptococcus spp.,N1	1	1	3
Favorable	1 (100.0)	0	3 (100.0)
Unfavorable or Indeterminate	0	1 (100.0)	0
Enterococcus faecalis,N1	4	0	4
Favorable	4 (100.0)	0	4 (100.0)
Unfavorable or Indeterminate	0	0	0
Staphylococcus haemolyticus,N1	0	2	0
Favorable	0	2 (100.0)	0
Unfavorable or Indeterminate	0	0	0
Streptococcus lugdunensis,N1	2	6	4
Favorable	2 (100.0)	6 (100.0)	4 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus agalactiae,N1	4	1	5
Favorable	4 (100.0)	1 (100.0)	5 (100.0)
Unfavorable or Indeterminate	0	0	0
Streptococcus dysgalactiae,N1	1	0	1
Favorable	1 (100.0)	0	1 (100.0)
Unfavorable or Indeterminate	0	0	0

Source: Post-text Table 42.5
Abbreviations: ABSSSI=acute bacterial skin and skin structure infection; ME=microbiologically evaluable; MRSA=methicillin-resistant *Staphylococcus aureus*; MSSA=methicillin-susceptible *Staphylococcus aureus*; PTE=post-therapy evaluation
Notes: N=number of patients in the ITT analysis set; N1=number of patients in the specific category with the specific pathogen isolated from the ABSSSI; n=number of patients in the specific category. Percentages are calculated as 100 × (n/N). Presumed eradication and presumed persistence is based on the programmatic determination of clinical response at the EOT Visit.

Correlation of MIC and Zone Diameter with Clinical and Microbiological Outcome

This section provides a summary of the investigator's assessment of TR-701 clinical response and favorable microbiological outcome by MIC and PTE for the microbiological evaluable population from the controlled studies. Table 110 provides a summary of MIC versus Investigator's Assessment of Clinical Outcome. A summary of MIC versus Microbiologic Favorable Response is shown in Table 111. A positive clinical response was observed for staphylococci, streptococci, and enterococci for MIC values up to 0.5, 0.25, and 0.5 mcg/mL, respectively.

T able 110: Summary of MIC versus Investigator's Assessment of Clinical Outcome at PTE; Microbiologically Evaluable Analysis Set

Positive Clinical Outcome (n/N1[a]) and Percent for Pathogen by TR-700 MIC (µg/mL) Value									
MIC (µg/mL)	***Staphylococcus aureus* (All)**	**MRSA**	**MSSA**	***Staphylococcus haemolyticus***	***Staphylococcus lugdunensis***	***Streptococcus pyogenes***	***Streptococcus anginosus* Group**	***Streptococcus agalactiae***	***Enterococcus faecalis***
≤0.015						1/1 (100)	2/2 (100)		
0.015									
0.03							2/2 (100)		
0.06						2/2 (100)	3/3 (100)		
0.12	4/5 (80)	4/5 (80)		2/2 (100)	3/3 (100)	19/20 (95.0)	7/7 (100)	2/2 (100)	
0.25	212/225 (94.2)	100/109 (91.7)	112/116 (96.6)	3/3 (100)	1/1 (100)	5/7 (71.4)	4/4 (100)	5/5 (100)	4/4 (100)
0.5	54/55 (98.2)	6/6 (100)	48/49 (98.0)						2/3 (66.7)
1									
Total	**270/285 (94.7)**	**110/120 (91.7)**	**160/165 (96.9)**	**5/5 (100)**	**4/4 (100)**	**27/30 (90.0)**	**18/18 (100)**	**7/7 (100)**	**6/7 (85.7)**

Source: Appendix 9 Summary Tables - Table 9.3
Abbreviations: PTE=post therapy evaluation; MIC=minimum inhibitory concentration; MRSA=methicillin-resistant *S. aureus*; MSSA=methicillin-susceptible *S. aureus*
[a]n, number of favorable microbiologic outcomes in the specific category; N1, number of pathogens in the specific category; Percentages are calculated as 100 x (n/N1)

Table 111: Summary of MIC versus Microbiologic Favorable Response at PTE; Microbiologically Evaluable Analysis Set

Microbiologic Favorable Response (n/N1[a]) and Percent for Pathogen by TR-700 MIC (µg/mL) Value									
MIC (µg/mL)	***Staphylococcus aureus* (All)**	**MRSA**	**MSSA**	***Staphylococcus haemolyticus***	***Staphylococcus lugdunensis***	***Streptococcus pyogenes***	***Streptococcus anginosus* Group**	***Streptococcus agalactiae***	***Enterococcus faecalis***
≤0.015						1/1 (100)	2/2 (100)		
0.015									
0.03							2/2 (100)		
0.06						2/2 (100)	3/3 (100)		
0.12	4/5 (80)	4/5 (80)		2/2 (100)	3/3 (100)	19/20 (95.0)	7/7 (100)	2/2 (100)	
0.25	212/225 (94.2)	100/109 (91.7)	112/116 (96.6)	3/3 (100)	1/1 (100)	5/7 (71.4)	4/4 (100)	5/5 (100)	4/4 (100)
0.5	54/55 (98.2)	6/6 (100)	48/49 (98.0)						2/3 (66.7)
1									
Total	**270/285 (94.7)**	**110/120 (91.7)**	**160/165 (96.9)**	**5/5 (100)**	**4/4 (100)**	**27/30 (90.0)**	**18/18 (100)**	**7/7 (100)**	**6/7 (85.7)**

Source: Appendix 9 Summary Tables - Table 8.2
Abbreviations: PTE=post therapy evaluation; MIC=minimum inhibitory concentration; MRSA=methicillin-resistant *S. aureus*; MSSA=methicillin-susceptible *S. aureus*
[a]n, number of favorable microbiologic outcomes in the specific category; N1, number of pathogens in the specific category; Percentages are calculated as 100 x (n/N1)

A summary of Zone Diameter versus Investigator's Assessment of Clinical Outcome is presented in Table 112. A positive clinical response was observed for zone diameter values as low as 16 mm for staphylococci, 18 mm for *S. pyogenes*, 17 mm for *S. anginosus* Group, 18 mm for *S. agalactiae*, and 19 mm for *E. faecalis*.

Table 112: Summary of Zone Diameter versus Investigator's Assessment of Clinical Outcome at PTE; Microbiologically Evaluable Analysis Set

Positive Clinical Outcome (n/N1[a]) and Percent for Pathogen by TR-700 Zone Diameter (mm) Value									
Zone Diameter (mm)	*Staphylococcus aureus* (All)	MRSA	MSSA	*Staphylococcus haemolyticus*	*Staphylococcus lugdunensis*	*Streptococcus pyogenes*	*Streptococcus anginosus* Group	*Streptococcus agalactiae*	*Enterococcus faecalis*
6	1/1 (100)	1/1 (100)							
15									
16	1/1 (100)		1/1 (100)						
17	1/1 (100)	1/1 (100)					1/1 (100)		
18						1/1 (100)	1/1 (100)	1/1 (100)	
19	0/2 (0.0)	0/1 (0.0)	0/1 (0.0)					1/1 (100)	2/2 (100)
20	7/7 (100)	4/4 (100)	3/3 (100)			4/4 (100)			1/1 (100)
21	5/5 (100)	2/2 (100)	3/3 (100)					1/1 (100)	1/2 (50.0)
22	7/8 (87.5)	1/2 (50.0)	6/6 (100)			2/4 (50.0)			1/1 (100)
23	13/13 (100)	9/9 (100)	4/4 (100)	1/1 (100)		4/4 (100)	1/1 (100)		1/1 (100)
24	26/27 (96.3)	10/10 (100)	16/17 (94.1)			7/7 (100)	1/1 (100)	3/3 (100)	
25	13/15 (86.7)	5/6 (83.3)	8/9 (88.9)			5/5 (100)	1/1 (100)		
26	32/34 (94.1)	14/15 (93.3)	18/19 (94.7)			2/3 (66.7)	4/4 (100)	1/1 (100)	
27	35/37 (94.6)	13/14 (92.9)	22/23 (95.7)	2/2 (100)	1/1 (100)	1/1 (100)	1/1 (100)		
28	45/47 (95.7)	17/19 (89.5)	28/28 (100)	1/1 (100)		1/1 (100)	1/1 (100)		
29	28/31 (90.3)	9/12 (75.0)	19/19 (100)				3/3 (100)		
≥30	55/55 (100)	23/23 (100)	32/32 (100)	1/1 (100)	3/3 (100)		4/4 (100)		
Total	**269/284 (94.7)**	**109/119 (91.5)**	**160/165 (97)**	**5/5 (100)**	**4/4 (100)**	**27/30 (90.0)**	**18/18 (100)**	**7/7 (100)**	**6/7 (85.7)**

Source: Appendix 9 Summary Tables - Table 9.6
Abbreviations: PTE=post therapy evaluation; MIC=minimum inhibitory concentration; MRSA=methicillin-resistant *S. aureus;* MSSA=methicillin-susceptible *S. aureus*
[a]n, number of favorable microbiologic outcomes in the specific category; N1, number of pathogens in the specific category; Percentages are calculated as 100 x (n/N1)

A summary of Zone Diameter versus Favorable Microbiologic Outcome is presented in Table 113. As observed for the comparison of Clinical Outcome and Favorable Microbiologic Response for MIC values, virtually identical results were observed when analyzing Favorable Microbiologic Response and Clinical Outcome for zone diameter.

Table 113 Summary of Zone Diameter versus Microbiologic Favorable Response at PTE; Microbiologically Evaluable Analysis Set

Microbiologic Favorable Response (n/N1[a]) and Percent for Pathogen by TR-700 Zone Diameter (mm) Value									
Zone Diameter (mm)	*S. aureus* (All)	MRSA	MSSA	*S. haemolyticus*	*S. lugdunensis*	*S. pyogenes*	*S. anginosus* Group	*S. agalactiae*	*E. faecalis*
6	1/1 (100)	1/1 (100)							
15									
16	1/1 (100)		1/1 (100)						
17	1/1 (100)	1/1 (100)					1/1 (100)		
18						1/1 (100)	1/1 (100)	1/1 (100)	
19	0/2 (0.0)	0/1 (0.0)	0/1 (0.0)					1/1 (100)	2/2 (100)
20	7/7 (100)	4/4 (100)	3/3 (100)			4/4 (100)			1/1 (100)
21	5/5 (100)	2/2 (100)	3/3 (100)					1/1 (100)	1/2 (50.0)
22	8/9 (88.9)	2/3 (66.7)	6/6 (100)			3/5 (60.0)			1/1 (100)
23	15/15 (100)	9/9 (100)	6/6 (100)	1/1 (100)		4/4 (100)	1/1 (100)		1/1 (100)
24	26/27 (96.3)	10/10 (100)	16/17 (94.1)			7/7 (100)	1/1 (100)	4/4 (100)	
25	14/17 (82.4)	5/6 (83.3)	9/11 (81.8)			6/6 (100)	1/1 (100)		
26	33/37 (89.2)	15/16 (93.8)	18/21 (85.5)			3/4 (75.0)	4/4 (100)	1/1 (100)	
27	37/41 (90.2)	13/15 (86.7)	24/26 (92.3)	2/2 (100)	1/1 (100)	1/1 (100)	1/1 (100)		
28	49/51 (96.1)	18/20 (90.0)	31/31 (100)	1/1 (100)		1/1 (100)	1/1 (100)		
29	29/33 (87.9)	9/13 (69.2)	20/20 (100)				3/3 (100)		
≥30	57/57 (100)	24/24 (100)	33/33 (100)	1/1 (100)	3/3 (100)		7/7 (100)		
Total	**283/304 (93.1)**	**113/125 (90.4)**	**170/179 (95)**	**5/5 (100)**	**4/4 (100)**	**30/33 (90.9)**	**21/21 (100)**	**8/8 (100)**	**6/7 (85.7)**

Source: Appendix 9 Summary Tables - Table 8.4
Abbreviations: *E. faecalis=Enterococcus faecalis;* PTE=post therapy evaluation; MIC=minimum inhibitory concentration; MRSA=methicillin-resistant *S. aureus;* MSSA=methicillin-susceptible *S. aureus; S. agalactiae=Streptococcus agalactiae; S. aureus=Staphylococcus aureus; S. anginosus=Streptococcus anginosus; S. haemolyticus=Staphylococcus haemolyticus; S. lugdunensis=Staphylococcus lugdunensis; S. pyogenes=Streptococcus pyogenes*
[a]n, number of favorable microbiologic outcomes in the specific category; N1, number of pathogens in the specific category; Percentages are calculated as 100 x (n/N1)

NDA: 205435/205436 DATE REVIEW COMPLETED: 02/24/2014
Tedizolid Phosphate (Sivextro)

SCATTER PLOTS SHOWING MIC AND DISK DIFFUSION METHODS:

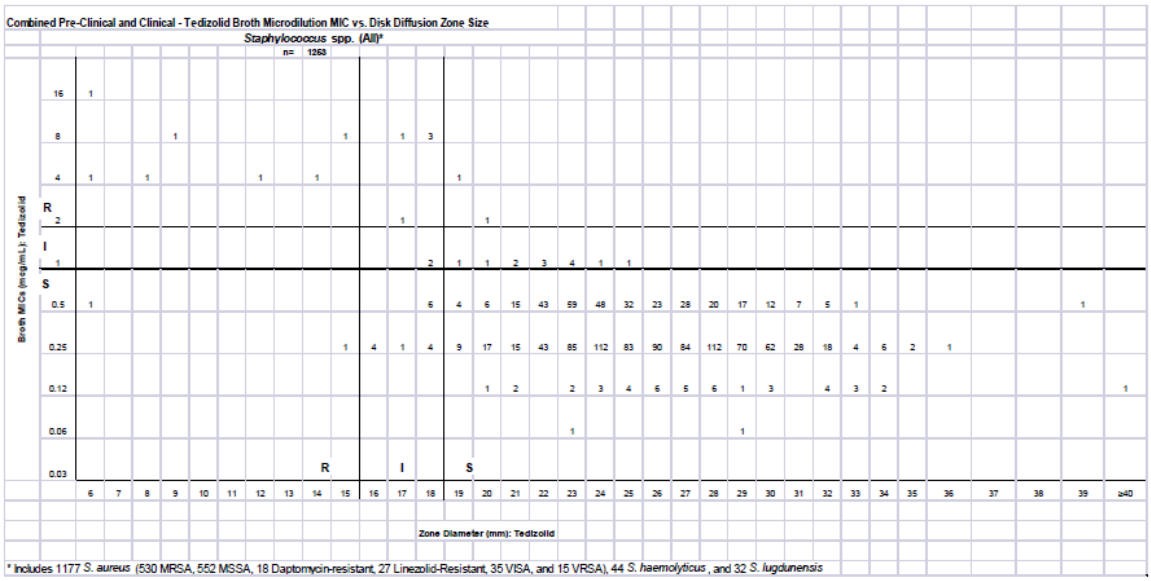
MIC susceptibility and resistance interpretive criteria are established by using three principles. The first is the MIC distribution patterns from large surveillance studies; second, is the observation of clinical response data with respect to the prescribed drug dose; third, is the PK/PD characteristics of the drug. The in vitro antibacterial effect of Tedizolid has been considered to be time-dependent. Based on information submitted by the Applicant, an MIC of up to 0.5 µg/mL is supported by PK/PD data.

The error-rate bounded method classification is used to show a correlation between the MIC and zone diameter of bacteria encountered in the clinical trial. The zone diameters used to classify bacteria as susceptible or resistant to antibiotics depended on clinically relevant MIC breakpoints from the bacteria encountered in the clinical trials, as well as reproducible methods with adequate quality controls. Scatter plots with error rates comparing MIC and disk diffusion methods for isolates encountered in the Phase 3 studies (ME population) and from surveillance studies are presented in the following Figures 50-64. Please note that TR-700 MIC and zone diameter results for baseline isolates of the MITT population from both treatment arms of clinical studies TR701-112 and TR701-113 and from TR701-104 were included in the assessment of MIC and zone diameter correlation. Error rates for each scatter plots were calculated using the error rate bounded method recommended by the CLSI M23 document. For staphylococci, the error rates were calculated on the basis of the application of an Intermediate range. For streptococci, no Intermediate range was required since isolates with elevated TR-700 MIC values were not observed. For enterococci, only vancomycin-susceptible isolates were considered due to the absence of resistant strains in the clinical program, and therefore no Intermediate category was employed.

Breakpoint analysis for *Staphylococcus* species:

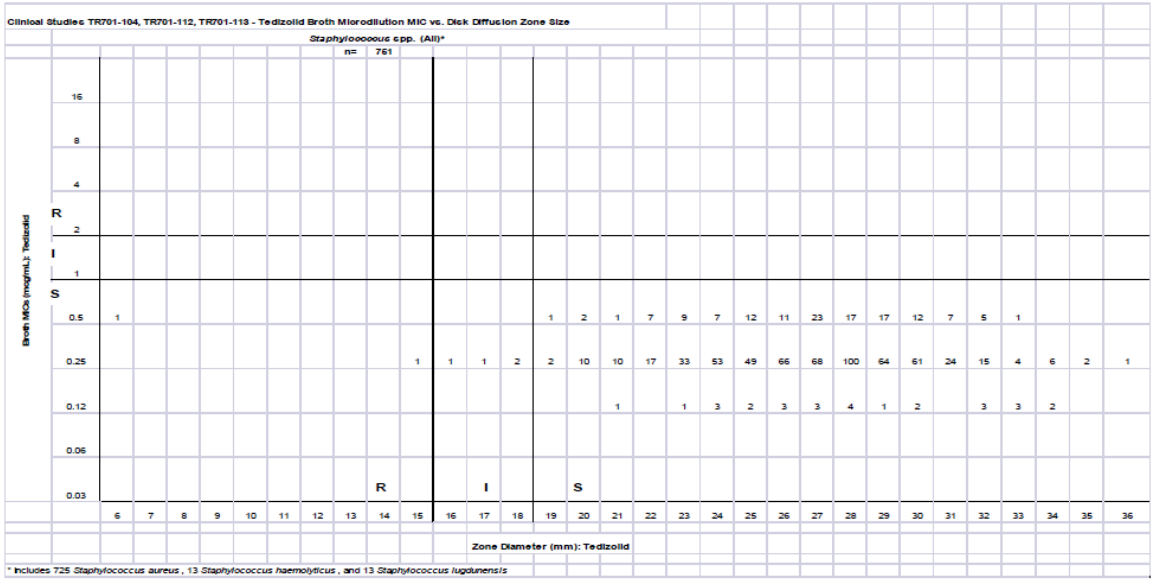
A scatter plot combining clinical and preclinical studies for 1253 *Staphylococcus* spp. (All), which includes 1177 *S. aureus* (530 MRSA, 552 MSSA, 18 daptomycin-resistant, 27 LRSA, 35 VISA, and 15 VRSA), 44 *S. haemolyticus*, and 32 *S. lugdunensis* isolates, is shown in Figure 50. Adjustment of the zone diameter breakpoints to minimize categorical errors resulted in the following breakpoints for *Staphylococcus* spp.: Susceptible (≤0.5 mcg/mL, ≥19 mm), Intermediate (1 mcg/mL, 16-18 mm), and Resistant (≥2 mcg/mL, ≤15 mm). These breakpoints resulted in the following total error rates: very major, 0.2%; major, 0.2%, minor, 2.6%.

Figure 50: TR-700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for *Staphylococcus spp.* (All) from Combined Clinical (MITT Population, Both Treatment Arms) and Preclinical Studies (n=1253)



MIC Range	Total N	Very Major	%Very Major	Major	%Major	Minor	%Minor
>I+2	12	1	8.3%	N/A	N/A	4	33.3%
I+1 to I-1	345	1	0.3%	1	0.3%	20	5.8%
<I-2	896	N/A	N/A	1	0.1%	9	1.0%
Total	1253	2	0.2%	2	0.2%	33	2.6%

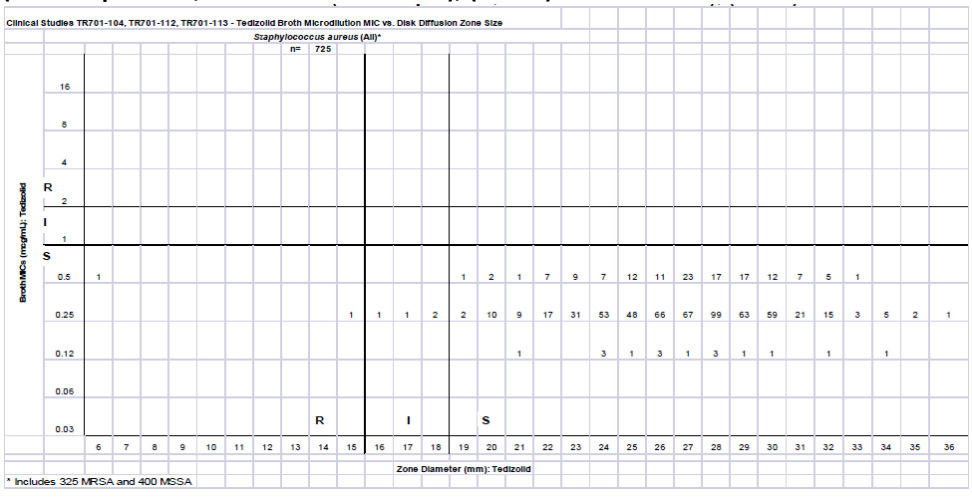
Figure 51 : subset of clinical isolate MIC and zone diameter values for 751 *Staphylococcus* species obtained from both treatment arms. The data show very low error rates that were observed in all MIC range categories.



MIC Range	Total N	Very Major	%Very Major	Major	%Major	Minor	%Minor
>I+2	0	0	0.0%	N/A	N/A	0	0.0%
I+1 to I-1	133	0	0.0%	1	0.8%	0	0.0%
<I-2	618	N/A	N/A	1	0.2%	4	0.6%
Total	751	0	0.0%	2	0.3%	4	0.5%

Figure 52 shows the subset of all 725 clinical isolates MIC and zone diameter values for S. aureus (including 325 MRSA and 400 MSSA) from both treatment arms. Once again, Very low total error rates were observed: very major, 0.0%; major, 0.3%; minor, 0.6%. In addition, acceptable error rates were observed in all MIC range categories.

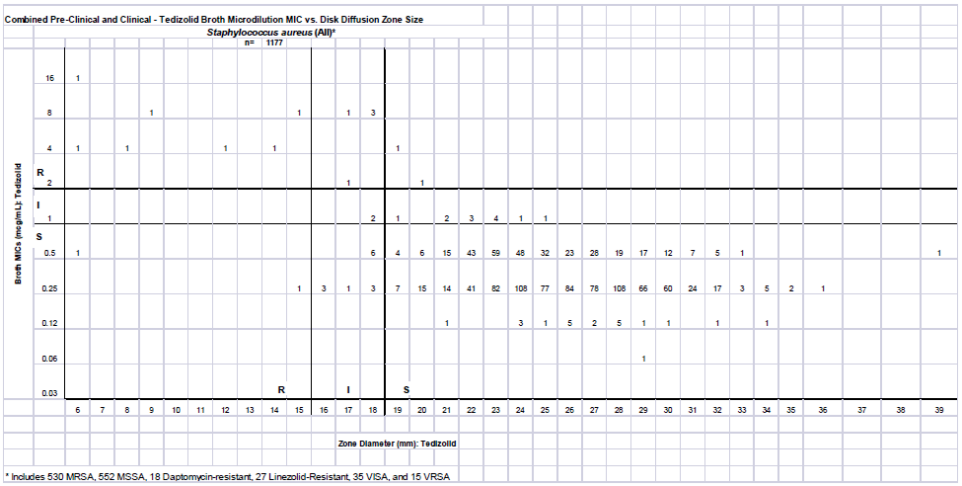
Figure 52: TR-700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for *Staphylococcus aureus* from Clinical Studies (MITT Population, Both Treatment Arms), (n=725)



MIC Range	Total N	Very Major	%Very Major	Major	%Major	Minor	%Minor
>I+2	0	0	0.0%	N/A	N/A	0	0.0%
I+1 to I-1	133	0	0.0%	1	0.8%	0	0.0%
<I-2	592	N/A	N/A	1	0.2%	4	0.7%
Total	725	0	0.0%	2	0.3%	4	0.6%

Figure 53 shows a scatter plot combining clinical and preclinical studies for 1177 *S. aureus* isolates includes 530 MRSA, 552 MSSA, 18 daptomycin-resistant, 27 linezolid-resistant, 35 VISA, and 15 VRSA. The proposed breakpoints resulted in the following total error rates, which were the same as those observed for *Staphylococcus* spp. (All): very major, 0.2%; major, 0.2%; minor, 2.5%.

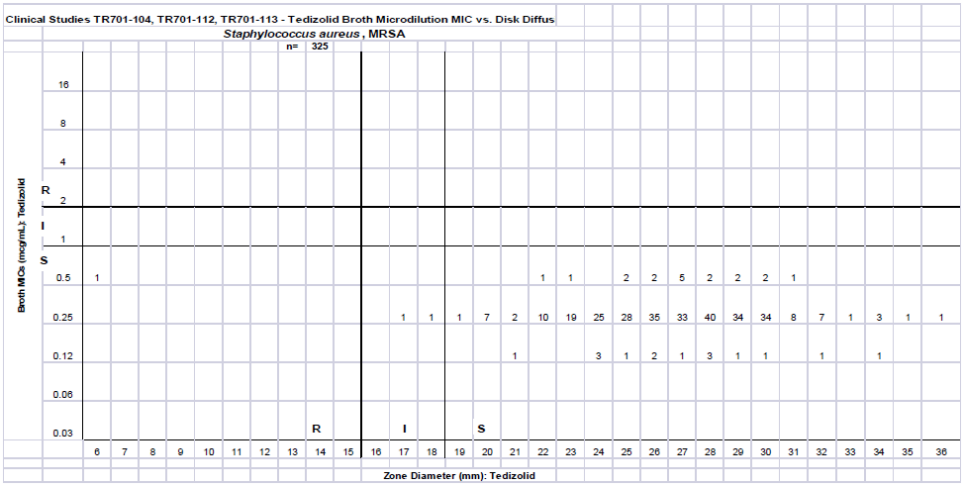
Figure 53: TR-700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for *Staphylococcus aureus* from Combined Clinical (MITT Population, Both Treatment Arms) and Preclinical Studies (n=1177)



MIC Range	Total N	Very Major	%Very Major	Major	%Major	Minor	%Minor
>I+2	12	1	8.3%	N/A	N/A	4	33.3%
I+1 to I-1	343	1	0.3%	1	0.3%	19	5.5%
<I-2	822	N/A	N/A	1	0.1%	7	0.9%
Total	1177	2	0.2%	2	0.2%	30	2.5%

Figure 54 shows the subset of clinical isolate MIC and zone diameter values for 325 MRSA isolates from both treatment arms. Very low total error rates were observed: very major, 0.0%; major, 0.3%; minor, 0.6%.

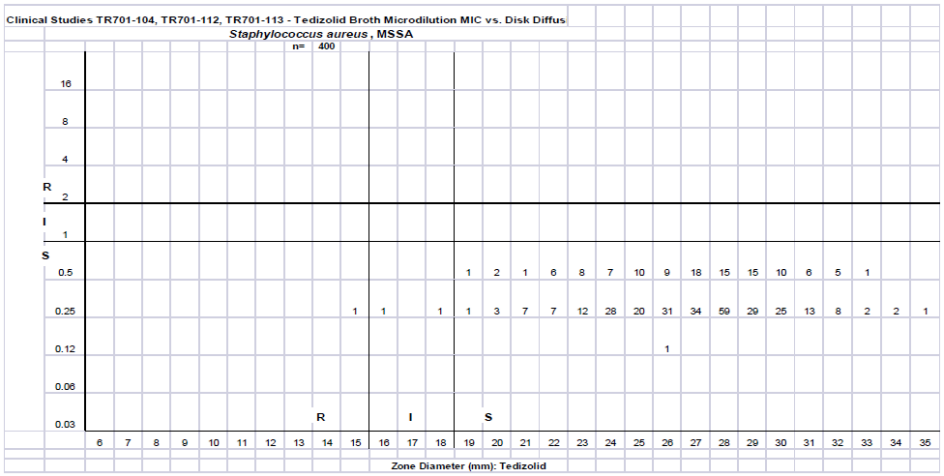
Figure 54: TR- 700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for MRSA from Clinical Studies (MITT Population, Both Treatment Arms) (n= 325)



MIC Range	Total N	Very Major	%Very Major	Major	%Major	Minor	%Minor
>I+2	0	0	0.0%	N/A	N/A	0	0.0%
I+1 to I-1	19	0	0.0%	1	5.3%	0	0.0%
<I-2	306	N/A	N/A	0	0.0%	2	0.7%
Total	325	0	0.0%	1	0.3%	2	0.6%

Figure 55 shows the subset of clinical isolate MIC and zone diameter values for 400 MSSA isolates from both treatment arms. Very low total error rates were observed: very major, 0.0%; major, 0.3%; minor, 0.5%. In addition, acceptable error rates were observed in all MIC range categories.

Figure 55: TR- 700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for MSSA from Clinical Studies (MITT Population, Both Treatment Arms) (n= 400)



MIC Range	Total N	Very Major	%Very Major	Major	%Major	Minor	%Minor
>I+2	0	0	0.0%	N/A	N/A	0	0.0%
I+1 to I-1	114	0	0.0%	0	0.0%	0	0.0%
<I-2	286	N/A	N/A	1	0.3%	2	0.7%

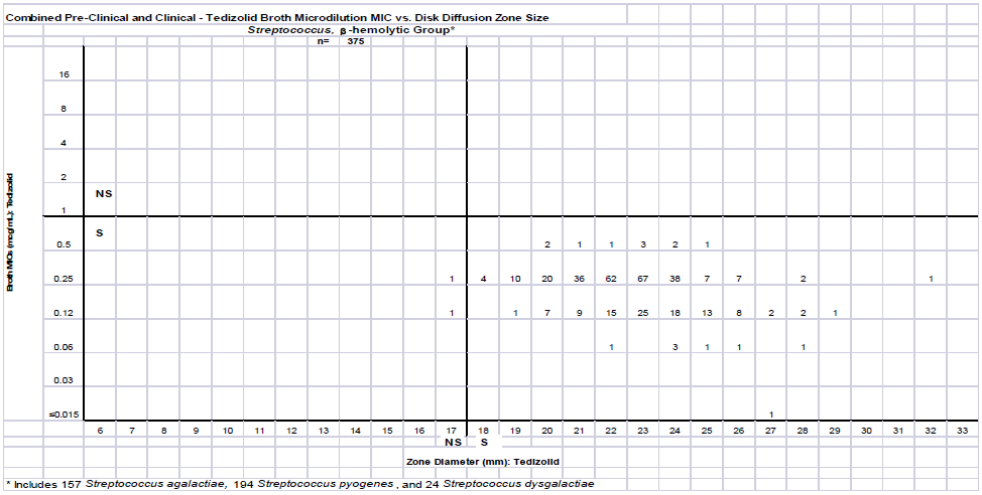
Total	400	0	0.0%	1	0.3%	2	0.5%

Please note that limited information was presented on *Staphylococcus haemolyticus* and *Staphylococcus lugdunensis.* Additionally, limited in vitro susceptibility information was presented for the above mentioned isolates.

Streptococcus spp. β-hemolytic Group

A scatter plot combining clinical and preclinical studies for 375 *Streptococcus* spp. *β*- hemolytic Group, which includes 157 *S. agalactiae,* 194 *S. pyogenes*, and 24 *S. dysgalactiae,* is shown in Figure 56. There were no clinical isolates of β- hemolytic *Streptococcus* spp. with elevated TR-700 MIC values. Since the Monte Carlo simulations demonstrate an acceptable target attainment percentage at an MIC value as high as 0.5 mcg/mL, the Applicant is proposing a susceptible breakpoint of ≤0.5 mcg/ml for this organism, in spite of the absence of clinical trial isolates with MIC values of 0.5 mcg/mL. At the proposed MIC susceptible breakpoint of ≤0.5 mcg/ml and a disk breakpoint of ≥18 mm the following total error rates were observed: very major, 0.0%; major, 0.5%. In addition, acceptable error rates were observed in all MIC range categories.

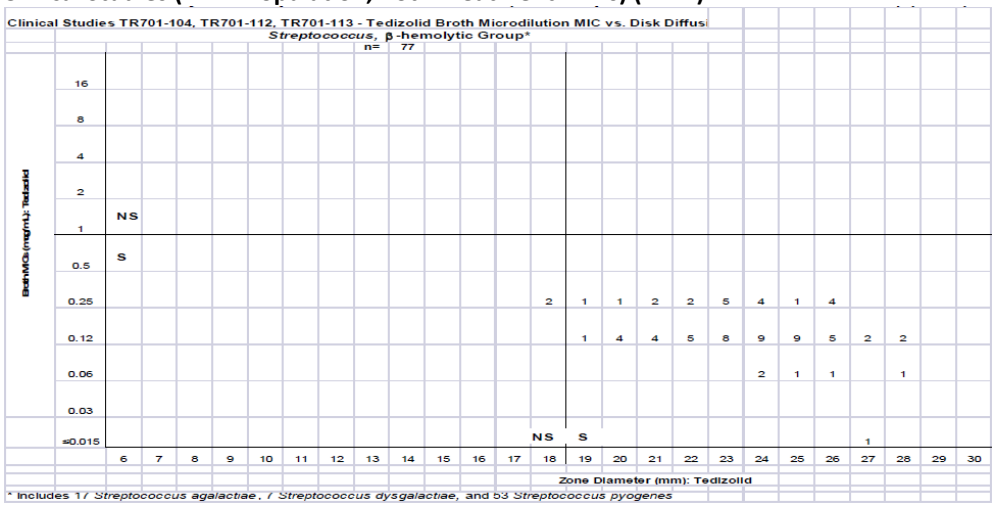
Figure 56: TR-700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for *Streptococcus* spp. *β*- hemolytic Group from Combined Clinical (MITT Population, Both Treatment Arms) and Preclinical Studies (n=375)



MIC Range	Total N	Very Major	%Very Major	Major	%Major
≥R+1	0	0	0.0%	N/A	N/A
R+S	10	0	0.0%	0	0.0%
≤S-1	365	N/A	N/A	2	0.5%
Total	375	0	0.0%	2	0.5%

Figure 57 shows the subset of clinical isolate MIC and zone diameter values for 77 isolates of *Streptococcus* spp. *β*-hemolytic Group which includes 17 *S. agalactiae*, 7 *S. dysgalactiae,* and 53 *S. pyogenes.* Very low total error rates were observed: very major, 0.0%; major, 2.6%. In addition, acceptable error rates were observed in all MIC range categories.

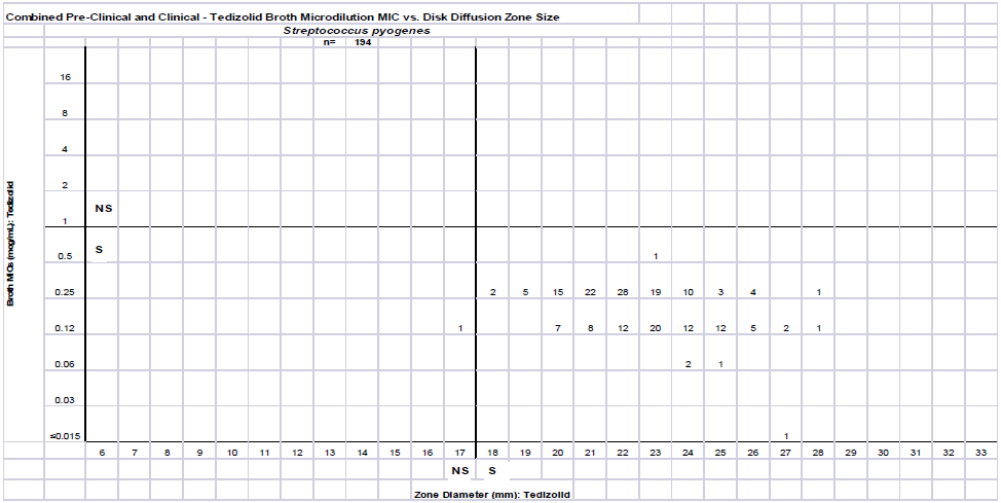
Figure 57: TR-700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for *Streptococcus* spp. *β*- hemolytic Group from Clinical Studies (MITT Population, Both Treatment Arms) (n=77)



MIC Range	Total N	Very Major	%Very Major	Major	%Major
≥R+1	0	0	0.0%	N/A	N/A
R+S	0	0	0.0%	0	0.0%
≤S-1	77	N/A	N/A	2	2.6%
Total	77	0	0.0%	2	2.6%

Figure 58 shows a scatter plot combining clinical and preclinical studies for *S. pyogenes* only, which includes data for 194 isolates. At the proposed MIC susceptible breakpoint of ≤0.5 mcg/ml and zone diameter breakpoint of ≥18 mm the following total error rates were observed: very major, 0.0%; major, 0.5%.

Figure 58: TR-700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for *Streptococcus pyogenes* from Combined Clinical (MITT Population, Both Treatment Arms) and Preclinical Studies (n=194)

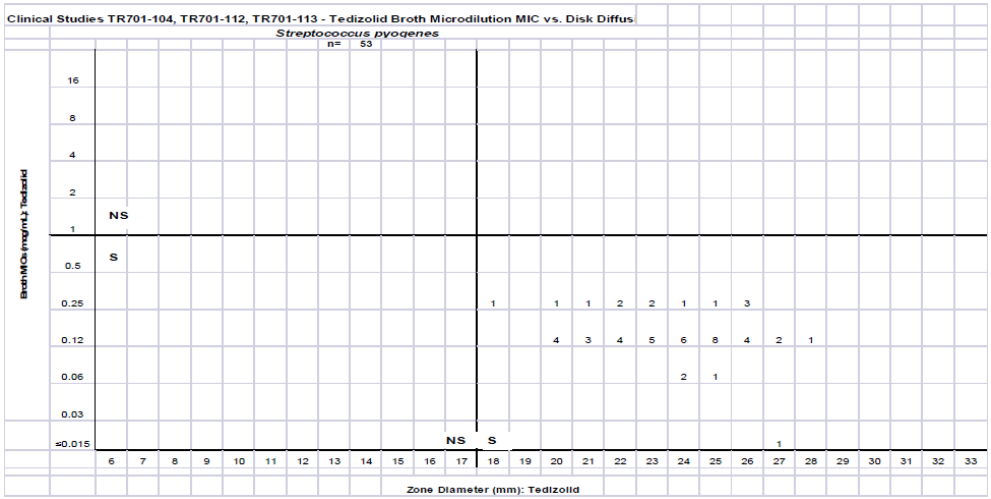


MIC Range	Total N	Very Major	%Very Major	Major	%Major
≥R+1	0	0	0.0%	N/A	N/A
R+S	1	0	0.0%	0	0.0%
≤S-1	193	N/A	N/A	1	0.5%

Total	194	0	0.0%	1	0.5%

Figure 59 shows the subset of clinical isolate MIC and zone diameter values for 53 isolates of *S. pyogenes*. Very low total error rates were observed: very major, 0.0%; major, 0.0%.

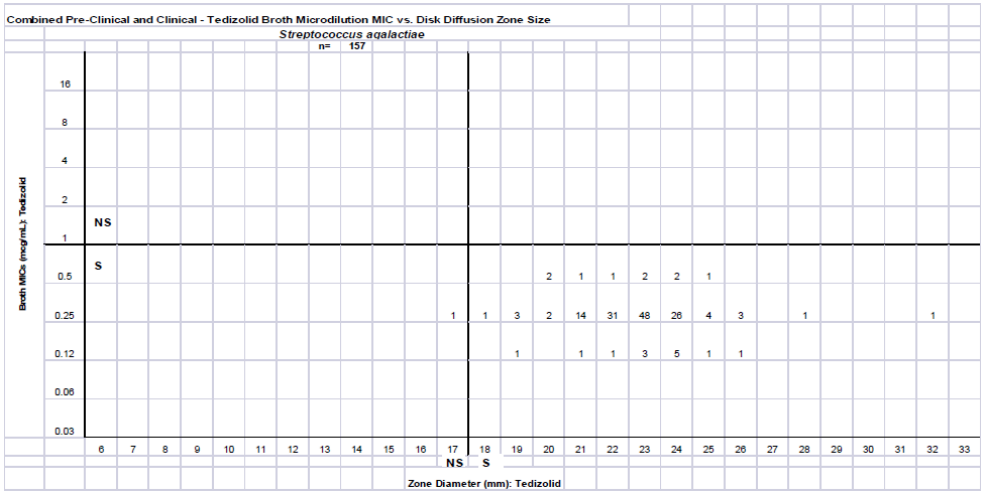
Figure 59: TR-700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for *Streptococcus pyogenes* from Clinical Studies (MITT Population, Both Treatment Arms) (n=53)



MIC Range	Total N	Very Major	%Very Major	Major	%Major
≥R+1	0	0	0.0%	N/A	N/A
R+S	0	0	0.0%	0	0.0%
≤S-1	53	N/A	N/A	0	0.0%
Total	53	0	0.0%	0	0.0%

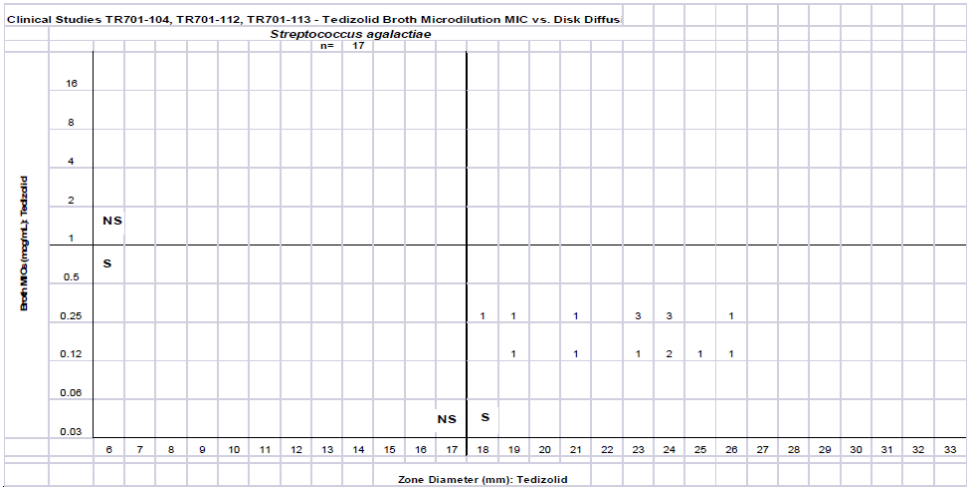
Figure 60 shows a scatter plot that combines the clinical and preclinical studies for 157 isolates of *S. agalactiae*. While Figure 61 shows the subset of clinical isolate MIC and zone diameter values for 17 isolates of *S. agalactiae*.

Figure 60: TR-700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for *Streptococcus agalactiae* from Combined Clinical (MITT Population, Both Treatment Arms) and Preclinical Studies (n=157)



MIC Range	Total N	Very Major	%Very Major	Major	%Major
≥R+1	0	0	0.0%	N/A	N/A
R+S	9	0	0.0%	0	0.0%
≤S-1	148	N/A	N/A	1	0.7%
Total	157	0	0.0%	1	0.6%

Figure 61: TR-700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for *Streptococcus agalactiae* from Clinical Studies (MITT Population, Both Treatment Arms) (n=17)

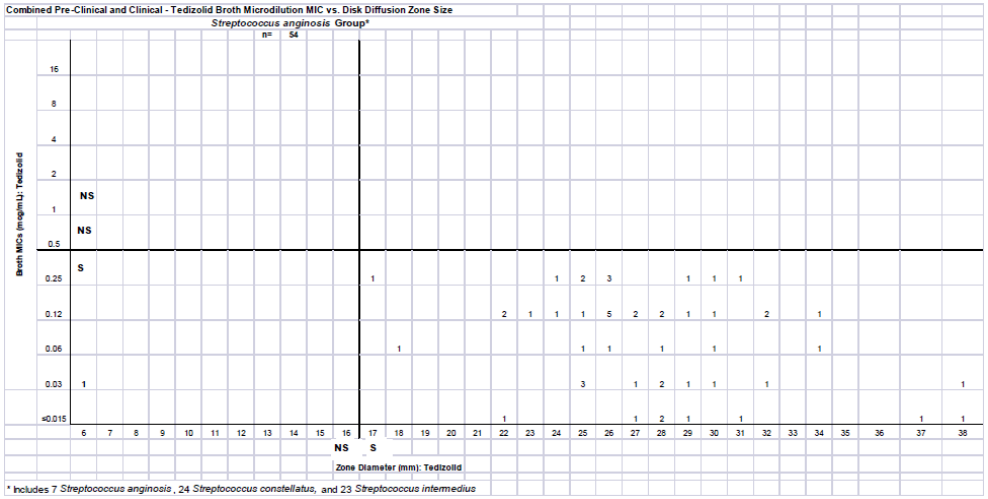


MIC Range	Total N	Very Major	%Very Major	Major	%Major
≥R+1	0	0	0.0%	N/A	N/A
R+S	0	0	0.0%	0	0.0%
≤S-1	17	N/A	N/A	0	0.0%
Total	17	0	0.0%	0	0.0%

Streptococcus anginosus Group

Figure 62 shows a scatter plot for the *S. anginosus* Group clinical isolates which includes 7 *S. anginosus*, 24 *S. constellatus,* and 23 *S. intermedius*. The two preclinical studies did not contribute data for the analysis of this organism group. There was an absence of clinical isolates of *S. anginosus* Group with elevated TR-700 MIC values; therefore, only a susceptible breakpoint can be defined. The Applicant proposes a susceptible MIC breakpoint of ≤0.25 mcg/ml and a zone diameter breakpoint of ≥17 mm for this organism group and at the proposed breakpoints, the following total error rates were observed: very major, 0.0%; major, 1.9%.

Figure 62: TR-700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for *Streptococcus anginosus* Group from Combined Clinical (MITT Population, Both Treatment Arms) and Preclinical Studies (n=54)

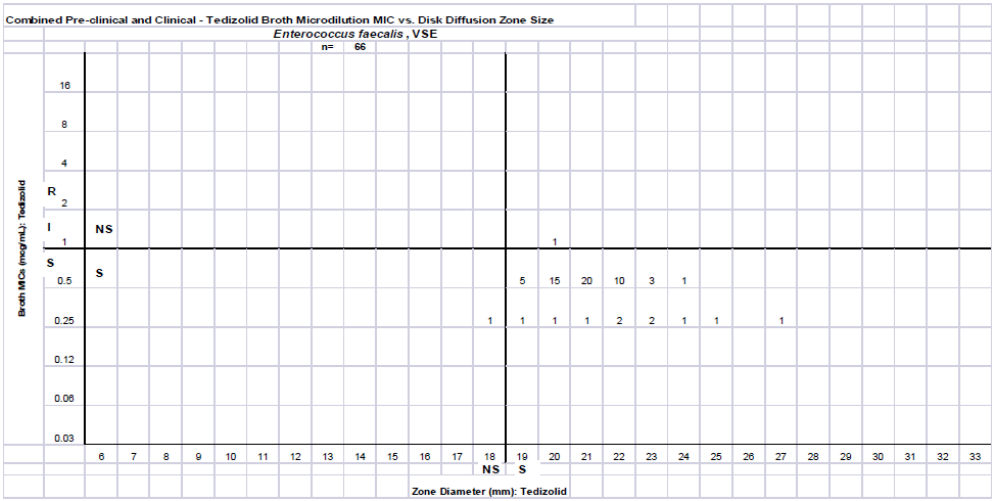


MIC Range	Total N	Very Major	%Very Major	Major	%Major
≥R+1	0	0	0.0%	N/A	N/A
R+S	10	0	0.0%	0	0.0%
≤S-1	44	N/A	N/A	1	2.3%
Total	54	0	0.0%	1	1.9%

Enterococcus faecalis (vancomycin-susceptible isolates only)

Figure 63 shows a scatter plot combining clinical and preclinical studies for 66 *E. faecalis* isolates (vancomycin-susceptible only). There were an absence of clinical isolates with elevated TR-700 MIC values; therefore, only a susceptible breakpoint can be defined. At the proposed MIC susceptible breakpoint of ≤0.5 mcg/ml and zone diameter breakpoint of ≥19 mm, the following total error rates were observed: very major, 1.5%; major, 1.5%. In the ≤ S-1 category there was a relatively high major error rate of 9.1%, but this was based upon 1 error in a total 11 isolates.

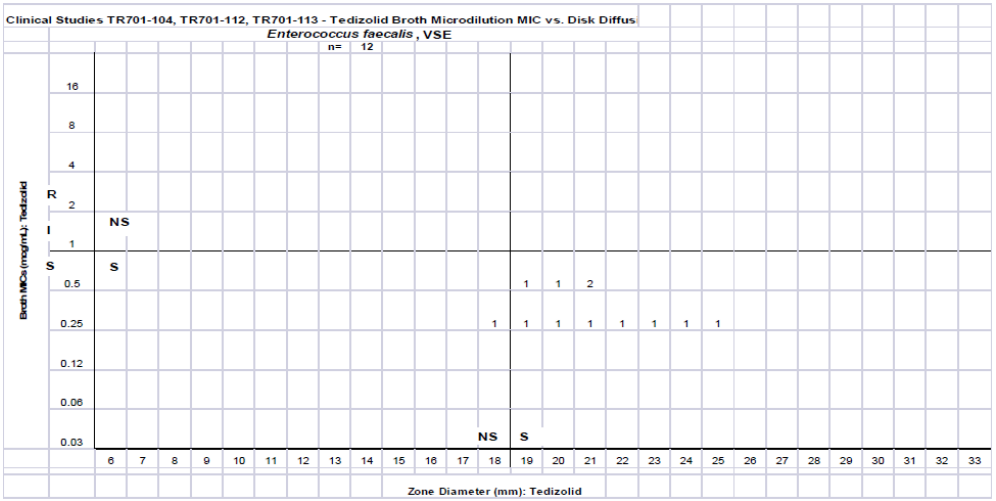
Figure 63: TR-700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for *Enterococcus faecalis* (vancomycin-susceptible isolates) from Combined Clinical (MITT Population, Both Treatment Arms) and Preclinical Studies (n=66)



MIC Range	Total N	Very Major	%Very Major	Major	%Major
≥R+1	0	0	0.0%	N/A	N/A
R+S	55	1	1.8%	0	0.0%
≤S-1	11	N/A	N/A	1	9.1%
Total	66	1	1.5%	1	1.5%

Figure 64 shows the subset of clinical isolate MIC and zone diameter values for 12 isolates of *E. faecalis*. There were no very major errors but in the ≤S-1 category; however, there was a major error rate of 12.5%. Please note that this value is based upon a very small numbers of isolates where a single error may result in higher percentages. Adjusting the zone diameter breakpoint line to ≥18 mm may lower the error; however, this may be problematic since in the clinical outcome data there were no positive outcomes demonstrated at 18 mm in the ME population.

Figure 64: TR-700 Broth Microdilution MIC versus Disk Diffusion Zone Diameter for *Enterococcus faecalis* (vancomycin-susceptible isolates) from Clinical Studies (MITT Population, Both Treatment Arms) (n=12)



MIC Range	Total N	Very Major	%Very Major	Major	%Major
≥R+1	0	0	0.0%	N/A	N/A
R+S	4	0	0.0%	0	0.0%
≤S-1	8	N/A	N/A	1	12.5%
Total	12	0	0.0%	1	8.3%

The Applicant's Proposed TR-700 Interpretive Criteria

Table 114 shows a summary of the Applicant's proposed interpretive criteria for the targeted isolates.

Table 114: Proposed TR-700 MIC and Zone Diameter Interpretive Breakpoints

Organism	MIC (µg/mL)	Zone Diameter[a] (mm)
Staphylococcus aureus (including methicillin-resistant and methicillin susceptible strains) *Staphylococcus haemolyticus* *Staphylococcus lugdunensis*	≤0.5 Susceptible 1 Intermediate ≥2 Resistant	≥19 Susceptible 16-18 Intermediate ≤15 Resistant
Streptococcus pyogenes *Streptococcus agalactiae*	≤0.5 Susceptible (b) (4)	≥18 Susceptible
Streptococcus anginosus Group (including *S. anginosus, S. intermedius and S. constellatus*)	≤0.25 Susceptible (b) (4)	≥17 Susceptible
Enterococcus faecalis (vancomycin-susceptible isolates only)	≤0.5 Susceptible (b) (4)	≥19 Susceptible

[a] **20 mcg TR-700 disk.**

NDA: 205435/205436 DATE REVIEW COMPLETED: 02/24/2014
Tedizolid Phosphate (Sivextro)

Sponsor's version of the microbiology section of the label:

12.4 Microbiology

Mechanism of Action

The antibacterial activity of tedizolid is mediated by binding to the 50S subunit of the bacterial ribosome resulting in inhibition of protein synthesis. (b) (4)

(b) (4)

Mechanism of Resistance

(b) (4)

Frequency of Resistance

Spontaneous mutations conferring reduced susceptibility to tedizolid occur *in vitro* at a frequency rate of approximately 10^{-10}. (b) (4)

Interaction with Other Antimicrobials

In vitro drug combination studies with tedizolid and aztreonam, ceftriaxone, ceftazidime, imipenem, rifampin, trimethoprim/sulfamethoxazole, minocycline, clindamycin, ciprofloxacin, daptomycin, vancomycin, gentamicin, amphotericin B, ketoconazole, and terbinafine demonstrate neither synergy nor antagonism.

Spectrum of Activity

Tedizolid has been shown to be active against most isolates of the following bacteria, both *in vitro* and in clinical infections, as described in (b) (4) *Indications and Usage (1)* (b) (4)

Aerobic and Facultative Gram-positive Microorganisms

- *Staphylococcus aureus* (including methicillin-resistant [MRSA] and methicillin-susceptible [MSSA] isolates)

(b) (4)

- *Streptococcus pyogenes*
- *Streptococcus agalactiae*
- *Streptococcus anginosus* Group (including *S. anginosus*, *S. intermedius*, and *S. constellatus*)
- *Enterococcus faecalis* (b) (4)

The following *in vitro* data are available, but their clinical significance has not been established. At least 90% of the following microorganisms exhibit an *in vitro* minimum inhibitory concentration (MIC) less than or equal to (b) (4) for tedizolid. However, the safety and effectiveness of TRADENAME in treating clinical infections due to these microorganisms have not been established in adequate and well-controlled clinical trials.

Aerobic and Facultative Anaerobic Gram-positive Microorganisms

- *Staphylococcus epidermidis* (including methicillin-susceptible and methicillin-resistant strains)

(b) (4)

- *Enterococcus faecium* (b) (4)

Susceptibility Test Methods

When available, the clinical microbiology laboratory should provide cumulative results of the *in vitro* susceptibility test results for antibacterial drugs used in local hospitals and practice areas to the physician as periodic reports that describe the susceptibility profile of nosocomial and community-acquired pathogens. These reports should aid the physician in selecting the most effective antibacterial drug product for treatment.

Dilution Techniques

Quantitative methods are used to determine antibacterial minimum inhibitory concentrations (MICs). These MIC values provide estimates of the susceptibility of bacteria to antibacterial compounds. The MIC values should be determined using a standardized procedure based on dilution methods (broth, agar, or microdilution)[1,3] or equivalent using standardized inoculum and concentrations of tedizolid. The MIC values should be interpreted according to the criteria provided in Table 3.

Table 3 Susceptibility Interpretive Criteria for TRADENAME

Pathogen	Minimum Inhibitory Concentrations (µg/mL)			Disk Diffusion Zone Diameter (mm)		
	S	I	R	S	I	R
Staphylococcus aureus (methicillin-resistant and methicillin-susceptible isolates)	≤0.5	1	≥2	≥19	16-18	≤15
(b) (4)						
Streptococcus pyogenes	≤0.5	-	-	≥18	-	-
Streptococcus agalactiae	≤0.5	-	-	≥18	-	-
Streptococcus anginosus Group*	≤0.25	-	-	≥17	-	-
Enterococcus faecalis	≤0.5	-	-	≥19	-	-

S=susceptible, I=intermediate, R=resistant
*Includes *S. anginosus*, *S. intermedius*, *S. constellatus*

Diffusion techniques

Quantitative methods that require measurement of zone diameters also provide reproducible estimates of the susceptibility of bacteria to antibacterial compounds. The standardized procedure[2,3] requires the use of standardized inoculum concentrations. This procedure uses paper disks impregnated with 20 mcg tedizolid to test the susceptibility of microorganisms to tedizolid. Reports from the laboratory providing results of the standard single-disk susceptibility test with a 20 mcg tedizolid disk should be interpreted according to the criteria in Table 3.

A report of "Susceptible" indicates that the (b) (4) is likely to inhibit growth of the pathogen if the antibacterial (b) (4) reaches the concentration (b) (4) A report of "Intermediate" indicates that the result should be considered equivocal, and if the microorganism is not fully susceptible to alternative (b) (4) drugs, the test should be repeated. This category implies possible clinical (b) (4) in body sites where the drug is physiologically concentrated. This category also provides a buffer zone that prevents small uncontrolled technical factors from causing major discrepancies in interpretation. A report of "Resistant" indicates that the (b) (4) is not likely to inhibit growth of the pathogen if the (b) (4) reaches the concentrations usually achievable at the infection site; other therapy should be selected.

Quality Control

Standardized susceptibility test procedures require the use of laboratory control microorganisms to monitor and ensure the accuracy and precision of supplies and reagents used in the assay, and the techniques of the individuals performing the test.[1, 2, 3] Standardized tedizolid powder should provide the following range of MIC

values noted in Table 4. For the diffusion technique using the 20 mcg tedizolid disk, results within the ranges specified in Table 4 should be observed.

Table 4 Acceptable Quality Control Ranges for Susceptibility Testing

Quality Control Organism	Minimum Inhibitory Concentrations (µg/mL)	Disk Diffusion (zone diameter in mm)
Staphylococcus aureus ATCC 29213	0.25 - 1	Not Applicable
Staphylococcus aureus ATCC 25923	Not Applicable	22 - 29
Enterococcus faecalis ATCC 29212	0.25 - 1	Not Applicable
Streptococcus pneumoniae ATCC 49619	0.12 - 0.5	24 - 30

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DIVISION OF ANTI-INFECTIVE PRODUCTS CLINICAL MICROBIOLOGY NDA REVIEW

NDA: 205435/205436 DATE REVIEW COMPLETED: 02/24/2014
Tedizolid Phosphate (Sivextro)

Concurrence:
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Clinical Microbiology Team Leader, DAIP
4 May 2014

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