CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205436Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY REVIEW

NDA:	205,435 (oral) & 205,436 (intravenous [IV])
Drug	Tedizolid Phosphate
Trade Name	SIVEXTRO
OCP Reviewer	Zhixia (Grace) Yan, Ph.D.
OCP Team Leader	Kimberly L. Bergman, Pharm.D.
OCP Division	DCP4
OND Division	DAIP
Sponsor	Trius Therapeutics, Inc., San Diego, CA
Relevant IND(s)	77,872 (oral) & 106,307 (IV)
Submission Type; Code	Original New Drug Application (New Molecular Entity), Priority
Formulation; Strength(s)	Tedizolid phosphate 200 mg tablet: a yellow film-coated oval tablet; each tablet is debossed with "TZD" on one side and "200" on the other side. Tedizolid phosphate 200 mg for injection: a sterile white to off-white lyophilized powder for injection in a single-use vial containing (b) (4) tedizolid phosphate to allow delivery of 200 mg after reconstitution with 4 ml of Sterile Water for Injection.
Indication	For the treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by susceptible isolates of Gram-positive microorganisms.
Dosage and Administration	200 mg administered once-daily orally or as an intravenous (IV) infusion over 1 hour for six (6) days

BACKGROUND

Trius Therapeutics, Inc. submitted a New Drug Application (NDA) for tedizolid phosphate 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 pyogenes*, *Streptococcus agalactiae*, *Streptococcus anginosus* Group (including *Streptococcus anginosus*, *Streptococcus intermedius* and *Streptococcus constellatus*), and *Enterococcus faecalis*. The proposed clinical dose 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)

The following detailed labeling recommendations are provided as an addendum to the Clinical Pharmacology Review (DARRTS dated 03/24/2014).

DETAILED LABELING RECOMMENDATIONS

Sponsor's draft label version: 10/18/2013

The following proposed package insert has been marked by revisions made by the Reviewer, indicated with ~~red strikethrough font~~ for deleted text and underlined blue font for inserted text. Affected sections include **Highlights**, **Dosage and administration (2)**, **Warnings and Precautions (5)**, **Use in Specific Populations (8)**, **Overdosage (10)**, and **Clinical Pharmacology (12)**.

22 Page(s) of Draft Labeling has been Withheld in Full as B4 (CCI/TS) immediately following this page

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/s/

ZHIXIA YAN
06/18/2014

KIMBERLY L BERGMAN
06/18/2014

CLINICAL PHARMACOLOGY REVIEW

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OCP Team Leader	Kimberly L. Bergman, Pharm.D.
PM Reviewer	Fang Li, Ph.D.
PM Team Leader	Jeffry A. Florian, Ph.D.
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Indication	For the treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by susceptible isolates of Gram-positive microorganisms.
Dosage and Administration	200 mg administered once daily for six (6) days either orally (with or without food) or as a 1-hour IV infusion in patients (b) (4)

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Abbreviations

ABSSSI, acute bacterial skin and skin structure infections
Ae, cumulative amount excreted in urine
AUC, area under plasma concentration-time curve
AUC_{0-24}, area under plasma concentration-time curve over 24 hours
$AUC_{0-\infty}$, area under plasma concentration-time curve from time 0 to infinity
C_{max}, maximum observed plasma concentration
C_{min}, minimum observed plasma concentration
CI, confidence interval
CL, plasma clearance
CLcr, creatinine clearance
CL_R, renal clearance
cSSSI, complicated skin and skin structure infections
CV, coefficient of variation
CYP, cytochrome P450
ESRD, end-stage renal disease
f, free unbound (i.e., microbiologically active) drug
HD, hemodialysis
HPLC, high performance liquid chromatography
IRT, Interdisciplinary Review Team for QT Studies
IV, intravenous
LC-MS/MS, liquid chromatography with tandem mass spectrometry
LLOQ, lower limit of quantification
MAO, monoamine oxidase
MIC, minimum inhibitory concentration
MIC_{50}, minimum inhibitory concentration for 50% of bacterial population
MIC_{90}, minimum inhibitory concentration for 90% of bacterial population
MRSA, methicillin-resistant *Staphylococcus aureus*
MSSA, methicillin-susceptible *Staphylococcus aureus*
NDA, new drug application
P-gp, P-glycoprotein
PAE, post-antibiotic effect
PD, pharmacodynamics
PK, pharmacokinetics
PK-PD, pharmacokinetics-pharmacodynamics
PTE, Post-Therapy Evaluation
Q12h, every 12 hours
Q24h, every 24 hours
QC, quality control
SAE, serious adverse event
SD, standard deviation
$t_{1/2}$, elimination half-life
T_{max}, time of maximum observed plasma concentration
TEAE, treatment-emergent adverse event
V_{ss}, apparent steady-state volume of distribution

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1. EXECUTIVE SUMMARY

Trius Therapeutics, Inc. submitted a New Drug Application (NDA) for tedizolid phosphate 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 pyogenes*, *Streptococcus agalactiae*, *Streptococcus anginosus* Group (including *Streptococcus anginosus*, *Streptococcus intermedius* and *Streptococcus constellatus*), and *Enterococcus faecalis*. The proposed clinical dose 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)

Components of the tedizolid phosphate NDA are summarized as follows:

- Fourteen *in vitro* studies with human biomaterials were submitted, evaluating plasma protein binding, biotransformation of prodrug in plasma, metabolism in hepatic microsomes, and inhibition/induction of cytochrome P450 (CYP) enzymes and membrane transporters.
- Sixteen Phase 1 studies evaluating pharmacokinetics of tedizolid phosphate and its active moiety, tedizolid, were submitted. Studies included single- and multiple-dose pharmacokinetics (oral and IV); metabolism and elimination via mass balance and metabolite profiling; effect of food, hepatic impairment (moderate and severe), renal impairment (severe and end-stage renal disease [ESRD] on intermittent hemodialysis [HD]), age (elderly and adolescent); effect on the pressor response of tyramine and pseudoephedrine; and impact on QT prolongation.
- Two supportive Phase 2 trials evaluating safety/efficacy of tedizolid phosphate were submitted. TR701-104 was a multicenter, randomized, double-blind, non-controlled study comparing the safety, tolerance, and efficacy of tedizolid phosphate at 200, 300, 400 mg (oral) once daily for 5-7 days in patients with complicated skin and skin structure infections (cSSSI); TR701-126 was an open-label, multicenter study of tedizolid phosphate 200 mg (oral) once daily for 6 days in patients with major cutaneous abscess or cellulitis/erysipelas.
- Two pivotal Phase 3 trials evaluating safety/efficacy of tedizolid phosphate versus active comparator, linezolid, in the treatment of ABSSSI were submitted. TR701-112 was a randomized, double-blind, double-dummy, multicenter Phase 3 study in ABSSSI patients given oral tedizolid phosphate 200 mg once daily for 6 days or oral Zyvox® (linezolid) 600 mg every 12 hours for 10 days; TR701-113 was a randomized, double-blind, double-dummy, multicenter Phase 3 study in ABSSSI patients given IV to oral tedizolid phosphate 200 mg once daily for 6 days or IV to oral Zyvox® (linezolid) 600 mg every 12 hours for 10 days.

1.1 Recommendations

The Office of Clinical Pharmacology, Division of Clinical Pharmacology 4 has reviewed NDAs 205,435 and 205,436, and they are acceptable from a clinical pharmacology perspective.

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1.2 Phase 4 Commitments/Requirements

None.

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

1.3.1 Pharmacokinetics

Tedizolid phosphate (TR-701) is a prodrug that is rapidly and extensively converted by phosphatases to tedizolid (TR-700), the microbiologically-active moiety, following oral and IV administration. Only the pharmacokinetic profile of tedizolid is discussed further. Following multiple once-daily oral or IV administration, steady-state concentrations are achieved within approximately three days with moderate drug accumulation of approximately 30%. Pharmacokinetic (PK) parameters of tedizolid following oral and IV administration of 200 mg once daily tedizolid phosphate are shown in **Table 1.3.2-1**.

Table 1.3.2-1 Mean (SD) tedizolid pharmacokinetic parameters following single and multiple oral/IV administration of 200 mg once daily tedizolid phosphate in healthy adults.

Pharmacokinetic Parameters of Tedizolid	Oral		IV	
	Single Dose	Steady State	Single Dose	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 (0.7)	2.2 (0.6)	2.3 (0.6)	3.0 (0.7)
C_{min} (µg/mL)	not applicable	0.44 (0.19)	not applicable	0.36 (0.09)
T_{max}[b] (h)	2.5 (1.0 – 8.0)	3.5 (1.0 – 6.0)	1.1 (0.9 – 1.5)	1.2 (0.9 - 1.5)
CL or CL/F (L/hr)	6.9 (1.7)	8.4 (2.1)	6.4 (1.2)	5.9 (1.4)

[a]*AUC is $AUC_{0-\infty}$ for single administration and AUC_{0-24} for multiple administration.*

[b]*Median (minimum, maximum) presented for T_{max}.*

Note: Adapted from Module 2.5 Clinical Overview, Table 2.

Absorption

Peak plasma tedizolid concentrations are achieved at approximately 3 hours following oral administration under fasting condition or at the end of the 1 hour IV infusion of tedizolid phosphate. The absolute bioavailability is approximately 91% and no dose adjustment is necessary between IV and oral administration. Tedizolid phosphate (oral) may be administered with or without food as total systemic exposure ($AUC_{0-\infty}$) is unchanged between fasted and fed conditions [*note: AUC/MIC was shown to best correlate with activity in animal infection models*].

Distribution

Protein binding of tedizolid to human plasma proteins is approximately 70 to 90%. The mean steady state volume of distribution of tedizolid in healthy adults following a single IV dose of tedizolid phosphate 200 mg ranged from 67 to 80 L (approximately twice total body water). Tedizolid penetrates into the interstitial space fluid of adipose and skeletal muscle tissue with exposure similar to free drug exposure in plasma.

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Metabolism
Other than tedizolid, which accounts for approximately 95% of the total radiocarbon AUC in plasma, there are no other significant circulating metabolites in humans.

Excretion
Following single oral administration of ^{14}C-labeled tedizolid phosphate, 82% of the radioactive dose was recovered in feces and 18% in urine primarily as a non-circulating and microbiologically inactive sulfate conjugate, with most of the elimination (>85%) occurring within 96 hours. Less than 3% of tedizolid phosphate administered dose is excreted as tedizolid.

1.3.2 Intrinsic Factors

Clinical studies demonstrated that no dose adjustment is necessary in the following specific populations: adolescent or elderly patients, males or females, patients with severe renal impairment, patients on hemodialysis, and patients with moderate to severe hepatic impairment.

The population pharmacokinetic analysis identified ideal body weight and total bilirubin as significant covariates for tedizolid exposure. However, the impact of these two covariates (ideal body weight and total bilirubin) as well as all other evaluated covariates (age, gender, race, ethnicity, renal or liver function) did not result in any clinically relevant changes (>20%) in tedizolid exposure.

1.3.3 Extrinsic Factors

Drug Metabolizing Enzymes
In vitro, neither tedizolid phosphate nor tedizolid was shown to be a substrate/inhibitor/inducer of major cytochrome P450 enzymes (CYP1A2, 2B6, 2D6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4).

Membrane Transporters
In vitro, neither tedizolid nor tedizolid phosphate was shown to be a substrate or inhibiter of major membrane transporters (OAT1, OAT3, OATP1B1, OATP1B3, OCT1, OCT2, and P-gp) except for BCRP. Tedizolid inhibited BCRP with an IC_{50} of 51.1 μM; however, this is unlikely to be of clinical significance as $C_{max,ss}$ of tedizolid ranged from 6-8 μM after oral and IV administration of 200 mg tedizolid phosphate once daily and tedizolid phosphate is not intended for chronic administration (i.e. treatment duration not to exceed 6 days).

Monoamine Oxidase Inhibition
Tedizolid is a weak and reversible inhibitor of monoamine oxidase (MAO) in vitro. Drug interaction studies with 200 mg once daily oral tedizolid phosphate showed no meaningful changes in blood pressure or heart rate with pseudoephedrine, and no clinically relevant increase in tyramine sensitivity. Therefore, no restrictions are necessary on concomitant use of drugs with adrenergic and serotonergic activity or food containing tyramine.

1.3.4 Exposure-Response Analysis for Efficacy

Animal Models of Infection

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The unbound AUC0-24 hours to MIC ratio (AUC/MIC) was the PK/PD parameter best associated with in vivo efficacy for tedizolid based on studies in the neutropenic mouse thigh infection model. In an MRSA infection produced in neutropenic mice, bacterial stasis was achieved at a *f*AUC/MIC ratio of approximately 50, corresponding to a total AUC/MIC ratio of approximately 250 for protein binding of 80%. In a study conducted in non-neutropenic animals, tedizolid activity against MRSA was enhanced by the presence of granulocytes by a factor of 25-fold on average (range from 16- to 35-fold) in non-neutropenic versus neutropenic animals. Based on this finding, a target unbound AUC/MIC ratio (adjusted for the previously obtained AUC/MIC ratio from neutropenic mice by a factor of 16-fold) of 3 was identified, corresponding to a total AUC/MIC ratio of 15 (using an 80% protein binding factor).

Analyses of Clinical Data

A flat exposure-response relationship was identified between tedizolid exposure (AUC_{ss}, AUC_{ss}/MIC) and clinical response using combined data from the two Phase 3 studies. This observed exposure-response relationship may in part be due to the clinical response rates (79.5%) observed from tedizolid treatment and the limited range of exposures resulting from the fixed 200 mg daily dose used in the Phase 3 studies. No efficacy differences were noted in the Phase 2 Study at tedizolid doses of 200 mg, 300 mg, or 400 mg. Thus, it is likely that the exposures achieved with the dose level administered in these Phase 3 trials are on the plateau of the exposure-response curve.

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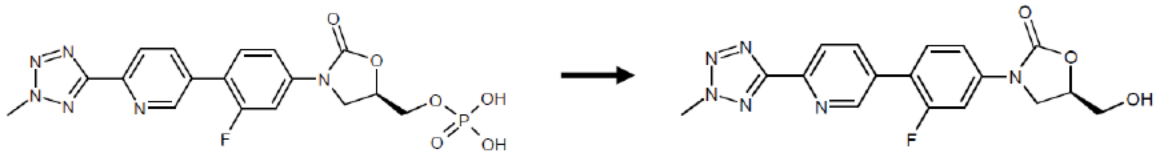
2. Question-Based Review

2.1 General Attributes of the Drug

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to the clinical pharmacology and biopharmaceutics review?

Tedizolid phosphate is a novel oxazolidinone prodrug antibiotic that is converted in vivo by phosphatases to the microbiologically active moiety tedizolid (**Figure 2.1.1-1**). The prodrug approach was used, in part, because tedizolid is only sparingly soluble in aqueous media and therefore ill-suited for IV and oral administration.

Figure 2.1.1-1. Chemical structures of tedizolid phosphate ($C_{17}H_{16}FN_6O_6P$; MW= 450.32) and tedizolid ($C_{17}H_{15}FN_6O_3$; MW= 370.34)



Oral

Tedizolid phosphate tablets, 200 mg, are available as immediate-release, film coated yellow oval-shaped tablets debossed with "TZD" on the obverse and "200" on the reverse side. (b) (4) [redacted]

Intestinal alkaline phosphatase enzymes dephosphorylate tedizolid phosphate to generate the highly permeable, pharmacologically active moiety, tedizolid. Tedizolid phosphate is technically a Biopharmaceutical Classification System (BCS) Class IV compound, with poor solubility below pH 4 and poor permeability of the phosphate prodrug moiety. However, at intestinal pH, tedizolid phosphate together with the active moiety, tedizolid, behave similarly to a BCS Class I drug, with high solubility of the prodrug and high permeability of the active metabolite at the site of absorption. This physical-chemical property was supported by the clinically observed high oral bioavailability of tedizolid following oral administration of tedizolid phosphate of greater than 90%.

IV

Tedizolid phosphate for injection is a sterile white to off-white lyophilized powder for injection in a single-use vial containing [redacted] (b) (4) tedizolid phosphate to allow delivery of 200 mg after reconstitution with 4 ml of Sterile Water for Injection [redacted] (b) (4). The reconstituted dose volume (4 ml) is to be added to an IV bag containing 0.9% Sodium Chloride Injection, USP.

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2.1.2 What are the proposed mechanism(s) of action and therapeutic indications(s)?

Tedizolid belongs to the oxazolidinone class of antibiotics and shares the largely bacteriostatic mechanism of action of linezolid, the first-in-class oxazolidinone approved in the US in 2000. Tedizolid is differentiated from linezolid by the substitution of a hydroxymethyl group at C-5 and the addition of pyridine (C-ring) and methyltetrazole (D-ring) moieties at the terminal ends of the molecule. Tedizolid is a protein synthesis inhibitor that interacts with the 50S subunit of the bacterial ribosome. The increased potency of tedizolid relative to linezolid in vitro is largely due to additional ribosome binding interactions of the C- and D-ring systems.

The proposed indication for tedizolid phosphate is 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 pyogenes*, *Streptococcus agalactiae*, *Streptococcus anginosus* Group (including *Streptococcus anginosus*, *Streptococcus intermedius* and *Streptococcus constellatus*)
- *Enterococcus faecalis*

2.1.3 What are the proposed dosage(s) and route(s) of administration?

The proposed clinical dose 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) for the treatment of ABSSSI.

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2.2 General Clinical Pharmacology

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

In total, there were fourteen in vitro studies and fifteen Phase 1 studies (12 reviewed) evaluating the clinical pharmacology of tedizolid phosphate and tedizolid. Studies included single- and multiple-dose pharmacokinetics (oral and IV); metabolism and elimination via mass balance and metabolite profiling; effect of food, hepatic impairment (moderate and severe), renal impairment (severe and end-stage renal disease [ESRD] on intermittent hemodialysis [HD]), age (elderly and adolescent); effect on the pressor response of tyramine and pseudoephedrine; and impact on QT prolongation. Data from Phase 1 studies were also used along with pharmacokinetic data obtained from two Phase 2 trials and two Phase 3 trials for development of population pharmacokinetic (PK) models.

Efficacy and safety of tedizolid phosphate in the treatment of ABSSSI was assessed in two pivotal Phase 3 trials (TR701-112 [oral] and TR701-113 [IV to oral]) and two supportive Phase 2 trials (TR701-104 [oral] and TR701-126 [oral]) as summarized in **Table 2.2.1-1**.

Table 2.2.1-1 Overview of Clinical Trials for Tedizolid Phosphate

Study No.	Design	Tedizolid Phosphate Regimen	Comparator Linezolid Regimen	Population Size
Phase 3				
TR701-112	Multicenter, randomized, double-blind, comparative study (ABSSSI)	200 mg once daily for 6 days (oral)	600 mg q12h for 10 days (oral)	Tedizolid Phosphate N = 332 Linezolid N = 335
TR701-113		200 mg once daily for 6 days (IV to oral)	600 mg q12h for 10 days (IV to oral)	Tedizolid Phosphate N = 332 Linezolid N = 334
Phase 2				
TR701-104	Multicenter, randomized, double-blind, noncontrolled study (cSSSI)	200, 300, and 400 mg once daily for 5-7 days (oral) *Note: disodium salt form of prodrug in capsules was used.*	N/A	200 mg N = 63 300 mg N = 63 400 mg N = 62
TR701-126	Multicenter, open-label, safety and exploratory skin lesion measurement study (major cutaneous abscess or cellulitis/erysipelas)	200 mg once daily for 6 days (oral)	N/A	N = 200

2.2.2 What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics (PD)) and how are they measured in clinical pharmacology and clinical studies?

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The primary efficacy endpoint used in Phase 3 studies was early clinical response, defined as cessation of spread and absence of fever in TR701-112 and ≥20% reduction in lesion area in TR701-113, at the 48 to 72 Hour Visit in the ITT Analysis Set (**Table 2.2.2-1**).

Table 2.2.2-1 Early Clinical Response of Tedizolid Phosphate in Phase 3 trials for ABSSSI

Phase 3 Clinical Trial	Efficacy Endpoint Measure At 48-72 hour Visit	Tedizolid Phosphate n (%)	Linezolid n (%)	Difference (%) (95% Confidence Interval)
TR701-112	cessation of spread and absence of fever	264 (79.5)	266 (79.4)	0.1 (-6.1, 6.2)
	≥20% reduction in lesion area*	259 (78.0)	255 (76.1)	1.9 (-4.5, 8.3)
TR701-113	≥20% reduction in lesion area	283 (85.2)	276 (82.6)	2.6 (-3.0, 8.2)

Note: Adapted from Module 2.5, Clinical Overview.
**The analysis for ≥20% reduction in lesion area was included for consistency across studies.*

Analysis populations and endpoints for ABSSSI were based on FDA final Guidance for Industry Acute Bacterial Skin and Skin Structure Infections: Developing Drugs for Treatment (October 2013) and past meetings between the Sponsor and the Division. Please refer to reviews by Statistical and Medical Reviewers for complete analysis of tedizolid efficacy in ABSSSI.

2.2.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Bioanalytical methods were developed and validated to support the quantification of tedizolid phosphate (prodrug) and tedizolid (active moiety) in samples generated from clinical studies. Details regarding validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays for quantification of tedizolid phosphate and tedizolid in plasma, urine, feces, and other biological matrices were reported and acceptable. Additionally, a non-validated high-performance liquid chromatography (HPLC) assay was used for metabolite profiling in plasma, urine, and feces in the mass balance study (TR701-106). (See **Section 2.6** for details.)

2.2.4 Exposure-response

2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy? If relevant, indicate the time to the onset and offset of the desirable pharmacological response or clinical endpoint.

The exposure-response relationship for tedizolid has been evaluated using in vitro microbiological studies, in vivo animal models of infection, population PK-PD analysis, and target attainment analysis using Monte Carlo simulations.

***In Vitro* Susceptibility:** The in vitro spectrum and potency of tedizolid against clinical isolates has been determined in a variety of studies conducted during preclinical and clinical development (**Table 2.2.4.1-1**). Profiling and surveillance studies demonstrate that, as expected for an oxazolidinone,

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the spectrum of tedizolid is largely limited to Gram-positive organisms and overlapped that of linezolid. Tedizolid demonstrated potent activity against staphylococci (*S. aureus*, *S. epidermidis*, other coagulase-negative staphylococci), enterococci (*E. faecium*, *E. faecalis* and other enterococci), and streptococci (*S. pyogenes*, *S. agalactiae*, Group C/F/G streptococci, *S. anginosus* Group streptococci, and *S. pneumoniae*).

Table 2.2.4.1-1 Summary of Weighted Average MIC Values for Tedizolid against Selected Pathogens

Organism	N	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Staphylococcus aureus			
Methicillin-susceptible	1389	0.28	0.46
Methicillin-resistant	1588	0.33	0.52
Coagulase-negative staphylococci			
Methicillin-susceptible	165	0.29	0.44
Methicillin-resistant	319	0.27	0.37
S. pneumoniae			
Penicillin-susceptible	91	0.25	0.25
Penicillin-intermediate	63	0.25	0.35
Penicillin-resistant	89	0.25	0.25
Enterococcus faecalis			
Vancomycin-susceptible	329	0.35	0.61
Vancomycin-resistant	106	0.47	0.50
Streptococcus pyogenes	268	0.17	0.35
Streptococcus agalactiae	127	0.25	0.38

Note: Adapted from Module 2.7.2.4, Special Studies (Microbiology), Table 27
To account for the variation in the volume of isolates tested across several studies, weighted average MIC_{50} and MIC_{90} values were calculated based on the following formula:
Weighted Average MICxx = Sum (wi ×MICxxi)/Sum(wi), where wi = number of isolates for the ith study in the group and MICxxi = either the MIC_{50} or MIC_{90} value for the ith study in the species group.

Time-Kill Kinetics: Tedizolid showed bacteriostatic effects (i.e., ≤2-log kill) within 24 hours against *S. aureus*, coagulase-negative staphylococci, and penicillin-resistant *S. pneumoniae*. The decrease in viable bacteria rarely exceeded 1-log at 24 hours for both tedizolid and the comparator, linezolid, against the evaluated isolates of *E. faecalis* and *E. faecium*, including vancomycin-resistant isolates, regardless of the concentration evaluated.

In studies comparing the intracellular accumulation of tedizolid and intracellular antimicrobial activity of tedizolid and linezolid against phagocytized *S. aureus*, *L. monocytogenes*, and *L. pneumophila*, tedizolid and linezolid penetrated human macrophages. Tedizolid exhibited an intracellular to extracellular ratio of 10 to 14 (versus a ratio of 1 to 2 was observed for linezolid) and dose-dependent static activity against phagocytized *S. aureus* and intracellular pathogens *L. monocytogenes* and *L. pneumophila*, including linezolid-resistant isolates.

Post-Antibiotic Effect (PAE): The PAE for tedizolid was found to be slightly shorter than the PAE for linezolid. The PAE of tedizolid ranged from 0.05 to 0.7 hours against staphylococci compared with linezolid PAE of 0.1 to 1.3 hours. The PAE of tedizolid ranged from 0. 15 to 1.05 hours against enterococci compared with linezolid PAE of 0.65 to 1.05 hours (**Table 2.2.4.1-2**).

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Table 2.2.4.1-2 Post-Antibiotic Effect of Tedizolid and Linezolid against Selected Pathogens

Organism	MIC (µg/mL)		PAE (h)	
	Tedizolid	Linezolid	Tedizolid	Linezolid
S. aureus				
Methicillin-susceptible	0.5	2	0.05	0.10
Methicillin-resistant	0.5	2	0.25-0.63	0.60-1.30
Coagulase-negative staphylococci				
Methicillin-susceptible	0.5	2	0.7	1
Methicillin-resistant	0.25	1	0.30	0.56
Enterococcus faecium				
Vancomycin-resistant	0.25	1	0.15-1.05	0.65-1.05
Enterococcus faecalis	0.5	2	0.57	0.77

Note: Adapted from Module 2.7.2.4, Special Studies (Microbiology), Table 88

Animal Infection Models: In vivo efficacy of tedizolid has been evaluated in various animal infection models, of which the pharmacokinetic-pharmacodynamic (PK-PD) relationship was characterized in neutropenic and non-neutropenic murine thigh models of infection. The *f*AUC/MIC ratio versus the $\log_{10}$ (CFU/g) for tedizolid phosphate dose intervals of 6, 12, or 24 hours in dose fractionation studies indicated that once-daily dose administration provided the same efficacy as giving the same total daily dose as multiple, equally divided doses each day.

In an MRSA infection produced in neutropenic mice, bacterial stasis was achieved at a *f*AUC/MIC ratio of approximately 50, corresponding to a total AUC/MIC ratio of approximately 250 for protein binding of 80%. In a study conducted in non-neutropenic animals, tedizolid activity against MRSA was enhanced by the presence of granulocytes by a factor of 25-fold on average (range from 16- to 35-fold) in non-neutropenic versus neutropenic animals. Based on this finding, a target unbound AUC/MIC ratio (adjusted for the previously obtained AUC/MIC ratio from neutropenic mice by a factor of 16-fold) of 3 was identified, corresponding to a total AUC/MIC ratio of 15 (using an 80% protein binding factor).

Granulocytes are a critical element in the body's response to ABSSSIs. The magnitude of granulocyte-mediated enhancement on tedizolid activity has not been seen with other antibiotics. The literature shows that granulocytes decreased the AUC/MIC target value by 2- to 4-fold when a quinolone was used for cohorts of normal versus neutropenic mice (*Drusano DL et. al, Antimicrobial Agents and Chemotherapy, 2011, p. 5300–5305*). The exact mechanism of granulocyte-mediated enhancement on tedizolid activity is unknown. As noted previously, tedizolid exhibited an intracellular to extracellular ratio of 10 to 14 (versus a ratio of 1 to 2 was observed for linezolid). Patients with recent history of opportunistic infections (e.g., leukemia, transplant, acquired immunodeficiency syndrome [AIDS]), or CD4 count <200 cells/mm^3 in patients with AIDS, or neutropenia with absolute neutrophil count (ANC)<1000 cells/mm^3, were excluded from the pivotal Phase 3 studies. The efficacy of the proposed 200 mg dose in neutropenic patients is unknown.

Population PK-PD:
Overall, PK/PD analyses of Phase 3 data showed a flat exposure-response relationship for efficacy endpoints such as such as clinical response at post treatment (PTE), early clinical

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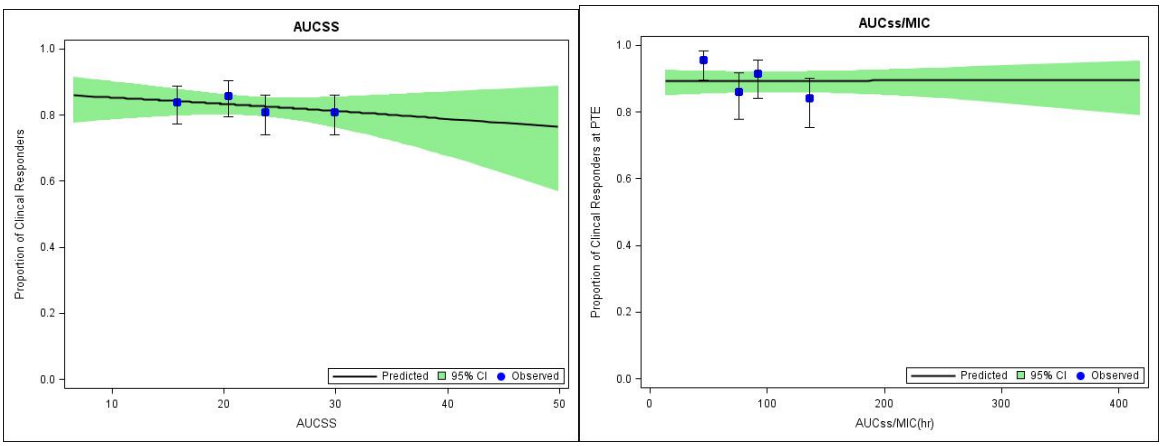
response at 48-72 hour, Early Clinical Response with ≥20% decrease in lesion, and microbiological response at post treatment. This may be due to the limited range of exposure resulting from a fixed 200 mg daily dose. In addition, exposures achieved in Phase 3 trials may be on the plateau of the exposure-response curve as a Phase 3 trial evaluating daily doses of 200, 300, and 400 mg also did not demonstrate a dose-response relationship.

Population PK analysis was conducted using concentration data obtained from four Phase 1 studies (full profile concentration data), one Phase 2 dose-escalation study and two pivotal Phase 3 studies (sparse sampling concentration data) in patients with ABSSSI. A total of 5066 tedizolid plasma concentrations collected from 648 individuals in these studies were utilized in the population PK analysis. The PK of tedizolid was described by a 2-compartment model with nonlinear absorption and linear elimination.

Individual tedizolid exposures for patients in the Phase 3 trials were estimated based on the final population PK model. Estimated tedizolid exposure metrics for each patient, included AUC_{0-24}, AUC_{ss}, and C_{max}. In addition, as the susceptibility of the pathogen is associated with response, both MIC and ratio of exposure/susceptibility measures (AUC_{ss}/MIC, AUC_{0-24}/MIC) were calculated and evaluated as independent measures for predicting treatment outcome. Efficacy endpoints, such as clinical response at post treatment (PTE), microbiologic response at end of treatment (EOT), were evaluated as dependent response measures using logistic regression analyses and the previously mentioned tedizolid exposure and exposure/MIC ratio measures. No evidence of an exposure-response relationship between these measures and the selected endpoints was identified for efficacy, as shown in **Figure 2.2.4.1-3**.

Figure 2.2.4.1-3 Relationship between proportion of patients who were clinical responders at post treatment and tedizolid exposure (AUC_{ss} and AUC_{ss}/MIC).



Note: The blue points are observed quartile mean (proportion of responders in each quartile (95% CI) plotted at the mean value within each quartile). The solid black line and green shaded areas are model-estimated 95% CI based on a univariate logistic analysis.

Please refer to the Pharmacometric review for complete analysis of tedizolid population PK/PD for efficacy.

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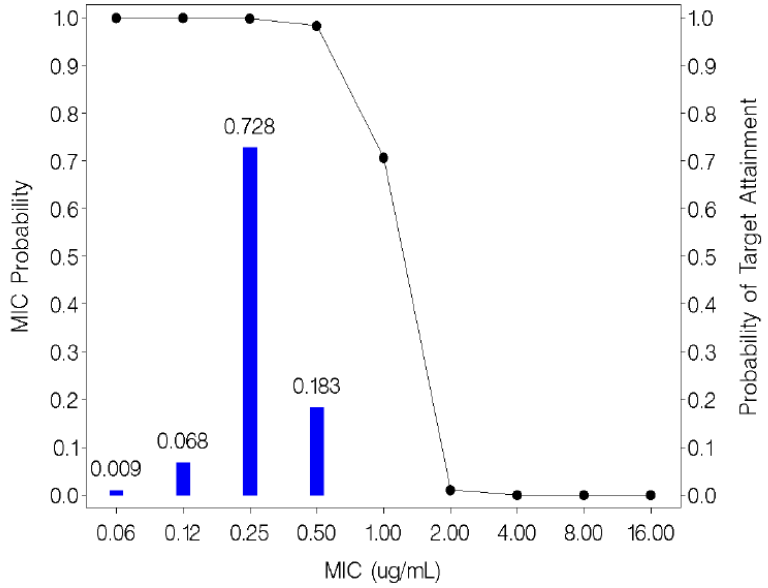
PK-PD Target Attainment: A tedizolid AUC_{0-24}/MIC ratio of 15 was determined as the PK/PD target associated with the activity against *S. aureus* in the non-neutropenic mouse thigh model of infection. Target attainment analysis was performed to estimate the probability of attaining this PK/PD target measure to support the definition of MIC interpretive breakpoints for *S. aureus*.

Based on the final population PK model and the distributions of the demographic covariates (determined to be significant predictors of tedizolid PK, including total bilirubin and ideal body weight) of ABSSSI patients enrolled in Phase 3 studies (TR701-112 and TR701-113), individual estimates of AUC_{0-24} on Day 1 and Day 6 (at steady state) were simulated by Monte Carlo simulation for a total of 100 trials of 1000 virtual patients each (100,000 patients total). MIC values between 0.06 and 16 µg/mL were considered to encompass a broad range of susceptibility for the target attainment analysis. The distribution of the percentage of patients with AUC/MIC observations above the target value of 15 for each MIC value was examined. The mean percentage of patients with simulated AUC_{0-24}/MIC ratios above the target, across all 100 datasets, was reported as the PK/PD target attainment at each MIC value. The MIC susceptibility breakpoint was determined to be the highest clinically relevant MIC value with a probability of PK/PD target attainment of at least 0.9.

The estimated mean target attainment for the AUC_{0-24}/MIC ratio of 15 was 100%, 100%, 99.84%, 98.31%, and 70.7% for MIC values of 0.06, 0.12, 0.25, 0.5, and 1 µg/mL, respectively (**Figure 2.2.4.1-4**). The simulated probabilities of target attainment at an MIC value of ≥2 µg/mL approached zero. In the ABSSSI clinical studies, 87% of early clinical response (with ≥20% decrease in lesion area) was observed at MIC values of 0.5 µg/mL, as compared to 82% early clinical response at MIC values less than 0.5 µg/mL. Based on these considerations, a susceptibility breakpoint of at least 0.5 µg/mL for *S. aureus* for tedizolid is proposed by the sponsor.

Figure 2.2.4.1-4 Probability of PK/PD Target Attainment for Tedizolid at the AUC_{0-24}/MIC Ratio of 15.



Note: Adapted from Module 2.7.2.4, Special Studies (Microbiology), Figure 71.

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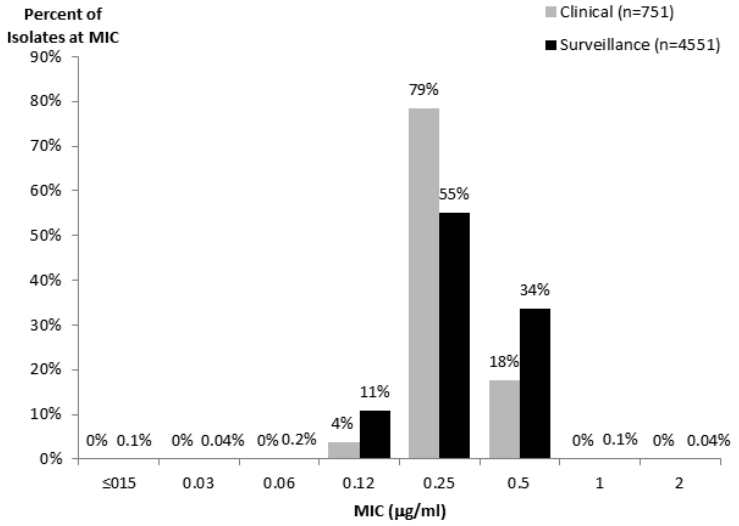
Interpretive Susceptibility Criteria:
Regarding determination of interpretive susceptibility criteria, the following pieces of information are considered: MIC distributions in clinical trials and surveillance programs, nonclinical PK-PD target attainment data, and analysis of clinical PK-PD relationships.

For *S. aureus*, the distribution of tedizolid MIC values for the clinical trial isolates (n=725) was similar to that of the surveillance study isolates (n=4499), as presented in **Figure 2.2.4.1-5.**

Figure 2.2.4.1-5 MIC Population Distribution for Clinical Trial and Surveillance Isolates for *S. aureus*



Note: Adapted from Module 2.7.2.4, Special Studies (Microbiology), Figure 61.

The target attainment analysis was performed to estimate the probability of attaining the nonclinical PK/PD target measure (AUC_{0-24}/MIC ratio of 15) for *S. aureus* using the simulated AUC_{0-24} under the 200-mg dose regimen and MIC population distribution data. The results suggested a susceptibility breakpoint of at least 0.5 µg/mL for *S. aureus* for tedizolid (**Figure 2.2.4.1-4)**.

As noted previously, in the ABSSSI clinical studies, 87% of early clinical response (with ≥20% decrease in lesion area) was observed at MIC values of 0.5 µg/mL, as compared to 82% early clinical response at MIC values less than 0.5 µg/mL. The PK/PD analyses of Phase 3 data together with results from the dose-ranging Phase 2 trial suggest that exposures achieved with the dose level administered in these Phase 3 trials (200 mg once daily) may be on the plateau of the exposure-response curve. In addition, across a range of AUC/MIC ratios observed in Phase 3 studies, shown on the x-axis in **Figure 2.2.4.1-6**, there was no apparent drop in efficacy (assessed by the number of subjects with a positive microbiological response, shown on the y axis).

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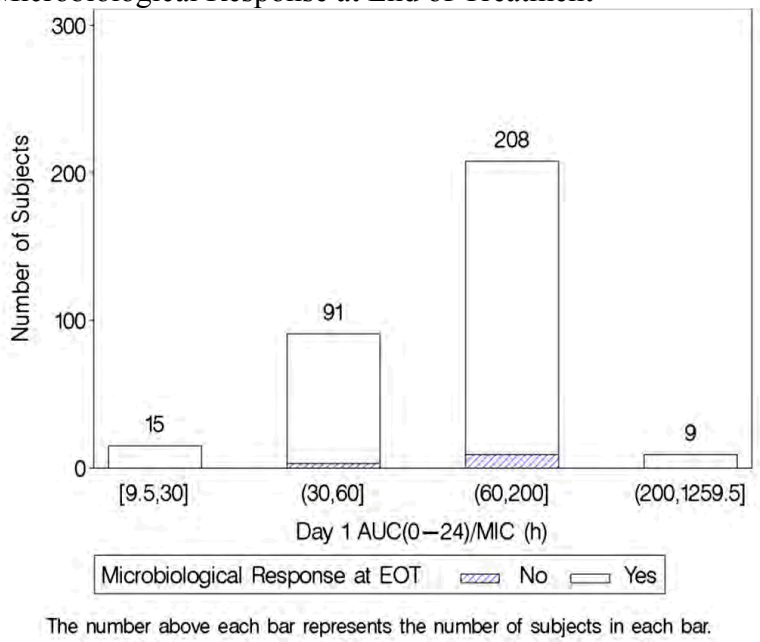
Figure 2.2.4.1-6 Frequency Distribution of Grouped Day 1 AUC_{0-24}/MIC Ratios, by Microbiological Response at End of Treatment



Note: Adapted from Module 2.7.2.4, Special Studies (Microbiology), Figure 70.

Based on the reviewer's assessment of MIC distributions in clinical trials and surveillance programs, nonclinical PK-PD target attainment data and simulations conducted by the sponsor, and an analysis of clinical PK-PD relationships, the sponsor's proposal of a susceptibility breakpoint of 0.5 µg/mL is appropriate from a clinical pharmacology perspective.
It should be noted that the determination of breakpoints involves multiple disciplines including clinical and microbiological perspectives in addition to the above nonclinical and clinical PK-PD considerations. The ultimate determination of the tedizolid breakpoint will depend on the totality of information provided by each discipline and continues to be assessed at the time of the completion of this review.

2.2.4.2 What are the characteristics of the exposure-response relationships (dose-response, concentration response) for safety? If relevant, indicate the time to the onset and offset of the undesirable pharmacological response or clinical endpoint.

The exposure-response analysis for safety that described the relationship between tedizolid exposure and the probability of any treatment-emergent adverse events (TEAEs), gastrointestinal AEs, and laboratory measurements was conducted using data from Phase 2 Study TR701-104 and Phase 3 Studies TR701-112 and TR701-113. The analysis dataset consisted of 1644 AE records from 822 patients, of which, 533 patients (65%) were male and 289 (35%) were female. A total of 686 patients (83.5%) in the pooled population were white; 13.6% of patients were black, and 0.9% of patients were Asian. The majority of patients (71.8%) were from North America, 20.0% were from Europe, 5.4% were from South Africa.

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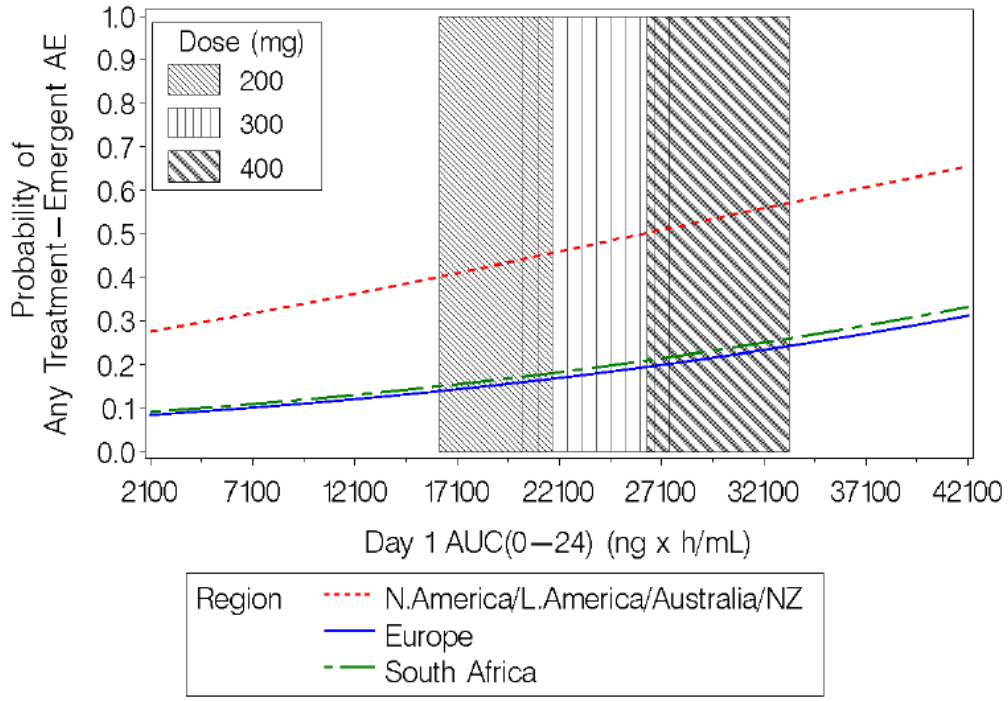
Treatment-emergent Adverse Events: A total of 305 patients (37%) had at least 1 occurrence of any treatment-emergent adverse event (TEAE) during the treatment period. The final model for the probability of any TEAE is the sum of the drug effect described by an increasing linear function of the Day 1 AUC_{0-24} and additive shifts for region. As Day 1 AUC_{0-24} increases, the predicted probability of experiencing any TEAE increases. Patients from North America, Latin America, Australia, and New Zealand have a higher predicted probability of any TEAE as compared to patients from Europe or South Africa (**Figure 2.2.4.2-1**). The lowest occurrence (33%) of any TEAE was observed in patients administered 200 mg tedizolid phosphate. The incidence of any TEAE increased with increasing tedizolid phosphate dose. There are no trends in the plots of minimum or maximum decrease in neutrophil counts or platelet counts versus Day 1 or steady-state tedizolid exposure measures, indicating no apparent exposure-related effects were observed in these laboratory measures.

Figure 2.2.4.2-1 Final Model-Predicted Probability of Any Treatment-emergent Adverse Event Versus Tedizolid Day 1 AUC_{0-24}, by Region.



Note: Adapted from study report CLN-13-0701-102, Figure 18.

Gastrointestinal AEs: A total of 147 patients (18%) had least 1 occurrence of a GI AE during the treatment period. The group of GI AEs accounted for approximately 33% of all TEAEs. As with any TEAE, most GI AEs (62%) occurred within the first two days of treatment with tedizolid phosphate (**Figure 2.2.4.2-2**). Patients with the occurrence of GI AEs were observed with a 9.2% higher median Day 1 AUC_{0-24} compared with those who did not experience GI AEs.

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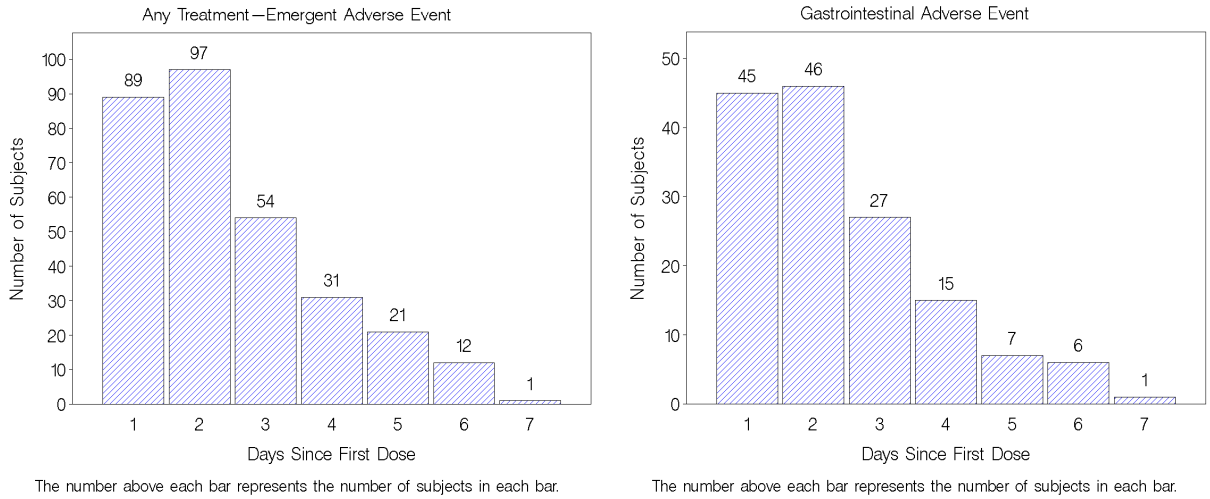
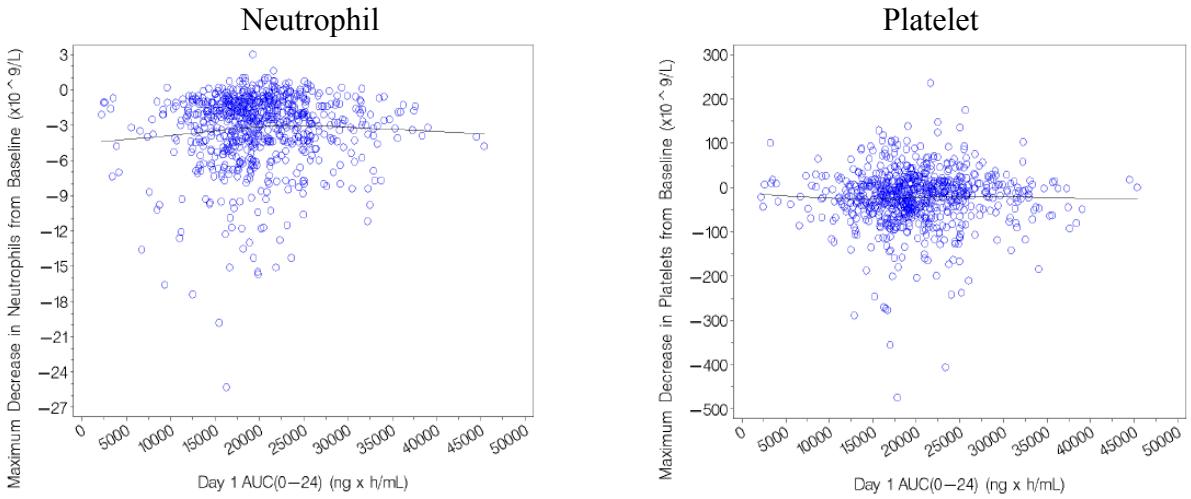
Figure 2.2.4.2-2 Frequency Distribution of the Time to the First Occurrence of AEs



Note: Adapted from the Sponsor's report CLN-13-0701-102, Figures 13 and 20.

Laboratory Measurements: Myelosuppression has been reported with the previously approved oxazolidinone linezolid, including anemia, leukopenia, pancytopenia, and thrombocytopenia. The relationships between tedizolid exposure and laboratory assessments of neutrophil and platelet counts were explored. There was no clear trend between decrease in neutrophil counts or platelet counts versus Day 1 or steady-state tedizolid exposure was observed (**Figure 2.2.4.2-3**).

Figure 2.2.4.2-3 Scatterplots of Maximum Decrease in Neutrophil and Platelet Counts from Baseline versus Tedizolid Exposure



Note: Adapted from the Sponsor's report CLN-13-0701-102, Figures 25 and 27.

Please refer to the Medical Officer review for complete analysis of tedizolid safety.

2.2.4.3 Does this drug prolong the QT or QTc interval

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No significant QTc prolongation effects of tedizolid phosphate at 200 mg and 1200 mg were detected in the thorough QT (TQT) study in healthy adults (TR-701-115). In this randomized, blinded, placebo-controlled crossover study, 48 healthy subjects received 200 mg and 1200 mg tedizolid phosphate, placebo, and 400 mg moxifloxacin. As shown in **Table 2.2.4.3-1**, the largest upper bounds of the 2-sided 90% CI for the mean differences between tedizolid phosphate 200 mg and placebo, and between tedizolid phosphate 1200 mg and placebo were below 10 ms, the threshold for regulatory concern as described in ICH E14 guidelines. The largest lower bound of the 2-sided 90% CI for the ΔΔQTcF for moxifloxacin was greater than 5 ms (**Table 2.2.4.3-1**), indicating that assay sensitivity was established.

Table 2.2.4.3-1 The Point Estimates and the 90% CIs Corresponding to the Largest Upper Bounds for tedizolid phosphate (200 mg and 1200 mg) and the Largest Lower Bound for Moxifloxacin (FDA Analysis).

Treatment	Time (hour)	ΔΔQTcF (ms)	90% CI (ms)
TR-701 FA 200 mg	14	3.6	(0.8, 6.3)
TR-701 FA 1200 mg	4	7.6	(5.4, 9.9)
Moxifloxacin 400 mg*	3.5	12.4	(10.1. 14.7)

** Multiple endpoint adjustment was not applied. The largest lower bound after Bonferroni adjustment for 4 timepoints is 9.2 ms. TR-701 FA denotes tedizolid phosphate free acid.*

The supratherapeutic dose (1200 mg) produces a mean C_{max} of tedizolid that is 4.7-fold higher than that for the therapeutic dose (200 mg) following a single oral dose of the drug. These concentrations are above those for the predicted worse-case scenario (47% increase in C_{max} in adolescents following IV administration relative to adults), and are higher than the concentrations of the maximum multiple doses (400 mg oral once daily for 21 days) tested. At these concentrations there are no detectable prolongations of the QT interval. While the drug is primary eliminated via biliary excretion, the C_{max} in patients with moderate and severe hepatic impairment was observed to be less than 10% higher than that in control subjects, and is below the exposure of the chosen supratherapeutic dose. No exposure-response relationship was evident between ΔΔQTcF and tedizolid concentrations (**Figure 2.2.4.3-1**).

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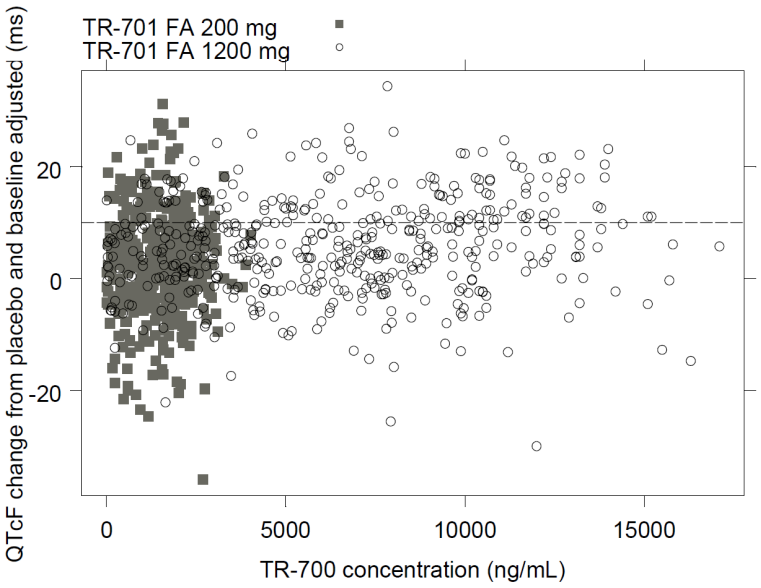
Figure 2.2.4.3-1 Exposure-Response relationship between ΔΔQTcF and Tedizolid Concentrations



TR-701 FA denotes tedizolid phosphate free acid.

Please refer to the review by the Interdisciplinary Review Team for QT Studies (DARRTS dated 01/14/2013) for complete analysis of tedizolid cardiovascular safety.

2.2.4.4 Is the dose and dosing regimen selected by the Sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The proposed dosing regimen of tedizolid phosphate 200 mg administered once daily for six days either orally (with or without food) or as an IV infusion for treatment of ABSSSI is supported by:

- Based on PK-PD target attainment analyses, requisite exposures of tedizolid for efficacy or bacteriostasis are estimated to be achieved at MIC ≤0.5 µg/mL against *S. aureus (>90% of target attainment at the target* $AUC_{0\text{-}24}$*/MIC of 15)*.
- In a Phase 2 dose-ranging study, doses of 200, 300, and 400 mg tedizolid phosphate once daily for 5 to 7 days demonstrated similar efficacy.
- The proposed regimen was evaluated for clinical and microbiological efficacy in pivotal Phase 3 trials.
- No significant safety concerns were observed with the proposed regimen in pooled clinical studies.

2.2.5 What are the PK characteristics of the drug and its major metabolite?

2.2.5.1 What are the single dose and multiple dose PK parameters?

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Oral
Pharmacokinetics of tedizolid were evaluated in healthy adults following single (200,400, 600, 800, and 1200 mg) and multiple (200, 300, and 400 mg once daily for 21 days) ascending dose of oral tedizolid phosphate (**Table 2.2.5.1-1** and **Table 2.2.5.1-2**).

Tedizolid Phosphate
After oral administration, tedizolid phosphate was rapidly and extensively converted to the active moiety tedizolid in plasma. Plasma concentrations of the prodrug were undetectable in almost all subjects. Tedizolid phosphate concentrations were quantifiable in only one subject at the 1200 mg dose level with a peak concentration of 16.9 ng/mL.

Tedizolid
After single dose administration of 200 to 1200 mg tedizolid phosphate, dose proportional increases in AUC, and less than dose proportional increases in C_{max}, were observed for tedizolid (**Table 2.2.5.1-1)**. Repeat 200 mg, 300 mg, and 400 mg tedizolid phosphate once-daily administration resulted in approximately dose proportional increases in tedizolid C_{max} and AUC (**Table 2.2.5.1-2**). The $t_{1/2}$, CL/F, and Vz/F values for tedizolid appeared independent of dose and duration of exposure (**Table 2.2.5.1-1** and **Table 2.2.5.1-2**). Approximately 31% accumulation of tedizolid AUC was observed at steady state for the therapeutic dose regimen of 200 mg once-daily tedizolid phosphate (**Table 2.2.5.1-2**). In both single- and multiple-dose studies, <1% of the tedizolid phosphate dose was excreted in urine as either tedizolid phosphate or tedizolid.

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Table 2.2.5.1-1 Mean (SD) Plasma and Urinary PK Parameters for Tedizolid After Single Ascending Dose Oral Administration of Tedizolid Phosphate (Disodium Salt form in Capsules) under fasted condition (Study TR701-101).

PK Parameter	200 mg TR-701	400 mg TR-701	600 mg TR-701	800 mg TR-701	1200 mg TR-701
C_{max} (µg/mL)	1.99 (0.43)	3.77 (0.96)	5.21 (0.73)	5.52 (1.19)	9.49 (1.92)
t_{max} (hr)[a]	3.01 (1.0, 4.0)	3.50 (2.0, 4.0)	2.50 (2.0, 4.0)	4.00 (2.0, 8.0)	4.00 (2.0, 4.0)
$AUC_{0-\infty}$ (µg•hr/mL)	25.35 (4.6)	56.10 (13.20)	79.26 (31.34)	91.80 (12.87)	123.10 (31.20)
$t_{½}$ (hr)	11.2 (3.59)	10.8 (0.80)	11.4 (2.57)	10.6 (1.29)	10.4 (1.43)
CL/F (L/hr)	6.08 (1.08)	5.58 (1.23)	6.58 (3.00)	6.65 (0.94)	7.77 (2.24)
V_z/F (L)	95.7 (23.5)	87.0 (18.0)	101 (23.5)	101 (14.0)	116 (29.2)
$f_{e\,(0-72)}$ (%)	0.638 (0.291)	0.876 (0.462)	0.664 (0.347)	0.518 (0.222)	0.660 (0.254)
CL_R (mL/hr)	41.5 (13.0)	54.4 (30.6)	41.0 (22.4)	36.7 (13.2)	53.8 (13.3)

Note: Adapted from Module 2.7.2. Summary of Clinical Pharmacology Studies, Table 7; TR-701 denotes tedizolid phosphate disodium salt in capsules.
[a]Median (minimum, maximum) presented for t_{max}.

Table 2.2.5.1-2 Mean (SD) Plasma PK Parameters for Tedizolid After Multiple Ascending Dose Oral Administration of Tedizolid Phosphate (Disodium Salt form in Capsules) under fasted condition (Study TR701-101).

	TR-701 Dose					
	200 mg QD[a]		300 mg QD		400 mg QD	
PK Parameter	Day 1	Day 15[b]	Day 1	Day 21	Day 1	Day 21
C_{max} (µg/mL)	1.8 (1.2)	1.8 (0.4)	2.1 (0.5)	2.7 (0.5)	4.2 (0.8)	4.7 (0.5)
C_{min} (µg/mL)	NA	0.41 (0.17)	NA	0.51 (0.14)	NA	0.85 (0.16)
t_{max} (hr)[c]	3 (1.5, 4)	3 (2, 4)	2 (1.5, 4)	3 (1.6, 8)	4 (1, 4)	2.5 (1.5, 4)
$AUC_{0-\tau}$ (µg•hr/mL)	16.7 (3.8)	22.5 (6.5)	24.1 (5.4)	31.2 (6.6)	46.0 (6.4)	52.0 (5.1)
$AUC_{0-\infty}$ (µg•hr/mL)	21.6 (6.5)	NC	29.6 (7.5)	NC	54.0 (8.2)	NC
$t_{½}$ (hr)	11.1 (1.20)	10.2 (2.05)	10.1 (1.36)	11.9 (1.67)	8.01 (1.17)	12.8 (1.42)
CL/F (L/hr)	7.48 (2.12)	7.16 (1.99)	7.98 (1.80)	7.51 (1.60)	5.66 (0.84)	5.82 (0.61)
V_z/F (L)	117 (21.9)	108 (38.1)	116 (27.8)	127 (22.0)	64.8 (9.96)	108 (20.40)

Note: Adapted from Module 2.7.2. Summary of Clinical Pharmacology Studies, Table 8; TR-701 denotes tedizolid phosphate disodium salt in capsules.
[a]Therapeutic dose.
bSteady state Day 15 and Day 21 PK results were comparable for most individuals;Day 15 shown for 200 mg dose due to one subject suspected to have missed the Day 21 administration;
[c]Median (minimum, maximum) presented for t_{max}.

IV

Pharmacokinetics of tedizolid were evaluated in healthy adults following single (100, 200, 300, and 400 mg) and multiple (200 and 300 mg once daily for 7 days) ascending dose of IV tedizolid phosphate (**Table 2.2.5.1-3** and **Table 2.2.5.1-4**). Concentration-time profiles of tedizolid following single and multiple once daily 200 mg IV administration are presented in **Figure**

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2.2.5.1-1. In the double-blind, single-ascending dose study, tedizolid phosphate or placebo was administered at a dose of 100 mg, 200 mg, or 400 mg in 3 different sequential administration regimens of increasing concentration: a 500 mL, 120 minute infusion, a 250 mL, 120 minute infusion, or a 250-mL, 60 minute infusion. In the double-blind, multiple-dose study, tedizolid phosphate (200 mg/day or 300 mg/day) or placebo was administered as a 250 mL, 60 minute infusion for 7 days. The 300 mg/day group was discontinued prior to Day 7 PK, due primarily to adverse events of injection site pain.

Tedizolid Phosphate

Following single-dose IV administration, plasma concentrations of tedizolid phosphate increased with dose, but were only detectable during infusion and briefly thereafter. The mean half-life of tedizolid phosphate of the 200 mg dose group was less than 10 min. There was no accumulation of the prodrug after 7 days of IV administration of 200 mg tedizolid phosphate. PK parameters of the prodrug for Day 1 and Day 7 were similar except that mean half-life increased to approximately 20 min.

Tedizolid

Following single-dose IV administration, plasma concentrations of tedizolid were detectable at the first sampling time point (15 min post start of infusion) and increased during the infusion. Tedizolid exposure (AUC and C_{max}) increased with ascending doses of the prodrug (up to 400 mg) (**Table 2.2.5.1-3**). Tedizolid T_{max} was generally achieved by the end of infusion and mean $t_{1/2}$ ranged from 9 to 13 hours for all doses (**Table 2.2.5.1-3**).

Following multiple-dose IV administration, apparent increase in tedizolid half-life was likely due to the longer sampling interval (72 hours postdose on Day 7 versus 24 hours on Day 1) (**Table 2.2.5.1-4**). Approximately 29% accumulation of tedizolid AUC was observed at steady state for the therapeutic dose regimen of 200 mg once-daily IV tedizolid phosphate (**Table 2.2.5.1-4**). The $t_{1/2}$, CL, and V_{ss} values for tedizolid appeared independent of dose and duration of exposure (**Table 2.2.5.1-3** and **Table 2.2.5.1-4**).In both single- and multiple-dose studies, ≤1% of the tedizolid phosphate dose was excreted in urine as either tedizolid phosphate or tedizolid.

Table 2.2.5.1-3 Mean (SD) Plasma PK Parameters for Tedizolid After Single Ascending Dose IV Administration of Tedizolid Phosphate (Study TR701-107).

PK Parameter	100 mg TR-701 FA	200 mg TR-701 FA	300 mg TR-701 FA	400 mg TR-701 FA
C_{max} (µ/mL)	1.16 (0.19)	2.62 (0.58)	4.53 (1.07)	5.13 (0.79)
AUC_{0-t} (µg•hr/mL)	17.02 (1.66)	29.99 (10.34)	43.40 (9.14)	58.19 (11.38)
$AUC_{0-\infty}$ (µg•hr/mL)	17.36 (1.8)	32.58 (8.30)	51.90 (11.20)	58.70 (11.59)
CL (L/hr)	4.77 (0.51)	5.41 (1.75)	4.92 (0.88)	5.79 (1.06)
V_{ss} (L)	74.5 (9.4)	67.1 (15.3)	61.2 (15.2)	67.5 (12.2)
$t_{½}$ (hr)	13.4 (1.14)	11.0 (0.76)	9.3 (1.44)	11.3 (1.23)

Note: Adapted from Module 2.7.2. Summary of Clinical Pharmacology Studies, Table 10; TR-701 FA denotes tedizolid phosphate free acid.

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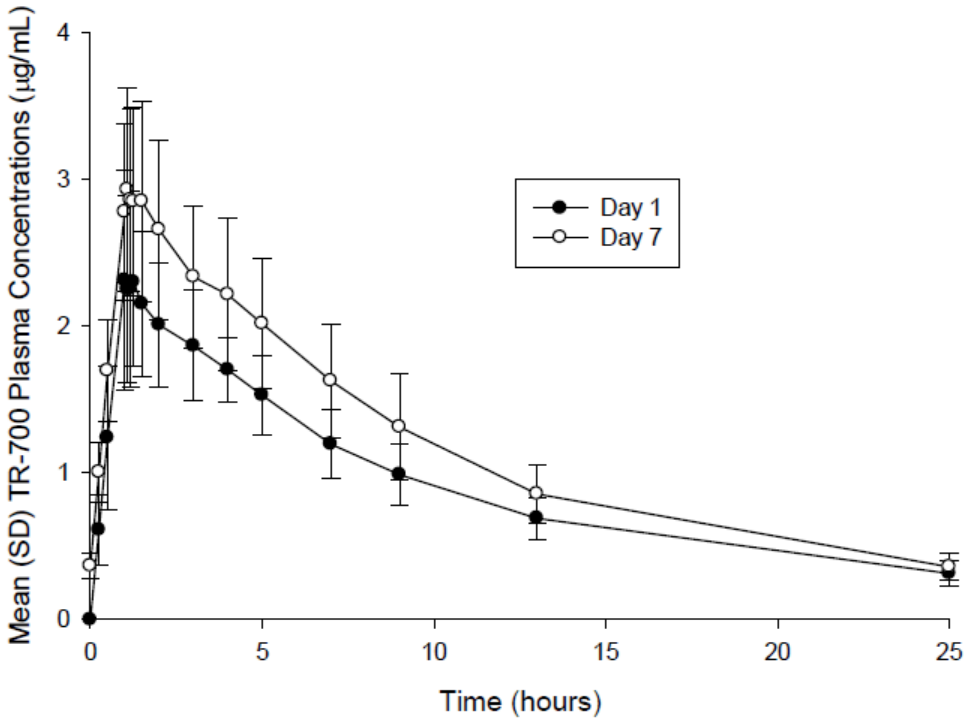
Table 2.2.5.1-4 Mean (SD) Plasma and Urinary PK Data for Tedizolid Phosphate and Tedizolid Following Single and Once-daily 200 mg Tedizolid Phosphate IV Infusion (Study TR701-107).

PK Parameter	TR-701		TR-700	
	Day 1	Day 7	Day 1	Day 7
C_{max} (µg/mL)	1.7 (0.5)	1.8 (0.4)	2.34 (0.64)	3.01 (0.66)
t_{max} (hr)[a]	0.25 (0.250, 0.917)	0.50 (0.250, 0.917)	1.08 (0.917, 1.50)	1.17 (0.917, 1.50)
AUC_{0-24} (µg•hr/mL)	1.4 (0.5)	1.5 (0.3)	22.29 (4.24)	29.18 (6.22)
$AUC_{0-\infty}$ (µg•hr/mL)	1.4 (0.46)	NA	26.59 (5.18)	NA
CL (L/hr)	160 (49.9)	135 (26.9)	6.37 (1.19)	5.87 (1.41)
V_{ss} (L)	12.1 (3.77)	16.6 (9.70)	77.6 (15.9)	80.1 (21.0)
$t_{½}$ (hr)	0.16 (0.08)	0.39 (0.34)	9.33 (1.50)	12.4 (1.25)
A_{e0-24} (mg)	0.00087 (0.00261)	0.00232 (0.00362)	1.72 (0.547)	1.95 (0.785)
CL_R (L/hr)	0.00072 (0.00215)	0.00208 (0.00308)	0.0779 (0.0234)	0.0662 (0.0220)
f_{e0-24} (%)	0.00044 (0.00131)	0.00116 (0.00181)	1.05 (0.332)	1.18 (0.477)

Note: Adapted from Module 2.7.2. Summary of Clinical Pharmacology Studies, Table 11; TR-701 FA denotes tedizolid phosphate free acid and TR-700 denotes tedizolid.
[a]Median (minimum, maximum) presented for t_{max}.

Figure 2.2.5.1-1 Mean (SD) Tedizolid Plasma Concentrations Following Single and Multiple Once-Daily 200 mg Intravenous Administration of Tedizolid Phosphate



Note: Adapted from Module 2.7.2. Summary of Clinical Pharmacology Studies, Figure 3.

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2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

A comparison of exposure to tedizolid between healthy subjects in Phase 1 studies (Study TR701-109, 123, 124) and patients in Phase 2/3 studies (Study TR701-104, 112, 113) following 200 mg dose is summarized in **Table 2.2.5.2-1**. The ABSSSI patients were predicted to have a 16% lower steady-state AUC compared with healthy volunteers. Based on the population PK analysis, ideal body weight and total bilirubin are two significant predictors of tedizolid PK. Tedizolid clearance increases with ideal body weight and decreases with total bilirubin. The difference between healthy subjects and patients may be partly explained by the difference in total bilirubin between the two groups. The total bilirubin in the ABSSSI patients was only about half (51%) of the healthy volunteers.

Table 2.2.5.2-1 Comparison of Tedizolid Exposures in Healthy Subjects versus ABSSSI Patients following 200 mg QD Tedizolid Phosphate

Parameter	**Healthy Subjects (n=84)**	**ABSSSI PK Patients (n=822)**
	Study 109, 123, 124	Study 104, 112, 113
Ideal Body Weight	54.4±13.4	43.0±15.2
Total Bilirubin (mg/dL)	0.91±0.77	0.47±0.29
CL/F (L/h)	5.9±1.7	7.0±2.1
V_c (L)	64.8±14.4	68.7±11.3
$AUC_{ss(0-24)}$ (µg*h/mL)	37.0 ± 12.3	31.0 ± 8.5

2.2.5.3 What are the characteristics of drug absorption?

Following a single 1-hour IV infusion or oral administration under fasting conditions (crossover design; TR701-107) of 200 mg tedizolid phosphate, median T_{max} values for tedizolid of 1.17 and 3.50 hours were observed, respectively. The mean plasma concentration-time profiles of tedizolid following IV and oral administration of 200 mg tedizolid phosphate are illustrated in **Figure 2.2.5.3-1**. The absolute bioavailability of tedizolid following oral administration of 200 mg tedizolid phosphate was determined to be approximately 91% (based on $AUC_{0-\infty}$) **(Table 2.2.5.3-1)**.

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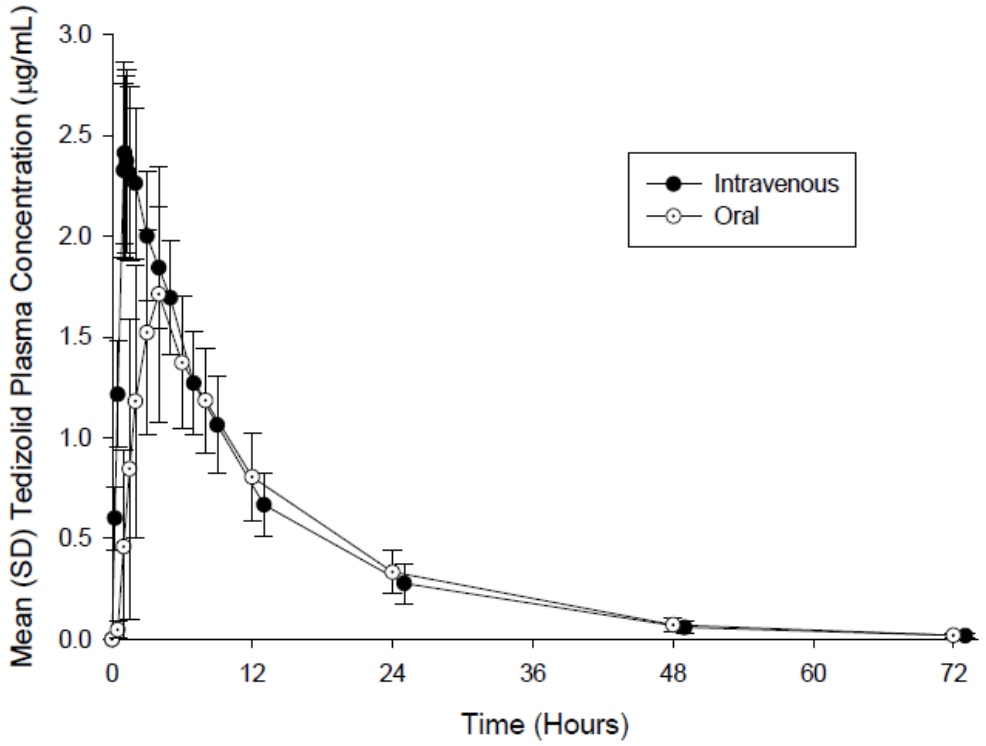
Figure 2.2.5.3-1. Mean (SD) Plasma Concentration-Time Profiles for Tedizolid after IV and Oral Administration of 200 mg Tedizolid Phosphate



Note: Adapted from report of Study TR701-107

Table 2.2.5.3-1 Absolute Bioavailability of Tedizolid following Oral Administration of 200 mg Tedizolid Phosphate Tablet (Relative to Intravenous Infusion)

Parameter (Units)	N[a]	Test Mean[b] (Oral Tablet)	N[a]	Reference Mean[b] (Intravenous)	Test/Reference[c]	90% Confidence Interval[d]
AUC_{0-t} (ng*hr/mL)[e]	8	25649	8	28094	91.30	(86.71 , 96.12)
$AUC_{0-\infty}$ (ng*hr/mL)	8	25972	8	28393	91.47	(86.81 , 96.38)

Note: Adapted from report of Study TR701-107
[a] *N was the number of subjects with evaluable PK data for that treatment.*
[b] *Least squares mean from ANOVA. Natural log parameter means calculated by transforming the natural log means back to the linear scale (ie, geometric means).*
[c] *Ratio of parameter means for natural-log transformed parameter (expressed as a percent). Natural log transformed ratios transformed back to the linear scale.*
[d] *90% confidence interval for ratio of parameter means of natural-log transformed parameter (expressed as a percent). Natural-log transformed confidence limits transformed back to the linear scale.*
[e] *IV duration is 1 hour longer than oral.*

Given the absolute bioavailability of 91% (based on $AUC_{0-\infty}$), dose adjustment is unnecessary for intravenous to oral switch.

2.2.5.4 What are the characteristics of drug distribution?

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Plasma protein binding of tedizolid was measured in vitro across species and ex vivo in samples obtained from clinical studies. Tedizolid protein binding in human plasma ranged from 70% to 90% (primarily bound to albumin) and binding appeared to be independent of concentration. Overall, human plasma protein binding is approximately 80%. There were no marked changes in tedizolid plasma protein binding in patients with renal and hepatic impairment and patients on hemodialysis. Following a single 200 mg oral dose of ^{14}C-labelled tedizolid phosphate, the mean whole blood to plasma total radioactivity $AUC_{0-\infty}$ ratio (AUC_b:AUC_p) was approximately 0.925 (of note, tedizolid accounts for approximately 95% of the total radiocarbon AUC in plasma), suggesting that tedizolid is equally distributed between plasma and red blood cells.

Penetration of tedizolid into the interstitial space fluid of adipose and skeletal muscle tissue was studied via a subcutaneous microdialysis probe in 15 healthy subjects following a single 600 mg oral dose of tedizolid phosphate capsules (TR701-102). Tedizolid concentrations in interstitial space fluid of subcutaneous adipose and skeletal muscle tissue were comparable to free plasma concentrations of tedizolid. Mean calculated ratios $AUC_{tissue}/fAUC_{plasma}$ were 1.1 and 1.2 for adipose and muscle tissues, respectively (**Table 2.2.5.4-1)**.

Table 2.2.5.4-1 Mean (SD) Tedizolid Pharmacokinetic Parameters for Plasma, Adipose Tissue, and Muscle Tissue following a Single 600 mg Oral Dose of Tedizolid Phosphate Capsules

PK Parameter	Plasma	Adipose Tissue[a]	Muscle Tissue[a]
C_{max} (µg/mL)	5.37 (1.51)	0.66 (0.16)	0.74 (0.16)
t_{max} (hr)[b]	2 (1-4)	3.33 (2.33-10.3)	3.67 (1.67-7)
AUC_{0-12} (µg•hr/mL)	38.81 (7.55)	5.27 (1.28)	5.95 (1.11)
$t_{½}$ (hr)[b]	8.13 (5.94-12.8)	9.22 (5.98-85.9)	9.59 (6.15-48.2)
$AUC_{tissue}/fAUC_{plasma}$[c]	NA	1.08 (0.22)	1.22 (0.18)

Note: Adapted from Module 2.7.2. Summary of Clinical Pharmacology Studies, Table 13.
[a]*In tissues, all concentrations are considered as free, unbound to proteins.*
[b]*Median (minimum, maximum) presented for t_{max}.*
[c]*Ratio of AUC0-12 was determined after adjusting total plasma concentration for ~87% protein binding.*

2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

Hepatic excretion appeared to be the major elimination pathway for tedizolid and its metabolites and renal excretion was a minor pathway.

2.2.5.6 What are the characteristics of drug metabolism?

Conversion of tedizolid phosphate to tedizolid appears to be mediated by in vivo phosphatases. A total of 8 metabolites of tedizolid phosphate were identified in plasma, urine, and feces (TR701-106; **Figure 2.2.5.6-1**). Of these metabolites, tedizolid was the only significant circulating metabolite found in plasma (96.3% of sample radioactivity). The sulfate analog of tedizolid was the main metabolite found in the feces (69% of administered dose) and urine (10% of administered dose), but was only detected in trace quantities in plasma in 1 subject (n=6).

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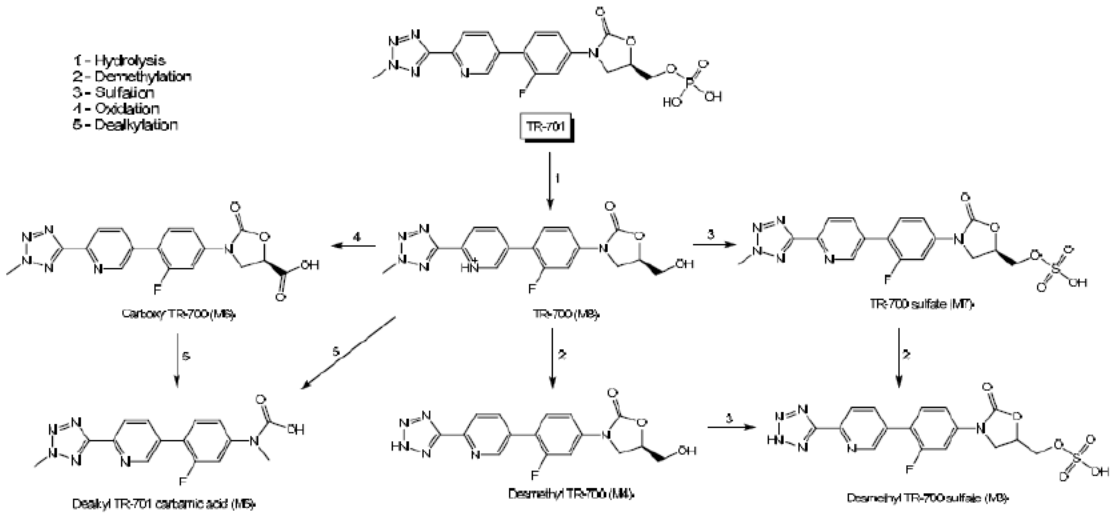
Other metabolites (desmethyl and carboxy tedizolid) were found in small amounts in urine and urine/feces.

Figure 2.2.5.6-1 Proposed biotransformation pathway of [^{14}C]-Tedizolid Phosphate in humans



Note: Adapted from Module 2.7.2 Summary of Clinical Pharmacology Studies, Figure 9.

In vitro, there was no degradation of tedizolid in human liver microsomes indicating tedizolid is unlikely to be a substrate for hepatic P450 enzymes.

2.2.5.7 What are the characteristics of drug excretion?

Following single oral administration of ^{14}C-labeled tedizolid phosphate (TR701-106), 82% of the radioactive dose was recovered in feces and 18% in urine primarily as a non-circulating and microbiologically inactive sulfate conjugate, with most of the elimination (>85%) occurring within 96 hours (**Figure 2.2.5.7**). Less than 3% of tedizolid phosphate administered dose is excreted as tedizolid.

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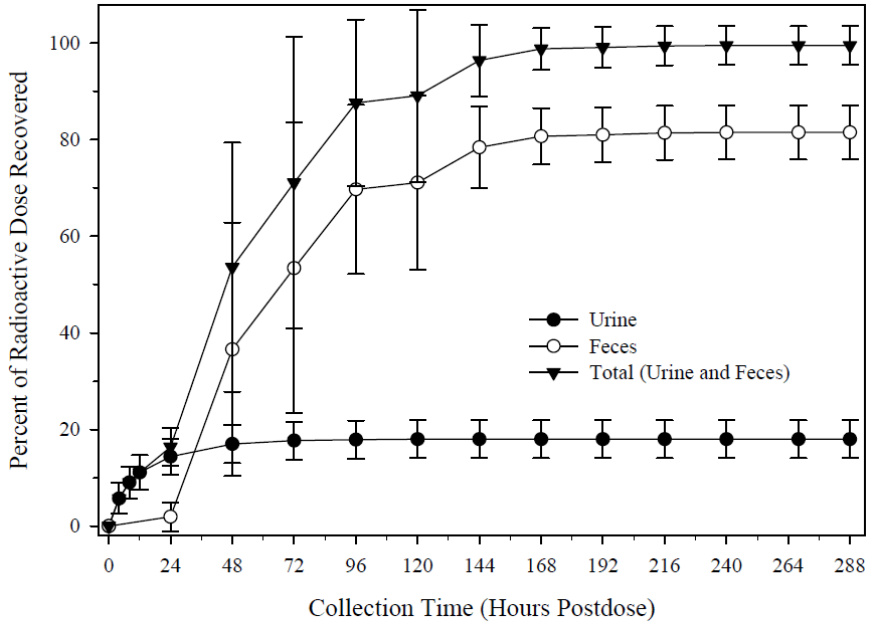
Figure 2.2.5.7 Mean Cumulative Percent of Radioactive Dose Recovered in Urine and Feces at Specified Intervals After a Single 204-mg (100-µCi) Oral Dose of [^{14}C]-Tedizolid Phosphate to Healthy Male Subjects



Note: Adapted from the Sponsor's report for Study TR701-106, Figure 11-2.

2.2.5.8 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

Tedizolid exhibits linear pharmacokinetics with approximately dose-proportional increase in exposure at tedizolid phosphate doses up to 400 mg (IV) and 1200 mg (oral). The PK parameters of tedizolid appear independent of dose.

2.2.5.9 How do the PK parameters change with time following chronic dosing?

Pharmacokinetic parameters (i.e., $t_{1/2}$, CL/F [CL], V_z/F [V_{ss}]) of tedizolid did not differ significantly between Day 1 and Day 15 or 7 following multiple oral and IV doses of tedizolid phosphate 200 mg once daily in healthy adults (**Table 2.2.5.1-2** and **Table 2.2.5.1-4**). Steady-state concentrations achieved within approximately 3 days with drug accumulation of approximately 30% (tedizolid half-life is ~12 hours).

2.2.5.10 What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

Inter-subject variability, expressed as percent coefficient of variation (%CV), was modest for tedizolid clearance (CL, 31.0% CV) and volume of distribution of the central compartment (V_c, 13.4% CV), based on the population PK analysis. The variability of CL and V_c is similar among healthy volunteers and patients (CL: 28.2% CV and 30.0% CV, respectively; V_c: 22.2 CV% vs. 16.4% CV, respectively). Ideal body weight and total bilirubin are two covariates that contributed significantly to the variability of CL; ideal body weight is the covariate that contributed significantly to the variability of V_c. Tedizolid CL increases with ideal body weight

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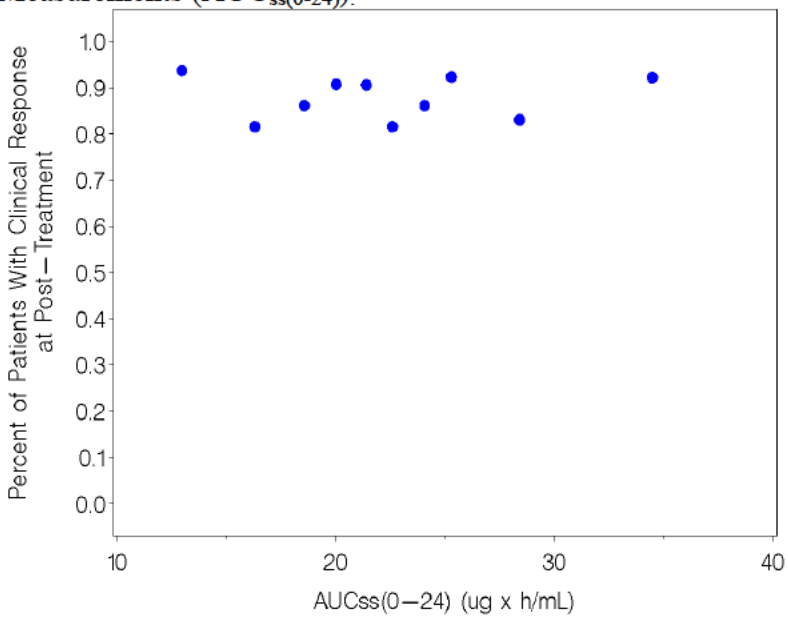
according to a power function, but decreases in proportion to total bilirubin. Tedizolid V_c increases with increasing ideal body weight.

PK/PD analyses of Phase 3 data showed a similar percentage of patients with a clinical response at PTE across the range of tedizolid therapeutic exposures (as measured by $AUC_{ss(0-24)}$ of 22.5 with a range of 6.57 to 49.86 µg*hr/ml) (**Figure 2.2.5.10**). Therefore, the magnitude of variability in tedizolid PK parameters (i.e., CL or V_c) is not likely to be clinically relevant.

Figure 2.2.5.10 Percent of Patients With Clinical Response at Post-Therapy Evaluation Versus Exposure Measurements ($AUC_{ss(0-24)}$).



Note: Adapted from the Sponsor's report CLN-13-0701-102, Figure 8
Circles represent the median exposure of 10th percentile and associated observed probabilities.

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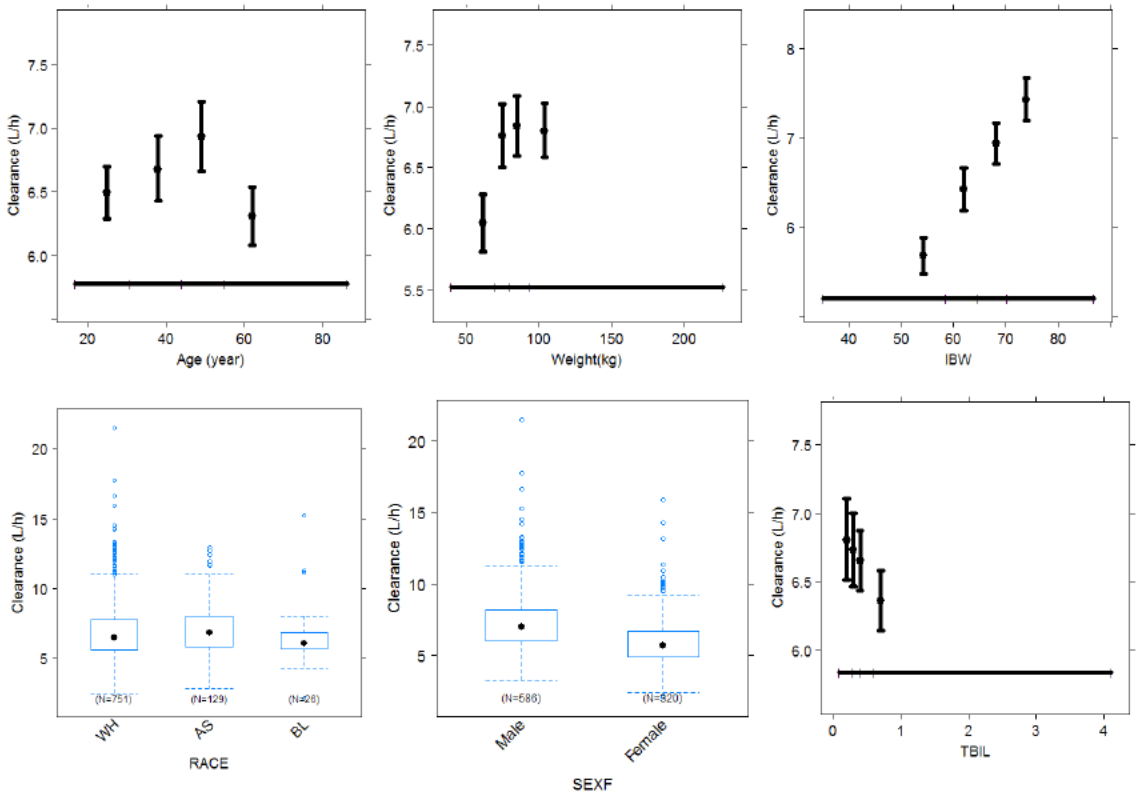
2.3 Intrinsic Factors

2.3.1 What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

Clinical studies demonstrated that no dose adjustment was necessary in the following specific populations: adolescent or elderly patients, males or females, patients with severe renal impairment, patients on hemodialysis, and patients with moderate to severe hepatic impairment.

Effects of the following intrinsic factors on the pharmacokinetics of tedizolid were evaluated in the population PK analysis: age, body weight, BMI, ideal body weight, race, ALT, AST, total bilirubin, and creatinine clearance (CLcr). Among the covariates evaluated, only ideal body weight and total bilirubin were identified to be significant predictors of tedizolid clearance and exposure (**Figure 2.3.1-1**). Tedizolid clearance increases with increasing ideal body weight but decreases with increasing total bilirubin. However, dose adjustment based on values of ideal body weight and total bilirubin is not recommended, due to flat exposure-response relationships for efficacy and safety. Other intrinsic factors have no significant effect on tedizolid clearance.

Figure 2.3.1-1 Tedizolid PK parameter-covariates relationships. The box plots and black circles are based on empirical Bayesian individual estimates.



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2.3.2 Based upon what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations, what dosage regimen adjustments, if any, are recommended for each of these groups? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative bases for the recommendation.

2.3.2.1 Elderly

Clinical Study: Pharmacokinetics of tedizolid were evaluated in healthy elderly subjects (age 65 years and older, with at least 5 subjects at least 75 years old) and younger control subjects (age 18 to 45 years), following a single 200 mg oral dose of tedizolid phosphate (TR701-109). There were no clinically meaningful differences in tedizolid PK parameters between the elderly group and the younger control group (**Table 2.3.2.1-1**). No dose adjustment is necessary in elderly subjects.

Table 2.3.2.1-1 Tedizolid PK Parameters in Elderly Subjects and Younger Control Subjects Following a Single 200 mg Oral Dose of Tedizolid Phosphate

Group	Statistic	C_{max} (µg/mL)	t_{max}[a] (hr)	AUC_{0-t} (µg•hr/mL)	$AUC_{0-\infty}$ (µg•hr/mL)	$t_{½}$ (hr)
Elderly	N	14	14	14	14	14
	Mean	2.62	3.00	34.16	34.68	12.3
	SD	0.73	1.50-8.00	10.35	10.59	1.25
	CV	0.278	0.593	0.303	0.305	0.101
Younger Control	N	14	14	14	14	14
	Mean	2.36	3.00	29.53	29.90	11.8
	SD	0.48	1.5-8.00	5.70	5.87	1.01
	CV	0.206	0.538	0.193	0.196	0.086

Note: Adapted from Module 2.7.2. Summary of Clinical Pharmacology Studies, Table 21.
[a]*Median (minimum, maximum) presented for t_{max}.*

Population PK Analysis:
Based on results of the population PK analysis, there was no significant different in tedizolid AUC in patients < 65 years old (n=812, 31.3 ± 9.0 µg*h/mL) and those >= 65 years old (n=94, 33.7 ± 9.0 µg*h/mL) (**Figure 2.3.2.1-1**). Thus, the population PK analysis supports that no dose adjustment for the elderly is recommended.

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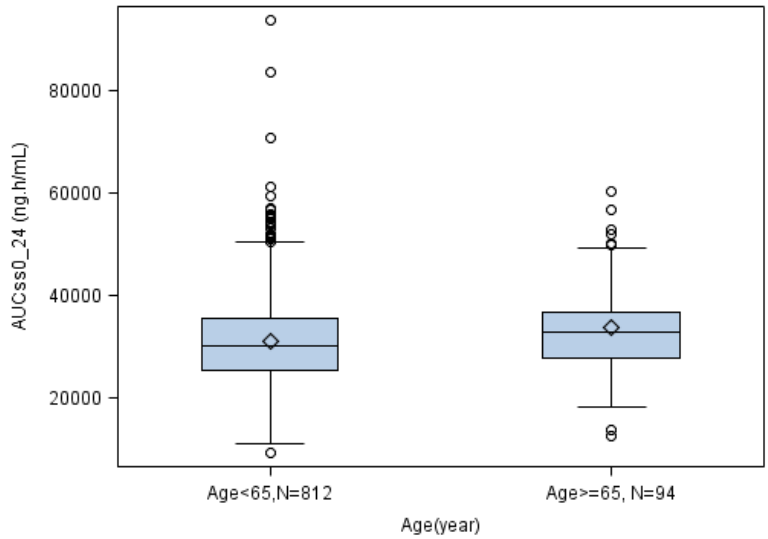
Figure 2.3.2.1-1 Tedizolid $AUC_{ss(0_24)}$ box plots by elderly age in the population PK dataset (n=906)



2.3.2.2 Pediatric patients

Clinical Study: Pharmacokinetics and safety of tedizolid were evaluated in adolescent (12-17 years of age) subjects (n=20) following a single oral dose (Part A) and a single IV infusion (Part B) of 200 mg tedizolid phosphate (TR701-111; parallel design).

Most PK parameters (i.e., AUC, T_{max}, CL) for tedizolid were similar following a single oral or IV infusion administration of 200 mg tedizolid phosphate between adolescent and adult subjects (**Table 2.3.2.2-1**, **Table 2.2.5.1-1** and **Table 2.2.5.1-3**). The C_{max} was 47% higher in adolescent subjects compared to adult subjects (3.85±1.5 vs. 2.62±0.58 µg/ml) following a single IV dose of 200 mg tedizolid phosphate (**Table 2.3.2.2-1** and **Table 2.2.5.1-3**). The absolute oral bioavailability was approximately 89% in adolescent subjects; similar to the absolute bioavailability of 91% observed in adults subjects. Less than 1% of administered dose was excreted as tedizolid in the urine.

Results from this trial showed that tedizolid phosphate, administered as an oral tablet or an IV infusion, was generally well-tolerated in adolescent patients (n=20). Treatment emergent adverse events (TEAEs) were mild, no deaths and no serious adverse events were reported, and no patient discontinued treatment due to a TEAE. Clinical laboratory evaluations, vital sign measurements, physical examinations, and 12-lead ECGs did not show clinically significant changes.

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Table 2.3.2.2-1 Mean (SD) Plasma PK Parameters for Tedizolid after single Oral and IV Administration of 200 mg Tedizolid Phosphate

PK Parameter	Oral (Part A) N	Oral (Part A) Mean (SD)	IV (Part B) N	IV (Part B) Mean (SD)
C_{max} (µg/mL)	10	2.23 (0.55)	10	3.85 (1.51)
t_{max}[a] (hr)	10	4.00 (1.00, 6.00)	10	1.02 (0.50, 2.02)
AUC_{0-24} (µg•hr/mL)	10	21.02 (6.49)	10	25.47 (6.37)
AUC_{0-t} (µg•hr/mL)	10	21.00 (6.50)	10	25.48 (6.38)
$AUC_{0-\infty}$ (µg•hr/mL)	10	25.20 (9.15)	10	27.82 (7.28)
$AUC_{ext\%}$ (%)	10	15.3 (7.14)	10	8.11 (2.12)
CL/F[b] (mL/hr)	10	7186 (2115)	10	6313 (1808)
V_{ss}/F[c] (mL)	10	90822 (23103)	10	54222 (10189)
V_z/F[d] (mL)	10	83498 (28194)	10	59278 (12208)
$t_{½}$ (hr)	10	8.26 (1.99)	10	6.64 (0.689)

Note: Adapted from Module 2.7.2. Summary of Clinical Pharmacology Studies, Table 23.
[a]*Median (minimum, maximum) presented for t_{max}.*
[b]*The parameter for IV is CL.*
[c]*The parameter for IV is V_{ss}.*
[d]*The parameter for IV is V_z.*

In this trial, tedizolid exposure (measured as AUC) in adolescents was comparable to that in adults following both oral and IV administration of 200 mg tedizolid phosphate. Based on this observation, it appears the fixed adult dose of 200 mg QD would be appropriate for adolescent patients in terms of efficacy. However, the safety of 200 mg tedizolid phosphate could not be demonstrated due to the limited enrollment of adolescent patients in this adolescent PK study (n=20) and the pivotal Phase 3 trials (n=1, age=17 years).

The Sponsor has requested deferral of pediatric PK and tolerability studies in the children, toddlers and infant age groups (<12 years) as well as safety and efficacy studies for all pediatric subpopulations, including adolescents (12-17 years). The Sponsor's proposal is currently pending review and agreement by the Agency.

Population PK Analysis: Because of limited enrollment of pediatric patients in the Phase 3 trial, population PK analysis on this covariate was not conducted.

2.3.2.3 Gender

Population PK Analysis:
In population PK analyses, no significant difference in tedizolid $AUC_{ss(0-24)}$ was observed between male and female patients in the PK population (n=586, 29.2 ± 7.7 µg*h/mL versus n=320, 35.7 ± 9.8 µg*h/mL). Female subjects displayed a 22% percent higher $AUC_{ss(0-24)}$ compared with male subjects (**Figure 2.3.2.3-1**). The difference is not expected to be clinically relevant. No dose adjustment is necessary based on gender.

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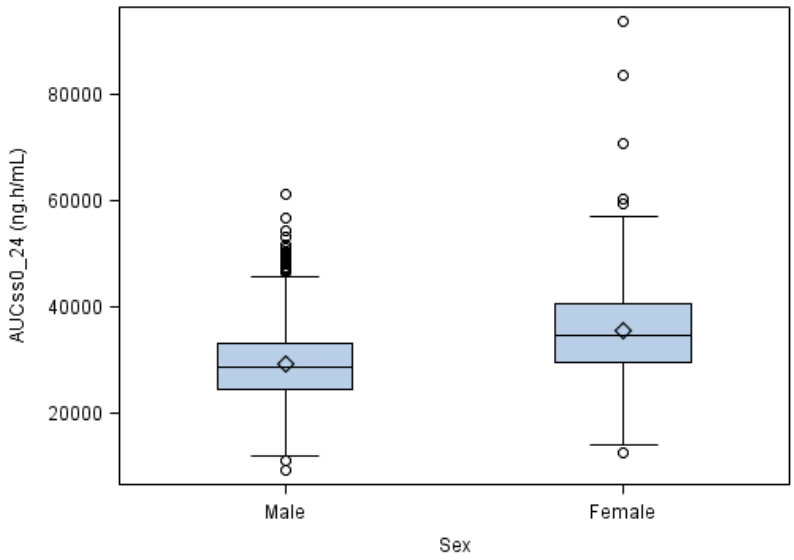
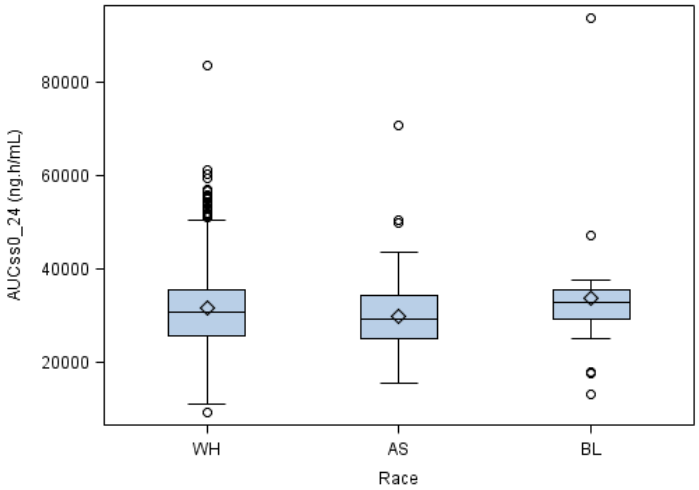
Figure 2.3.2.3-1 Tedizolid $AUC_{ss(0_24)}$ box plots by Gender in the population PK dataset (n=906)



2.3.2.4 Race

Population PK Analysis:
The effect of race on tedizolid exposure was assessed in population PK analyses. Tedizolid AUCss $_{(0\text{-}24)}$ were comparable between race groups for White (n=751, 31.7 ± 9.0 µg*h/mL), Asian (n=129, 29.9 ± 7.8 µg*h/mL), and patients classified as Black (n=26, 33.7 ± 14.1 µg*h/mL) (**Figure 2.3.2.4-1)**. No dose adjustment based on race is necessary.

Figure 2.3.2.4-1 Tedizolid AUCss $_{(0\text{-}24)}$ box plots by race in population PK population (n=906)



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2.3.2.5 Renal impairment

Clinical Study: Pharmacokinetics of tedizolid were evaluated in subjects with severe renal impairment not receiving hemodialysis (eGFR <30.0 mL/min/1.73 m^2; Nondialyzed Group) and subjects with end-stage renal disease (ESRD) receiving hemodialysis (eGFR <15.0 mL/min/1.73 m^2; Dialyzed Group) versus subjects with normal renal function (eGFR ≥80.0 mL/min/1.73 m^2), following a single IV dose of 200 mg tedizolid phosphate (TR701-123). Similar tedizolid PK results were demonstrated in dialyzed subjects pre- or postdialysis. Approximately 8% lower tedizolid AUC and nearly identical C_{max} values were observed in nondialyzed subjects with severe renal impairment relative to the matched control group. The disposition of tedizolid did not appear to be altered in patients with advanced renal impairment. Thus, no dose adjustment is necessary in subjects with renal impairment and subjects on hemodialysis.

Population PK Analysis:
Population PK analysis did not reveal significant relationship between tedizolid clearance and renal function (measured as creatinine clearance with a mean value of 121.72 ml/min and a range of 8.1 to 160.0 ml/min).

2.3.2.6 Hepatic impairment

Clinical Study: Pharmacokinetics of tedizolid were evaluated in subjects with moderate hepatic impairment (Child-Pugh classification B; score of 7–9) and subjects with severe hepatic impairment (Child-Pugh classification C; score of 10–15) versus subjects with normal hepatic function, following a single oral dose of 200 mg tedizolid phosphate under fasted condition (TR701-124). Generally, tedizolid PK were similar across all groups in this study. The largest differences in tedizolid PK parameters between subjects with hepatic impairment and matched controls were seen in AUC, which was approximately 34% higher in subjects with severe hepatic impairment compared with matched controls, and 22% higher in subjects with moderate hepatic impairment compared with matched controls. The C_{max} was 1% lower in subjects with severe hepatic impairment compared with matched controls and 9% higher in subjects with moderate hepatic impairment compared with matched controls (**Table 2.3.2.6**). There were no marked changes in unbound PK parameters relative to bound PK parameters. In all groups, mean excretion of tedizolid in urine over the 48 hour sampling period was less than 1%.

Despite the 22 and 34% mean increase in AUC in patients with moderate and severe hepatic impairment compared to the respective controls, this AUC is within the range of exposure observed at doses that demonstrated safety in Phase 1 studies (tedizolid AUC values range from 17.36 to 123.10 µg*hr/ml at 100-1200 mg of single prodrug dose [**Tables 2.2.5.1-1, 2, 3, and 4**]) and Phase 3 studies (mean tedizolid $AUC_{ss(0-24)}$ of 22 with a range of 6.57 to 49.86 µg*hr/ml at 200 mg QD prodrug dose). In addition, no safety issues were identified in this hepatic impairment trial. No dosage adjustment appears warranted for subjects with hepatic impairment.

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Table. 2.3.2.6 Mean (SD) Plasma PK Parameter Data for Tedizolid in Subjects with Moderate and Severe Hepatic Impairment versus Matched Control Subjects after single Oral Administration of 200 mg Tedizolid Phosphate

Group	Statistic	C_{max} (µg/mL)	t_{max} (hr)	AUC_{0-t} (µg*hr/mL)	$AUC_{0-\infty}$ (µg*hr/mL)	$t_{½}$ (hr)
Moderate Hepatic Impairment	n	8	8	8	8	8
	Mean	2.08	1.63	29.89	30.47	14.94
	Geometric Mean	1.97	NC	26.78	27.17	14.58
	SD	0.742	0.79	16.76	17.50	3.49
	Median	1.90	1.75	22.50	22.65	14.81
	Range	1.36–3.37	0.50 –3.00	16.49–65.93	16.62–68.10	10.3 –19.3
Moderate Match	n	8	8	8	8	8
	Mean	1.85	2.06	22.80	23.00	13.42
	Geometric Mean	1.80	NC	22.14	22.34	12.92
	SD	0.49	1.02	5.63	5.70	3.93
	Median	1.78	2.00	23.20	23.38	13.43
	Range	1.38–2.96	1.00–4.00	14.26–28.66	14.35–28.85	8.61–20.02
Severe Hepatic Impairment	n	8	8	8	8	8
	Mean	2.20	1.75	34.80	35.23	14.19
	Geometric Mean	2.00	NC	31.01	31.36	13.90
	SD	1.07	0.76	20.72	21.13	2.92
	Median	1.96	2.00	32.60	33.04	15.17
	Range	1.09–4.44	0.50–3.00	18.40–82.95	18.50–84.38	9.19–17.79
Severe Match	n	8	8	8	8	8
	Mean	2.12	3.25	24.37	24.56	13.68
	Geometric Mean	2.02	NC	23.19	23.38	13.20
	SD	0.80	2.19	8.03	8.04	3.71
	Median	1.96	3.00	23.97	24.17	13.36
	Range	1.32–4.01	1.00–8.00	13.06–38.88	13.16–39.01	7.04–20.59

Note: Adapted from Module 2.7.2. Summary of Clinical Pharmacology Studies, Table 28.

Population PK Analysis:

Effect of liver function [including ALT, AST, total bilirubin, indirect bilirubin]) on tedizolid PK was assessed in the Population PK analysis. ALT (mean: 27.14; range: 4.0, 284.0 U/L), AST (mean: 26.35; range: 6.0, 172.0 U/L), and indirect bilirubin (mean: 0.34; range: 0.0-2.2 mg/dL) were not found to have statistically significant influences on k_a, CL, or V_c. Tedizolid clearance was found to decrease in proportion to total bilirubin (mean: 0.51; range: 0.1-4.1 mg/dL). The change in total bilirubin from median to 5th percentile and to 95th percentile resulted in 3.7% decrease and 8.3% increase in tedizolid AUC_{ss}, respectively. The change in total bilirubin from 5th percentile to 95th percentile resulted in 12.4% increase in tedizolid AUC_{ss}. This magnitude of

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change is not considered to be clinically relevant. Thus, the population PK analysis supports the conclusion that no dose adjustment for hepatic impairment is recommended.

2.3.2.7 What pregnancy and lactation use information is there in the application?

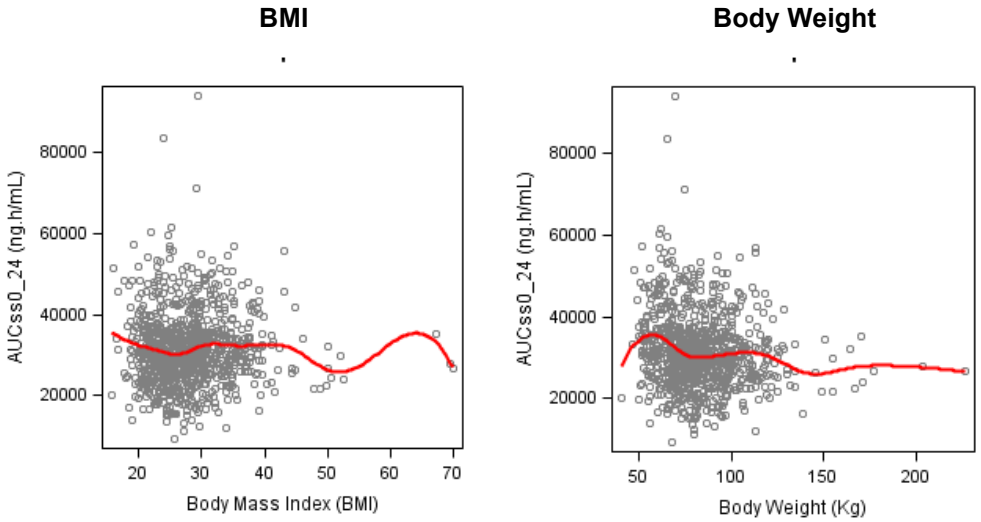
No studies with tedizolid phosphate have been performed in pregnant or lactating females. Information on pregnancy is limited to non-clinical data and is proposed by the Sponsor for Pregnancy Category C. The Sponsor recommends tedizolid phosphate should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Refer to the Pharmacology/Toxicology review for complete analysis of tedizolid toxicology.

2.3.2.8 Obesity

The effect of weight on tedizolid exposure ($AUCss_{(0-24)}$) was assessed in the population PK analysis. As shown in **Figure 2.3.2.8-1** below, no discernable trend was observed between body weight or BMI and tedizolid exposure.

Figure 2.3.2.8-1 Tedizolid $AUCss_{(0-24)}$ scatter plots by body weight and BMI in the pooled population PK population (n=219)



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2.4 Extrinsic Factors

2.4.1 Drug-drug interactions

2.4.1.1 Is there an in vitro basis to suspect *in vivo* drug-drug interactions?

In vitro metabolism, inhibition, and induction experiments suggest the potential for in vivo drug-drug interactions with tedizolid phosphate and tedizolid is low.

2.4.1.2 Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?

In vitro oxidative metabolism studies with human liver microsomes indicate tedizolid phosphate and tedizolid are not significant substrates of CYP enzymes. Little or no biotransformation of tedizolid phosphate and tedizolid was observed in human liver microsomes, with 88.7-116% of the compound remaining after up to 120-min incubation in pooled human liver microsomes.

Genetic information regarding the metabolism of tedizolid was not provided by the Sponsor.

2.4.1.3 Is the drug an inhibitor and/or inducer of CYP enzymes?

In vitro inhibition and induction studies indicate tedizolid is not an inhibitor or inducer of major CYP enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4).

2.4.1.4 Is the drug a substrate and/or inhibitor of membrane transporters?

In vitro studies indicate neither tedizolid nor tedizolid phosphate is a substrate or inhibiter of major membrane transporters (OAT1, OAT3, OATP1B1, OATP1B3, OCT1, OCT2, and P-gp) except for BCRP. Tedizolid inhibited BCRP with an IC_{50} of 51.1 μM; however, this is unlikely to be of clinical significance as $C_{max,ss}$ of tedizolid ranged from 6-8 μM after oral and IV administration of 200 mg tedizolid phosphate once daily and tedizolid phosphate is not intended for chronic administration (i.e., treatment duration not to exceed 6 days).

2.4.1.5 Are there other metabolic/transporter pathways that may be important?

No other in vitro metabolic/transporter experiments have been conducted.

2.4.1.6 Does the label specify co-administration of another drug, and if so, has the interaction potential between these drugs been evaluated?

Co-administration of another drug is not specified in the label.

2.4.1.7 What other co-medications are likely to be administered to the target patient population?

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Target patient populations of ABSSSI range from otherwise healthy patients to patients with significant co-morbidities. Tedizolid phosphate may be used with a wide variety of co-medications from different drug classes for many different therapeutic indications.

2.4.1.8 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

In vitro studies show that tedizolid is a reversible inhibitor of monoamine oxidase (MAO_A and MAO_B). Potent MAO_A inhibitors can produce increased blood pressure (pressor response) or a serotonin syndrome when coadministered with sympathomimetic or serotonergic agents, respectively. Enhancement of pressor responses to tyramine and pseudoephedrine was observed clinically with the previously approved oxazolidinone antibiotic, linezolid (a reversible, nonselective MAO inhibitor).

The relationship between free drug concentrations in plasma and IC_{50} for MAO inhibition is illustrated for tedizolid and linezolid in **Table 2.4.1.8-1**. The maximum tedizolid free concentration associated with 200 mg dose regimen is approximately 20-30% of the IC_{50} for MAO inhibition; whereas the maximum linezolid free concentration associated with the recommended dosage of 600 mg twice daily is approximately equal to the IC_{50} for MAO_A inhibition.

Table 2.4.1.8-1 Free Drug Concentrations in Plasma and IC_{50} for MAO Inhibition

Parameter		Tedizolid	Linezolid
C_{max}, µM		8.1[a]	62.8[b]
Protein binding		80%	31%
Calculated Free drug C_{max}, µM		1.62	43.4
MAO_A	IC_{50}, µM	8.7	46.0
	$C_{max\text{-}free}/IC_{50}$	0.186	0.94
MAO_B	IC_{50}, µM	5.7	2.1
	$C_{max\text{-}free}/IC_{50}$	0.284	21.0

Note: Adapted from Module 2.5 Clinical Overview, Table 3; C_{max}: the highest tedizolid C_{max} following 200 mg tedizolid phosphate once daily; highest linezolid C_{max} following 600 mg twice daily from Zyvox® PI.
[a]*Adjusted for tedizolid MW 370.34.*
[b]*Adjusted for linezolid MW 337.35.*

Nonclinical studies (rat tyramine challenge and 5-hydroxytryptophan mouse head twitch [serotonergic model]) showed no MAO interactions at multiples of up to ~30-fold above the human therapeutic peak tedizolid exposure.

Two placebo-controlled crossover studies were conducted to assess the potential of 200 mg/day tedizolid phosphate (at steady state) to enhance pressor responses to oral tyramine (TR701-105) or pseudoephedrine (TR701-114). In TR701-105, sensitivity to tyramine was determined by comparing the dose of tyramine (TYR_{30}) required to elicit a ≥30 mmHg increase in systolic blood pressure (SBP) when administered with placebo vs. tedizolid phosphate. The geometric mean tyramine sensitivity factor (TSF; placebo TYR_{30}/tedizolid phosphate TYR_{30}) was 1.3,

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which was below the predetermined TSF ≥2 indicative of a clinically relevant positive pressor response. In TR701-114, no significant difference in mean maximum increase in SBP was observed when pseudoephedrine was coadministered with tedizolid phosphate (11±5 mmHg) vs. placebo (12±6 mmHg).

Taken together, tedizolid does not appear to interact in vivo with adrenergic and serotonergic agents or dietary tyramine at therapeutic exposures.

2.4.1.9 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

There are no significant unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding for tedizolid phosphate or tedizolid.

2.4.2 What issues related to dose, dosing regimens, or administration are unresolved and represent significant omissions?

There are no unresolved issues related to dose, dosing regimens, or administration that represent significant omissions to this application.

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2.5 General Biopharmaceutics (Oral Tablet Formulation; NDA 205,435)

2.5.1 Based on the biopharmaceutic classification system principles, in what class is this drug and formulation? What solubility, permeability and dissolution data support this classification?

Tedizolid phosphate is a weak acid (pK_{a1} = 1.8, pK_{a2} = 6.5) and exhibits a pH-solubility profile (**Table 2.5.1-1**). (b) (4) [redacted] Dissolution volume testing was conducted (b) (4) [redacted] Therefore, a 0.05 M phosphate buffer solution at pH 6.8 was selected as the medium for dissolution testing of 200 mg tedizolid phosphate tablets. (b) (4) [redacted] Therefore, tedizolid phosphate is technically a Biopharmaceutical Classification System (BCS) Class IV compound, with poor solubility below pH 4 and poor permeability of the phosphate prodrug moiety.

Table 2.5.1-1. Kinetic Solubility of Tedizolid Phosphate in Aqueous Solutions

pH	Kinetic Solubility (mg/mL)	USP Solubility Rating
1.4	<0.00559	Practically insoluble
Water (pH 3.6)	0.235	Very slightly soluble
4.3	9.70	Slightly soluble
5.3	149	Freely soluble
6.1	144	Freely soluble
6.4	136	Freely soluble
6.5	141	Freely soluble
6.8	131	Freely soluble
7.6	130	Freely soluble

Note: Adapted from Module 3.2.P.2 Pharmaceutical Development, Table 2.

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Figure 2.5.1-1. Dissolution Profiles for 200 mg Tedizolid Phosphate Tablets Representative of Clinical and Registration Batches at Release (0.05 M phosphate buffer, pH 6.8, Paddles, 60 rpm, n=6 Tablets)

(b) (4)

Note: Adapted from Module 2.7.1 Summary of Biopharmaceutics and Associated Analytical Methods (Human), Figure 1.

(b) (4)

(b) (4)

Please refer to the ONDQA-Biopharmaceutics review for further information on solubility, permeability and dissolution characteristics of tedizolid.

2.5.2 How is the proposed to-be-marketed formulation of the drug linked to the clinically used formulation?

The proposed to-be-marketed formulation of tedizolid phosphate was used in the pivotal Phase 3 trials. Prior to initiation of the tedizolid phosphate Phase 3 program, most in vivo nonclinical and initial Phase 1 and 2 clinical studies (TR701-103 [food-effect], -108 [relative BA], and -104 [dose-ranging Phase 2]) were conducted using an earlier solid form of the prodrug designated as tedizolid phosphate disodium salt (TR-701). A free acid prodrug, TR-701 FA, was later formulated as an immediate release tablet for administration (b) (4)

Similar absorption of tedizolid (TR-700) from the 2 drug substances (TR-701 and TR-701 FA) was predicted based on the physicochemical properties of the prodrug/active moiety system.

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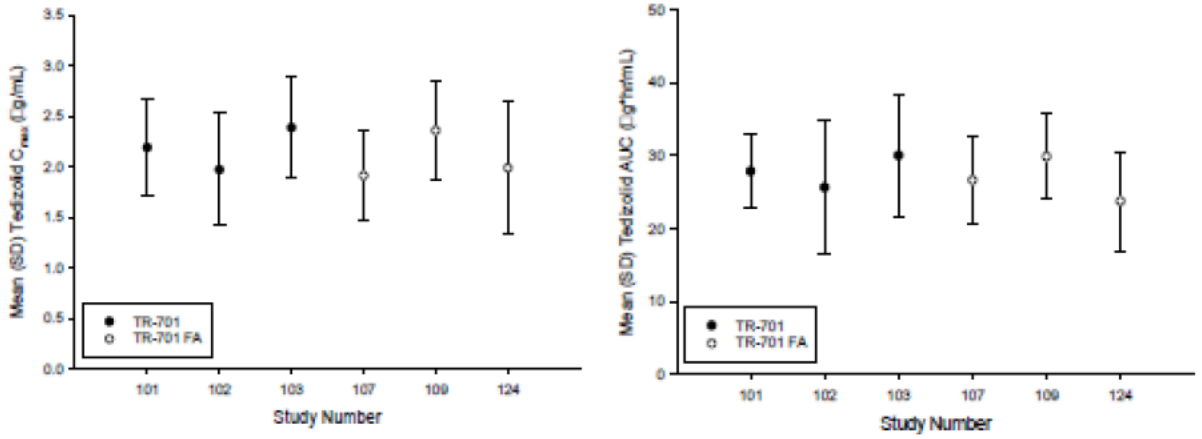
(b) (4)

A Phase 1 study (TR701-108) was conducted to assess the relative bioavailability between TR-701 and TR-701 FA in capsule formulations. Study results show that TR-700 exposure was similar following oral administration of the 2 prodrug substances; C_{max} and AUC ratios of TR700 for TR-701 FA administration relative to TR-701 administration were approximately 0.95 and 0.96, respectively, with 90% confidence intervals for both parameters within 80-120% bioequivalence limits.

No direct in vitro or in vivo comparison between the final TR-701 FA tablet drug product and TR-701 FA or TR-701 capsules was conducted. The nominal dose level of TR-701 FA 200 mg (164 mg TR-700 equivalents) was retained for simplicity for the Phase 3 dose, which resulted in a minor (<10%) increase in the dose of the active moiety compared to a 200 mg dose of TR-701 (150 mg TR-700 equivalents). Given this small change and demonstrated lack of PK differences, the TR-700 PK exposures from 200 mg of TR-701 FA were expected to be similar to those from 200 mg of TR-701, and well within the range of doses (200, 300, and 400 mg TR-701 in capsules) evaluated clinically in Phase 2.

A retrospective evaluation of PK results from studies completed in healthy volunteers with TR-701 capsules or TR-701 FA tablets show that TR-700 PK was similar for the two formulations (**Figure 2.5.2-1**).

Figure 2.5.1-1. Cross Study Comparison of Mean (SD) TR-700 C_{max} (left) and AUC (right) for Oral TR-701 capsules and TR-701 FA tablets.



Note: Adapted from Module 2.7.1 Summary of Biopharmaceutics and Associated Analytical Methods (Human), Figure 4. All TR-701 FA results are for 200 mg Tablets. Exposure parameters for TR-701 were normalized to a 200 mg dose of TR-701 FA from 200 mg TR-701 (TR701-101) or 600 mg TR-701 (TR701-102 and TR701-103). Tedizolid=TR-700; Tedizolid phosphate=TR-701; FA=free acid

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2.5.3 What is the effect of food on the bioavailability of the drug when administered as solution or as drug product?

A Phase 1 study was conducted to assess the effect of food on PK of tedizolid following single oral administration of 600 mg tedizolid phosphate disodium salt (200 mg TR-701 capsules) with standard high-fat, high-calorie meal. Results from this study show that food delayed the rate of absorption of (C_{max} decreased by 26%, T_{max} increased by 6 hours), but not extent (AUC unchanged) of exposure to tedizolid, relative to fasting state. (b) (4)

Of note, TR-701 capsules, instead of the to-be-marketed formulation (TR-701 FA tablets), and 600 mg dose, instead of the proposed clinical dose (200 mg) were used in this food effect study. Given 1) the physiochemical properties of this prodrug/active moiety pair (BCS I), 2) bioequivalence between TR701 and TR701 FA capsules, and 3) linear PK up to 1200 mg single dose, findings of this food effect study using TR-701 capsules (600 mg) appear to reflect what is expected with the to-be-marketed formulation, TR-701 FA tablets (200 mg).

Nonclinical PK/PD studies show that AUC/MIC is the relevant PK/PD parameter associated with the activity of tedizolid against *S. aureus*. The delayed the rate of absorption (C_{max} decreased by 26%, T_{max} increased by 6 hours) of tedizolid under the fed state is, therefore, not expected to be clinically relevant. Tedizolid exposure (as measured by AUC) was unchanged between fed and fasting states; thus, tedizolid phosphate tablets can be administered without regards to food. Of note, tedizolid phosphate was administered regardless of the timing of meals in the pivotal Phase 3 trials.

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2.6 Analytical Section

Overall, the validation and performance of the LC-MS/MS bioanalytical assays used for determination of the prodrug, tedizolid phosphate, and the active moiety, tedizolid, are deemed acceptable by the Clinical Pharmacology reviewer. An assessment of specific assay characteristics follows.

2.6.1 How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

Tedizolid phosphate and tedizolid in plasma, urine, feces and other matrices were quantified by validated LC-MS/MS assays. For the metabolic profiling in the mass balance study (TR701-106), a non-validated HPLC assay was used for quantification of metabolites in plasma, urine, and feces.

2.6.2 Which metabolites have been selected for analysis and why?

In addition to tedizolid phosphate (prodrug), tedizolid (active metabolite) was measured in plasma, urine, and dialysate fluid. Tedizolid is the microbiologically active moiety and accounts for approximately 95% of the total radiocarbon AUC in plasma. There are no other significant circulating metabolites in humans.

2.6.3 For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?

Total drug concentrations of tedizolid phosphate and tedizolid were measured in all clinical studies. Plasma protein binding of tedizolid was assessed separately with in vitro ultrafiltration experiments and was accounted for when assessing free (i.e., microbiologically active) exposures in renal and hepatic impairment studies (TR701-123 and TR701-124, respectively).

2.6.4 What bioanalytical methods are used to assess concentrations?

Multiple validated LC-MS/MS assays were used for quantification of tedizolid phosphate and tedizolid in various matrices. See **Table 2.6.4-1** (plasma)**, Table 2.6.4-3** (urine and feces)**,** and **Table 2.6.4-3** (other matrices) for details.

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Table 2.6.4-1 Summary of Analytical Methods for Quantification of Tedizolid Phosphate and Tedizolid in Plasma

Concentration in ng/mL	BAN-09-0701-007		BAN-09-0701-029	BAN-13-0701-101	BAN-13-0701-097	BAN-13-0701-098	
	TR-700	TR-701	TR-700	TR-700	TR-700	TR-700	TR-701
Linearity Range r^2	5-1000 ≥0.998		0.16-1000 ≥0.9994	0.16-1000 ≥0.99	1.6-1000 ≥0.99	5.00-1000 ≥0.9955	
LLOQ ULOQ	5 1000		0.16 1000	0.16 1000	1.6 1000	5.00 1000	
Inter- and intra-assay precision and accuracy based on	6 replicates in 3 assays		6 replicates in 4 assays	6 replicates	6 replicates	6 replicates in 2 assays	
LLOQ QC Low QC Medium QC High	5 15 150 750		0.256 (near LLOQ) 1.28 32 800	0.16 0.48 100 and 500 800	1.6 4.8 250 and 500 800	5 15.0 150 750	
Inter-assay Precision At LLOQ	≤2.2% 4.2%	≤5.3% 8.1%	≤5.3%	-		≤7.1% 8.3%	≤6.5% 6.9%
Inter-assay Accuracy At LLOQ	98.2-102.7% 98.2%	98.0-104.7% 98.0%	94.9-102% -	-		103.1-105.7% 110.4%	100.9-104.4% 111.0%
Intra-assay Precision At LLOQ	≤3.3% ≤4.5%	≤6.2% ≤10.2%	≤12.2% 16.0% in 1 batch at near LLOQ	≤8.79% 19.06%		≤5.5% 1.8% and 5.8%	≤5.0% 7.1% and 7.4%
Intra-assay Accuracy At LLOQ	95.0-103.3% 95.0-100.8%	91.2-109.3% 91.2-102.4%	90.4-107% -	89.25-103.60% 86.67%		98.0-111.3% 102.6% and 118.2%	97.3-109.0% 110.8% and 111.2%
Intra-day Precision At LLOQ					≤6.49% 6.49%		
Intra-day Accuracy At LLOQ					91.92-99.76% 91.92%		

Note: Precision quoted as RSD/%CV

Note: Adapted from Module 2.7.1 Summary of Biopharmaceutics and Associated Analytical Methods (Human), Table AP1- 1
The quantification of the prodrug TR-701 FA is labeled as TR-701 by convention.

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Table 2.6.4-2 Summary of Analytical Methods for Quantification of Tedizolid Phosphate and Tedizolid in Urine and Feces

	Concentration in ng/mL				Concentration in µg/g
	BAN-09-0701-008 Urine		BAN-13-0701-103 Urine		BAN-13-0701-099 Faeces
	TR-700	TR-701	TR-700	TR-701	TR-700
Linearity Range r^2	 5.00-1000 ≥0.998		 5.00-1000 ≥0.9992	 5.00-1000 ≥0.9985	 0.2-40
LLOQ ULOQ	5 1000		5 1000	5 1000	0.2 40
Inter- and intra-assay precision and accuracy based on	6 replicates in 3 batches		6 replicates in 2 validation runs		6 replicates
LLOQ QC Low QC Medium QC High ULOQ	5 15 75 150 -		5 15 150 750 1000		0.2 0.6 4 30 -
Inter-assay Precision At LLOQ	≤2.4% 2.7%	≤3.5% 4.1%	-	-	≤8.8% 13.6%
Inter-assay Accuracy At LLOQ	103.9-110.0% 103.4%	98.5-107.3% 101.2%	-	-	92.0-100.7% 96.0%
Intra-assay Precision At LLOQ	≤3.6% ≤3.6%	≤4.7% ≤4.7%	2.0-4.9% 6.2% and 6.0%	2.3-5.1% 5.0% and 6.7%	≤12.1% 13.2%
Intra-assay Accuracy At LLOQ	102.8-111.3% 102.8-104.8%	96.4-109.3% 98.6-104.4%	94.0-98.7% 95.2% and 92.6%	95.3-100.7% 98.4% and 86.8%	87.5-107.7% 84.5-107.5%

Note: Precision quoted as RSD/%CV

Note: Adapted from Module 2.7.1 Summary of Biopharmaceutics and Associated Analytical Methods (Human), Table AP1- 1
The quantification of the prodrug TR-701 FA is labeled as TR-701 by convention.

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Table 2.6.4-3 Summary of Analytical Methods for Quantification of Tedizolid Phosphate and Tedizolid in Other Matrices

Concentration in ng/mL	BAN-10-0701-014 Human Plasma Ultra Filtrate (PUF)	BAN-11-0701-018 BAL Supernatant	BAN-11-0701-019 BAL		BAN-13-0701-091 Human Dialysate		BAN-09-0701-021 Microdialysis
			BALF Cell Pellet	Surrogate Cell Pellet			
	TR-700	TR-700	TR-700		TR-700	TR-701	TR-700
Linearity Range r^2	 2.0-400 ≥0.9996	 10-1000 -	10-1000 -		5.0-1000 ≥0.9957		 50-3200 -
LLOQ ULOQ	2.0 400	10 1000	10 1000		5.00 1000		50 3200
Inter- and intra-assay precision and accuracy based on	6 replicates	6 replicates in 3 assays	6 replicates in 1 batch	6 replicates in 3 batches	6 replicates		6 replicates in 7 batches
LLOQ QC Low QC Medium QC High	2 6 60 300	10 30 150 750	10 30 150 750		5 15.0 150 750		50 100 400 and 1600 2400
Inter-assay Precision At LLOQ		≤3.3% 6.4%	-	≤1.9% 4.3%			≤8.59% 8.59%
Inter-assay Accuracy At LLOQ		95.3-96.3% 92.3%	-	103.3-104.9% 101.0%			94.63-104.28% 101.24%
Intra-assay Precision At LLOQ	≤3.8% 2.4%	≤4.6% ≤7.7%	≤3.0% 2.6%	≤2.8% ≤4.6%	≤3.0% 5.5%	≤1.7% 5.3%	≤7.34% ≤4.84%
Intra-assay Accuracy At LLOQ	97.3-100.7% 90.0%	94.0-98.4% 88.0-95.6%	104.8-107.7% 107.0%	102.7-105.5% 98.3-103.0%	99.3-102.0% 106.6%	102.0-104.0% 103.8%	85.11-110.82% 93.12-116.55%

Note: Precision quoted as RSD/%CV

Note: Adapted from Module 2.7.1 Summary of Biopharmaceutics and Associated Analytical Methods (Human), Table AP1- 1
The quantification of the prodrug TR-701 FA is labeled as TR-701 by convention.

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2.6.4.1 What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used?

See **Tables 2.6.4-1**, **2.6.4-2**, and **2.6.4-3**. When concentrations exceeded the standard curve range, samples were diluted, then assayed. Dilution integrity was verified within each clinical pharmacology study when samples dilutions were performed.

A linear least-squares regression ($y = mx + b$, where x is the concentration and y is the peak area ratio) was applied for all standard curves with a weighting factor of 1/concentration^2 ($1/x^2$).

2.6.4.2 What are the lower and upper limits of quantification (LLOQ/ULOQ)?

See **Tables 2.6.4-1**, **2.6.4-2**, and **2.6.4-3**. .

2.6.4.3 What are the accuracy, precision, and selectivity at these limits?

See **Tables 2.6.4-1**, **2.6.4-2**, and **2.6.4-3**.

2.6.4.4 What is the sample stability under the conditions used in the study (long-term, freeze-thaw, sample-handling, sample transport, autosampler)?

The sample stability in plasma, urine, and feces was determined to be at least 934, 378, and 93 days, respectively, at -60 to -80°C (feces samples stored at -80°C). Sample stability under short-term storage (bench top, room temperature) and the freeze-thaw conditions are summarized in **Table 2.6.4.4-1**.

Table 2.6.4.4-1 Room Temperature Stability and Freeze/Thaw Stability in Human Matrices

Matrix	Room Temperature Stability (hours)	Freeze/Thaw (number of cycles)
Plasma	44	5
Plasma	24	3
Urine	>24	3
Bronchoalveolar lavage supernatant	24	6
Bronchoalveolar lavage cell pellet	24	5
Dialysate	24	5
Microdialysis Samples	8	3
Feces	6	4

2.6.4.5 What is the QC sample plan?

See **Tables 2.6.4-1**, **2.6.4-2**, and **2.6.4-3**.

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3. DETAILED LABELING RECOMMENDATIONS

Detailed labeling recommendation will be provided later as an addendum.

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4. APPENDICES

4.1 Individual Clinical Pharmacology Study Report Reviews

Reference ID: 3476543

STUDY NO.: PDM-13-070-081

PROTEIN BINDING OF TR-701 AND TR-700 IN EDTA-TREATED MOUSE, RAT, DOG, AND HUMAN PLASMA

Date: 09/2007-10/2007
Laboratory Site: Dong-A Pharmaceuticals, Co. Ltd, Seoul, Korea

OBJECTIVE: To determine protein binding of TR-701 and TR-700 in mouse, rat, dog, and human EDTA-treated plasma at concentrations of 0.1-50 µg/ml.

METHODS

Study Procedures:
(1) A 5 µL aliquot of TR-701 or TR-700 stock solution (10, 100, 300, 1000, and 5000 µg/mL) was spiked into 0.5 mL of each EDTA-plasma to make final concentrations of 0.1, 1, 3, 10, and 50 µg/mL, respectively (in duplicate).
(2) After equilibrium for 30 min at 37 °C, a 50 µL aliquot of plasma (total before) was transferred to 1.5 mL Eppendorf tube containing a 100 µL aliquot of 1M HCI and was stored at -20 °C freezer until analysis.
(3) About a 0.4 mL aliquot of plasma was transferred to an ultrafiltration tube (Ultrafree-MC, 30000 MW-low binding regenerated cellulose).
(4) The tubes were centrifuged for 20 min (2000g, 37 °C), and each 50 µL aliquot of plasma (total after) and the filtrate (free) transferred to 1.5 mL Eppendorf tube containing a 100 µL aliquot of 1M HCI and was stored at -20°C freezer until analysis.

Analytical/Data Analysis: Quantification of TR-701 and TR-700 in plasma and filtrate was performed by LC-MS/MS analysis using linezolid as an internal standard.

RESULTS

TR-701 was stable in EDTA-treated plasma from all 4 species tested over the course of the experiment; formation of TR-700 was less than 1% of the spiked TR-701. TR-701 and TR-700 concentrations in plasma before and after centrifugation were not different. So, plasma protein binding was calculated using plasma concentration after centrifugation. The mean plasma protein binding of TR-701 and TR-700 in mouse, rat, dog, and human EDTA-treated plasma at the concentration ranges of 0.1-50 µg/mL was shown in the table below (N = 2, each).

Conc.	DA-7218				DA-7157			
(ug/mL)	Mouse	Rat	Dog	Human	Mouse	Rat	Dog	Human
0.1	75.6	95.8	78.0	80.0	97.1	NC	80.2	86.4
1	71.6	98.7	87.1	87.6	92.6	98.6	77.0	84.4
3	74.3	98.6	85.5	88.5	92.5	98.0	77.1	84.3
10	75.6	95.7	87.8	86.4	92.5	98.7	77.6	81.3
50	76.8	97.3	87.2	90.6	88.3	95.5	78.1	86.6
Mean	74.8	97.2	85.1	86.6	92.6	97.7	78.0	84.6
SD	1.99	1.43	4.07	4.01	3.10	1.50	1.31	2.15

Note: TR-701 and TR-700 were referred to as DA-7218 and DA-7157, respectively, in this study.

Reference ID: 3476543

SPONSOR'S CONCLUSIONS:
The protein binding of TR-701 and TR-700 in the EDTA-treated plasma of mouse, rat, dog, and human varied by species. Rank order for protein binding of TR-701 in EDTA-treated plasma was rat (97.2%) >human (86.6%) >dog (85.1%) >mouse (74.8%). For TR-700, rank order of protein binding was rat (97.7%) >mouse (92.6%) >human (84.6%) >dog (78.0%). The plasma protein binding of TR-701 and TR-700 was independent of concentration over the range tested (0.1 to 50 µg/mL).

REVIEWER ASSESSMENT: The Sponsor's conclusions appear appropriate based on study results.

Reference ID: 3476543

STUDY NO.: PDM-0701-07-028

IN VITRO METABOLIC STABILITY OF TR-701 AND TR-700

Laboratory Site: (b) (4) Dong-A Phannaceutical, Ltd. (Dong-A), Korea.

OBJECTIVE: The purpose of these studies was to evaluate the in vitro metabolic stability of the prodrug TR-701 (also referred to as DA-7218) and the microbiologically active moiety TR-700 (also referred to as DA-7157) in rat, dog, monkey and human liver microsomes; in the presence of heparin- and EDTA-treated rat and human blood; and in the plasma of mice, rats, dogs and humans.

METHODS

Study Procedures:

Metabolic Stability of TR-701 and TR-700 in Rat, Dog, Monkey and Human Liver Microsomes

TR-701 or TR-700 was incubated at 10 µM for 0, 15, 30, 45, 60, 90 and 120 min at 37 °C in an incubation mixture (final volume of 400 µL) containing KH_2PO_4 (50 mM, pH 7.4), liver microsomes of rat, dog, monkey or human (1.0 mg/mL) and an NADPH-generating system. The incubation mixture was pre-incubated for 5 min prior to addition of the compound. To terminate the reaction, 50 µL of the mixture was placed in a clean tube and ethylacetate (1.0 mL), 1 M HCl (10 µL) and internal standard (DA-7867; 2 µg/mL in acetonitrile) were added.

In Vitro Metabolic Stability of TR-701 in Mouse, Rat, Dog and Human Plasma

Blood was collected from male mice (abdominal aorta), Sprague-Dawley rats (abdominal aorta), beagle dogs (cephalic vein) and healthy, drug-free volunteers (arm vein) on the day of the experiment into heparinized tubes. Plasma was separated by centrifugation and pooled before use. Plasma was divided into aliquots of 200 µL (n = 3 for each species). TR-701 was added at 2 µg/mL and the samples were incubated at 37 °C for 0, 5, 10, 15, 20 and 30 min and 1, 2 and 4 h. Reactions were terminated at appropriate times by the addition of 200 µL of 1 M HCl and samples were kept on ice until analysis. Linezolid (50 µL of 1 µg/mL in 50% acetonitrile) was added in the mixture as an internal standard for analysis.

Metabolic Stability of TR-701 in Heparin- and EDTA-treated Rat, Dog and Human Blood

In the first study, TR-701 at 50, 500 or 5000 ng/mL was added to fresh heparinized or EDTA-treated blood from male SD rats and humans (0.5 mL) in duplicate. Samples were incubated on ice for 60 min. In the second study, TR-701 at 50, 500 or 5000 ng/mL was added to fresh heparinized or EDTA-treated blood from male SD rats, beagle dogs and humans (0.5 mL) in duplicate. Samples were incubated at room temperature for 60 min. Plasma was separated by centrifugation at 4 °C for 10 min. Plasma (200 µL) was transferred to a clean tube and TR-701 was stabilized with 200 µL of 1 M HCl. Linezolid (50 µL of 1 µg/mL in 50% acetonitrile) was added in the mixture as an internal standard for analysis.

Reference ID: 3476543

Analytical/Data Analysis: Quantification of TR-701 and TR-700 in liver microsomes, plasma and blood from tested species was performed by LC-MS/MS.

RESULTS

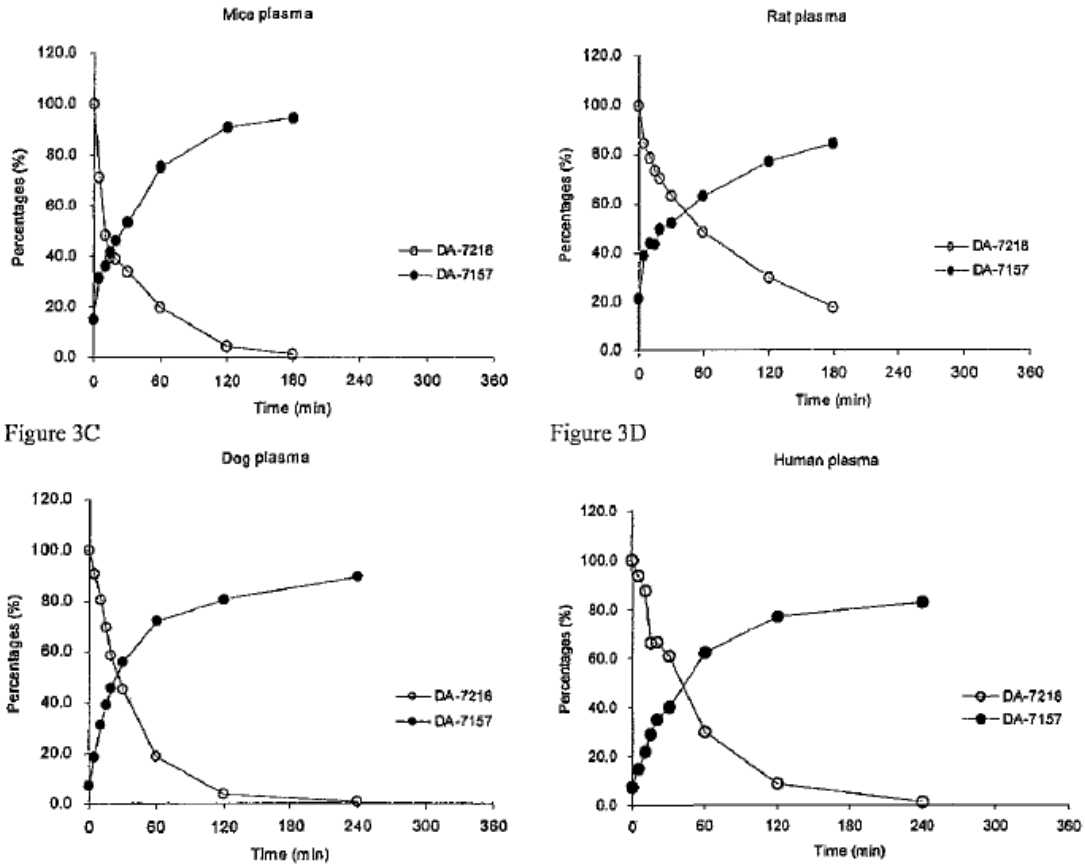
Metabolic Stability of TR-701 and TR-700 in Rat, Dog, Monkey and Human Liver Microsomes

TR-701 was stable in liver microsomes with 87.1, 92.7, 83.7, and 88.7% of TR-701 remaining after incubation for up to 120 min in rat, dog, monkey, and human liver microsomes, respectively. Formation of TR-700 ranged from 3.2-7.1% across species and was independent of NADPH. TR-700 was stable in liver microsomes, with 91.7, 105, 123, and 116% of TR-700 remaining after incubation in rat, dog, monkey, and human liver microsomes, respectively.

In Vitro Metabolic Stability of TR-701 in Mouse, Rat, Dog and Human Plasma

In mouse, rat, dog and human plasma at 37 °C, TR-701 at 2 µg/mL diminished rapidly, with half-lives of 28.8, 77.0, 28.3 and 36.1 min, respectively. After a 2-hour incubation, conversion to TR-700 was nearly complete in mice (4.1% of TR-70 1 remained), dog (3.4%) and human (8.5%), but not in rat (29.6%) (**Figure 1**).

Figure 1. Disappearance of TR-701 (DA-7218) and Formation of TR-700 (DA-7157) in Mouse, Rat, Dog and Human Plasma.



Reference ID: 3476543

Metabolic Stability of TR-701 in Heparin- and EDTA-treated Rat, Dog and Human Blood
EDTA prevented the conversion of TR-701 to TR-700 in rat, dog and human blood samples at 4 °C and at room temperature, but the inhibition of metabolism was greater at 4 °C (**Tables 1 and 2**). When human blood samples spiked with TR-701 were treated with heparin at room temperature, up to 46.7% of TR-700 was formed compared to 22.6% at 4 °C. In comparison, 2.48%, 2.14% and 4.43% of TR-700 was formed in rat, dog and human blood, respectively, after incubation with EDTA at room temperature, whereas only 0.58% and 0.54% of TR-700 was formed in rat and human blood, respectively, after incubation at 4 °C.

Table 1. Formation of TR-700 (mean±SD) in Heparin- or EDTA-treated Rat and Human Blood at 4°C

	Heparin-treated	EDTA-treated
Rat	3.26 ± 1.33	0.58 ± 0.08
Human	22.6 ± 4.3	0.54 ± 0.05

Table 2. Formation of TR-700 (mean±SD) in Heparin- or EDTA-treated Rat, Dog or Human Blood at 37°C

	Heparin-treated blood			EDTA-treated blood		
	TR-701 Concentration (ng/mL)			TR-701 Concentration (ng/mL)		
	50	500	5000	50	500	5000
Rat	-	8.18%	6.31%	-	2.48%	1.07%
Dog	19.5%	18.7%	17.6%	-	2.14%	1.42%
Human	46.7%	40.3%	26.5%	-	4.43%	2.33%

SPONSOR'S CONCLUSIONS: The prodrug TR-701 is stable in liver microsomes from rats, dogs, monkeys, and humans, but unstable in mouse, rat, dog and human blood/plasma. Using K_2-EDTA as an anticoagulant stabilizes the conversion of TR-701 to TR-700 in blood. The active compound, TR-700 was stable in liver microsomes from all tested species.

REVIEWER ASSESSMENT: The Sponsor's conclusions appear appropriate based on study results.

Reference ID: 3476543

STUDY NO.: PDM-07-0701-034

EVALUATING THE EFFECT OF TR-701 AND TR-700 IN VITRO ON CYTOCHROME P450 ENZYME ACTIVITY USING HUMAN LIVER MICROSOMES

Laboratory Site: (b) (4)

OBJECTIVE: The purpose of this study was to investigate the inhibitory potential of TR-701 (also referred to as DA-7218) and TR-700 (also referred to as DA-7157) on a panel of Cytochrome P450 (CYP) enzymes in human liver microsomes.

METHODS

Study Procedures: Stock solutions of TR-701 and TR-700 were prepared in distilled water and dimethyl sulfoxide, respectively. Both compounds were diluted to final assay concentrations of 0.05, 0.1, 1.0, 10, and 50 µM in incubation mixtures consisting of 0.25 mg/mL microsomal protein, 50 mM potassium phosphate pH 7.4, 10 mM MgCl, and a cocktail of substrates (**Table 1**) for cytochrome P450 enzymes CYP 1A2 (phenacetin), 2A6 (coumarin), 2C8 (paclitaxel), 2C9 (diclofenac), 2C19 (S-mephenytoin), 2D6 (bufuralol) and 3A4 (midazolam). Ketoconazole, sulfaphenzaole, and quinidine were tested in separate reactions as positive controls. After 5 minute incubation at 37 °C the reactions were initiated by the addition of 2 mM nicotinamide adenine dinucleotide phosphate (NADPH) and incubated an additional 20 minutes. Reactions were terminated by addition of acetonitrile containing 0.5 µM dextrophan (internal standard)

Table 1. Concentrations of probe substrates for respective CYP enzymes.

CYP450s	Substrate	Final concentration (uM)
CYP3A4	Midazolam	2.5
CYP2D6	Bufuralol	5
CYP2C9	Diclofenac	10
CYP1A2	Phenacetin	50
CYP2C19	S-Mephenytoin	40
CYP2A6	Coumarin	2.5
CYP2C8	Paclitaxel	10

Reviewer Comment: According to the literature and FDA draft Guidance for DDI studies, the selection of probe substrates and inhibitors, as well as corresponding concentrations for each test CYP enzyme appears appropriate.

Analytical Analysis: All analyses were performed using a validated LC-MS/MS method.

RESULTS AND CONCLUSIONS

Reference ID: 3476543

The prodrug TR-701 and its active metabolite TR-700 did not inhibited CYPs 1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 3A4 up to the concentration of 50 μM; whereas the positive control compounds inhibited the activity of selected CYP enzymes as expected (**Table 2**).

Table 2. Effects of selective CYP inhibitors, TR-701 (DA-7218) and TR-700 (DA-7157), on CYP enzymes.

	IC_{50} (uM)				
	Ketoconazole (CYP3A4)	Sulfaphenazole (CYP2C9)	Quinidine (CYP2D6)	DA-7218	DA-7157
CYP3A4	0.2	> 50	35	> 50	> 50
CYP2D6	25	> 50	3	> 50	> 50
CYP2C9	24	5	> 50	> 50	> 50
CYP1A2	30	> 50	50	> 50	> 50
CYP2C19	27	> 50	> 50	> 50	> 50
CYP2A6	> 50	> 50	50	> 50	> 50
CYP2C8	8	> 50	> 50	> 50	> 50

Reviewer Comment: Following administration of multiple doses of 200 mg TR-701 FA once daily in healthy human subjects, the mean C_{max} values of TR-700 were 2.2 and 3 μg/ml for oral and IV administration, respectively (equivalent to 5.9 and 8.1 μM, respectively). This C_{max} is 6-8.5-fold lower than the highest concentration of TR-700 tested (50 μM) in this study. Given that 1) IC_{50} is >50 μM; 2) protein binding of TR-700 in human plasma is high (70-90%); and 3) TR-701 is rapidly and extensively converted by phosphatases to its active metabolite TR-700 following oral or IV administration in humans, the inhibitory potential of TR-701 and TR700 on major CYP enzymes is expected to be very low in vivo in humans.

SPONSOR'S CONCLUSIONS: Neither TR-701 nor TR-700 inhibited activity of CYP enzymes tested at the concentration up to 50 μM.

REVIEWER ASSESSMENT: The Sponsor's conclusions appear appropriate based on study results.

Reference ID: 3476543

STUDY NO.: CPH-12-0701-050

DETERMINATION OF THE INHIBITORY POTENCY OF TR-700 AND TR-701 FA TOWARDS HUMAN CYP3A IN VITRO

Date: 01/2012-03/2012
Laboratory Site: Bayer HealthCare AG, Berlin, Germany

OBJECTIVE: The purpose of this study was to evaluate the potential of TR-700 and TR-701 FA as reversible and time-dependent inhibitors of CYP3A in human liver microsomes (HLM).

METHODS
Study Procedures: The metabolism of midazolam and testosterone to 1'-hydroxymidazolam and 6β-hydroxytestosterone, respectively, are primarily mediated by CYP3A (including CYP3A4 and 3A5); therefore, midazolam and testosterone were used as CYP3A probe substrates in this study. The inhibition potential of TR-700 and TR-701 FA towards CYP3A was evaluated with midazolam and testosterone in HLM by monitoring changes in the amount of 1'-hydroxymidazolam and 6β-hydroxytestosterone formed in the presence and absence of TR-700 or TR-701 FA. Metabolite formation studies were conducted under linear conditions with respect to protein concentration and incubation time.

***Reversible Inhibition Studies*:** HLM (at concentrations of 60 µg/mL for the midazolam study and 160 µg/mL for the testosterone study) was incubated with test compounds (TR-700 or TR-701 FA) at 12 different concentrations, evaluated in two sets of six concentrations (20, 10, 5, 2.5, 1.25, 0.63 µM and 50, 25, 12.5, 6.25, 3.13, 1.56 µM), NADPH-regenerating system (1 mM NADP, 5.0 mM glucose 6-phosphate, glucose 6-phosphate dehydrogenase (1.5 U/mL) and 1.0 mM EDTA in 50 mM potassium phosphate, pH 7.4, at 37°C for 6 minutes before initiating the reaction with 50 µM testosterone or 2.5 µM midazolam. The final volume was 200 µL of each incubation. After the addition of probe substrate, reactions were terminated with the addition of 100 µL acetonitrile containing the respective internal standard (**Table 1**). Incubations were performed on a Genesis Workstation in 96-well plates at 37°C. Stock solutions of probe substrates were prepared in water (midazolam 2.5 mM) or in methanol/acetonitrile 75:25 (testosterone, 50 mM).
Ketoconazole was used as positive control reversible inhibitor. The solvent (acetonitrile) percentage in the final incubation for the various test conditions was 0.8% (TR-701 FA, midazolam), 1% (TR-701 FA, testosterone) and 1.8% (TR-700, midazolam and testosterone). The reference samples (substrate, no TR-700 and TR-701 FA, respectively) were incubated in parallel in sextuplet and contained the same percentage of solvent as the test incubations.

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Table 1. Internal Standards

Inhibition Type	Assay	Internal Standard
Direct Acting	Midazolam	Omeprazole
Direct Acting	Testosterone	6β-Hydroxy [$^{2}H_3$]testosterone
Time Dependent	Midazolam	[$^{2}H_3$]Omeprazole
Time Dependent	Testosterone	6β-Hydroxy [$^{2}H_3$]testosterone

Time-dependent inhibition studies (TDI): TR-700 or TR-701 FA (20, 10, 5, 2.5, 1.25, 0.63 µM and 50, 25, 12.5, 6.25, 3.13, 1.56 µM) were pre-incubated with HLM, 60 µg/mL HLM for [$^{13}C_6$]-midazolam study or 160 µg/mL HLM for testosterone study, and NADPH-regenerating system in the absence of the probe substrate ([$^{13}C_6$]-midazolam or testosterone) for 30 min at 37°C. Following pre-incubation, probe substrate [$^{13}C_6$]-midazolam or testosterone, respectively, was added to initiate the reaction. The [$^{13}C_6$]-midazolam and testosterone assays proceeded for 10 and 15 min, respectively. Reactions were terminated by the addition of 100 µL acetonitrile containing the respective internal standard (**Table 1**). Mibefradil was included as the TDI positive control inhibitor. Of note, in the TDI experiment, stable label midazolam ([$^{13}C_6$]-midazolam) was utilized due to bioanalytical issues with unlabelled midazolam in this assay.

Analytical Analysis: All analyses were performed using a validated LC-MS/MS method.

RESULTS AND CONCLUSIONS

The inhibition data of TR-700 or TR-701 FA towards CYP3A probe substrates are summarized in Table 2 and Table 3. The IC_{50} values for positive control inhibitors (ketoconazole/competitive inhibitor and mibefradil/metabolism-based inhibitor) were in the range of values reported in the literature. IC_{50} values for TR-700 or TR-701 FA towards CYP3A could not be determined, as there was no significant inhibition observed at any test concentration. Additionally, TDI of CYP3A was not observed.

In summary, clinically relevant DDI of TR-700 or TR-701 FA due to inhibition of CYP3A is considered unlikely.

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Table 2. Summary of inhibition potential of TR-700 towards CYP3A mediated probe substrate metabolite formation (duplicate incubations, mean value) in HLM.

CYP isoform	Probe reaction	IC_{50} TR-700 [µM]	Positive control Inhibitor	IC_{50} [µM]	Literature value for CYP probe IC_{50}, reference [µM]
CYP3A	Midazolam 1'-hydroxylation	n.a.	Ketoconazole	0.02	0.0187[6] 0.0302[8] 0.05[10] 0.09[9]
	[$^{13}C_6$]Midazolam 1'-hydroxylation (30 min preincubation)	n.a.	Mibefradil	0.12	0.10*
CYP3A	Testosterone 6β-hydroxylation	n.a.	Ketoconazole	0.05	0.0261[6] 0.0451[8] 0.09[9]
	Testosterone 6β-hydroxylation (30 min preincubation)	n.a.	Mibefradil	0.20	0.3[11]

Table 3. Summary of inhibition potential of TR-701 FA towards CYP3A mediated probe substrate metabolite formation (duplicate incubations, mean value) in HLM.

CYP isoform	Probe reaction	IC_{50} TR-701 FA [µM]	Positive control Inhibitor	IC_{50} (Mean) [µM]	Literature value for CYP probe IC_{50}, reference [µM]
CYP3A	Midazolam 1'-hydroxylation	n.a.	Ketoconazole	0.02	0.0187[6] 0.0302[8] 0.05[10] 0.09[9]
	[$^{13}C_6$]Midazolam 1'-hydroxylation (30 min preincubation)	n.a.	Mibefradil	0.08	0.10*
CYP3A	Testosterone 6β-hydroxylation	n.a.	Ketoconazole	0.03	0.0187[6] 0.0302[8] 0.05[10] 0.09[9]
	Testosterone 6β-hydroxylation (30 min preincubation)	n.a.	Mibefradil	0.14	0.3[11]

REVIEWER ASSESSMENT: The Sponsor's conclusions appear appropriate based on study results.

Reference ID: 3476543

STUDY NO.: PDM-10-0701-012

INDUCTION OF TR-700 ON CYP ENZYME ACTIVITY IN HUMAN HEPATOCYTES

Date: 11/2010-02/2011
Laboratory Site: (b) (4)

OBJECTIVE: The purpose of this study was to investigate the *in vitro* induction effect of TR-700 towards 6 CYP enzymes in human hepatocytes.

METHODS
Study Procedures: The *in vitro* induction effect of TR-700 at 3 concentrations (0.1, 1, and 10 µM prepared in dimethyl sulfoxide [DMSO]) on 6 CYP enzyme activities (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4) in human hepatocytes from 3 human donors was evaluated. Fresh plated human hepatocytes (approximately 0.15 million cells/well in 48-well plates) were treated with 0.1, 1, and 10 µM TR-700 and incubated in a 95% air/5% CO_2 incubator at 37°C for 72 hours. Positive controls were treated in parallel with the induction media spiked with β-naphthoflavone at 10 µM for CYP1A2, phenobarbital at 1000 µM for CYP2B6, and rifampicin at 50 µM for CYP2C8, CYP2C9, CYP2C19, and CYP3A4 (**Table 1**). Vehicle controls were treated in parallel with the induction media containing 0.1% DMSO. All experiments were conducted in triplicate.

Table 1. CYP Probe Substrates, Metabolites, and Positive Chemical Inducers

CYP	Probe Substrate	Metabolite	Chemical Inducer
CYP1A2	7-Ethoxyresorufin (20 µM)	Resorufin	β-Naphthoflavone (BNF) (10 µM)
CYP2B6	Bupropion (250 µM)	Hydroxybupropion	Phenobarbital (PB) (1000 µM)
CYP2C8	Amodiaquine (20 µM)	Desethylamodiaquine	Rifampicin (RIF) (50 µM)
CYP2C9	Diclofenac (20 µM)	4'-Hydroxydiclofenac	Rifampicin (RIF) (50 µM)
CYP2C19	S-Mephenytoin (250 µM)	4'-Hydroxymephenytoin	Rifampicin (RIF) (50 µM)
CYP3A4	Testosterone (250 µM)	6β-Hydroxytestosterone	Rifampicin (RIF) (50 µM)

Reviewer Comment: According to the literature and FDA draft Guidance for DDI studies, the selection of probe substrates and inducers, as well as corresponding concentrations for each test CYP enzyme appears appropriate.

Analytical Analysis: CYP enzyme activities were measured by determining the formation of CYP-specific probe substrate metabolites using LC-MS/MS. The fold-inductions in enzyme activity compared with the vehicle controls or positive inducers were estimated. Cell viability was measured by analyzing the cellular conversion of a tetrazolium salt into a formazan produced by dehydrogenases, which are active only in viable cells. The absorbance of formazan, which is proportional to the number of viable cells, was measured spectrophotometrically.

Reference ID: 3476543

RESULTS

The induction effects of TR-700 and the selected inducers on CYP enzyme activities in fresh human hepatocytes are summarized in Table 2 (CYP1A2), Table 3 (CYP2B6), Table 4 (CYP2C8), Table 5 (CYP2C9), Table 6 (CYP2C19), and Table 7 (CYP3A4).

Table 2. Induction effects of β-naphthoflavone and TR-700 on CYP1A2.

Donor	Compound	Treatment	Resorufin Formation (pmol/well/min)[a]	Fold-Induction[b]	% of BNF-Treated Cells[c]
1	Control	Solvent	0.011 ± 0.001	1.0	0
		10 µM BNF	0.16 ± 0.04**	14	100
	TR-700	0.1 µM	0.013 (n=2)	1.1	0.70
		1 µM	0.020 ± 0.02	1.8	5.7
		10 µM	0.012 ± 0.004	1.0	0.34
2	Control	Solvent	0.041 ± 0.007	1.0	0
		10 µM BNF	0.49 ± 0.04***	12	100
	TR-700	0.1 µM	0.038 ± 0.01	0.94	-0.57
		1 µM	0.035 ± 0.005	0.86	-1.3
		10 µM	0.033 ± 0.007	0.82	-1.7
3	Control	Solvent	0.057 ± 0.004	1.0	0
		10 µM BNF	0.63 ± 0.03***	11	100
	TR-700	0.1 µM	0.050 ± 0.01	0.88	-1.2
		1 µM	0.052 ± 0.01	0.91	-0.93
		10 µM	0.055 ± 0.001	0.97	-0.30

a Data were expressed as the mean ± SD from three individual incubations and measurements (n=3).
b Fold-induction was calculated based on the enzyme activity (formation rate of the probe substrate metabolite) of the test compound or the positive inducer to that of the vehicle-treated cells (solvent control).
c Percentage of enzyme activity relative to the positive inducer-treated cells (USA FDA Draft Guidance: Drug Interaction Studies, September 2006; Hewitt NJ, et al., Chem Biol Interact, 2007, 168:51-56).

Reference ID: 3476543

Table 3. Induction effects of phenobarbital and TR-700 on CYP2B6.

Donor	Compound	Treatment	OH Bupropion Formation (pmol/well/min)[a]	Fold-Induction[b]	% of PB-Treated Cells[c]
1	Control	Solvent	0.024 (n=2)	1.0	0
		1000 µM PB	0.81 ± 0.07**	34	100
	TR-700	0.1 µM	0.019 ± 0.009	0.81	-0.58
		1 µM	0.021 ± 0.006	0.90	-0.30
		10 µM	0.032 ± 0.001	1.3	1.0
2	Control	Solvent	0.012 ± 0.002	1.0	0
		1000 µM PB	0.23 ± 0.02***	19	100
	TR-700	0.1 µM	0.014 ± 0.001	1.2	1.0
		1 µM	0.011 ± 0.001	0.92	-0.44
		10 µM	0.014 ± 0.001	1.1	0.78
3	Control	Solvent	0.090 ± 0.01	1.0	0
		1000 µM PB	2.0 ± 0.07***	23	100
	TR-700	0.1 µM	0.10 ± 0.03	1.1	0.55
		1 µM	0.071 ± 0.004	0.79	-0.97
		10 µM	0.084 ± 0.02	0.93	-0.31

Table 4. Induction effects of rifampin and TR-700 on CYP2C8.

Donor	Compound	Treatment	Desethylamodiaquine Formation (pmol/well/min)[a]	Fold-Induction[b]	% of RIF-Treated Cells[c]
1	Control	Solvent	0.44 ± 0.08	1.0	0
		50 µM RIF	2.6 ± 0.44**	5.9	100
	TR-700	0.1 µM	0.33 ± 0.08	0.76	-4.9
		1 µM	0.27 ± 0.11	0.60	-8.1
		10 µM	0.28 ± 0.08	0.64	-7.4
2	Control	Solvent	0.67 ± 0.09	1.0	0
		50 µM RIF	2.4 ± 0.27***	3.6	100
	TR-700	0.1 µM	0.68 ± 0.10	1.0	0.72
		1 µM	0.75 ± 0.07	1.1	5.0
		10 µM	0.93 ± 0.10	1.4	16
3	Control	Solvent	0.41 ± 0.09	1.0	0
		50 µM RIF	1.9 ± 0.53***	4.6	100
	TR-700	0.1 µM	0.30 ± 0.14	0.74	-7.4
		1 µM	0.32 ± 0.11	0.77	-6.4
		10 µM	0.29 ± 0.09	0.70	-8.4

Reference ID: 3476543

Table 5. Induction effects of rifampin and TR-700 on CYP2C9.

Donor	Compound	Treatment	4'-OH Diclofenac Formation (pmol/well/min)[a]	Fold-Induction[b]	% of RIF-Treated Cells[c]
1	Control	Solvent	3.7 ± 0.09	1.0	0
		50 µM RIF	13 ± 0.44**	3.4	100
	TR-700	0.1 µM	2.6 ± 0.84	0.71	-12
		1 µM	3.2 ± 0.64	0.87	-5.3
		10 µM	2.9 (n=2)	0.78	-9.2
2	Control	Solvent	8.4 ± 1.61	1.0	0
		50 µM RIF	17 ± 3.04**	2.1	100
	TR-700	0.1 µM	7.2 ± 1.72	0.86	-13
		1 µM	6.7 ± 1.67	0.80	-19
		10 µM	7.6 ± 2.57	0.90	-9.2
3	Control	Solvent	8.3 ± 1.1	1.0	0
		50 µM RIF	17 ± 2.1**	2.1	100
	TR-700	0.1 µM	8.2 ± 2.0	0.98	-1.5
		1 µM	8.4 ± 0.78	1.0	1.2
		10 µM	9.6 ± 3.5	1.2	14

Table 6. Induction effects of rifampin and TR-700 on CYP2C19.

Donor	Compound	Treatment	4'-OH Mephenytoin Formation (pmol/well/min)[a]	Fold-Induction[b]	% of RIF-Treated Cells[c]
1	Control	Solvent	0.082 (n=2)	1.0	0
		50 µM RIF	0.46 ± 0.11**	5.7	100
	TR-700	0.1 µM	0.10 ± 0.05	1.2	3.8
		1 µM	0.083 ± 0.02	1.0	0.4
		10 µM	0.16 (n=2)	2.0	21
2	Control	Solvent	1.6 ± 0.11	1.0	0
		50 µM RIF	4.1 ± 0.53***	2.6	100
	TR-700	0.1 µM	1.6 ± 0.21	1.0	0.34
		1 µM	1.7 ± 0.31	1.1	4.7
		10 µM	1.5 ± 0.15	1.0	-1.2
3	Control	Solvent	0.17 ± 0.02	1.0	0
		50 µM RIF	1.1 ± 0.09***	6.4	100
	TR-700	0.1 µM	0.22 ± 0.07	1.3	4.9
		1 µM	0.19 ± 0.05	1.1	2.0
		10 µM	0.13 ± 0.03	0.78	-4.1

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Table 7. Induction effects of rifampin and TR-700 on CYP3A4.

Donor	Compound	Treatment	6β-OH Testosterone Formation (pmol/well/min)[a]	Fold-Induction[b]	% of RIF-Treated Cells[c]
1	Control	Solvent	1.1 ± 0.12	1.0	0
		50 μM RIF	50 ± 5.65**	42	100
	TR-700	0.1 μM	1.2 ± 0.19	1.0	0.04
		1 μM	1.2 ± 0.06	1.0	0.11
		10 μM	1.2 ± 0.09	1.0	0.07
2	Control	Solvent	4.4 ± 0.69	1.0	0
		50 μM RIF	99 ± 6.36***	23	100
	TR-700	0.1 μM	3.9 ± 0.68	0.89	-0.49
		1 μM	4.1 ± 0.69	0.93	-0.30
		10 μM	4.5 ± 0.81	1.0	0.10
3	Control	Solvent	11 ± 0.87	1.0	0
		50 μM RIF	127 ± 21***	11	100
	TR-700	0.1 μM	13 ± 2.3	1.2	1.7
		1 μM	12 ± 2.6	1.1	0.82
		10 μM	14 ± 2.2	1.2	2.1

The positive inducers for each CYP exhibited significant induction of CYP enzyme activities with acceptable fold induction values (fold-induction in enzyme activity ⩾2), indicating that the fresh human hepatocytes used in this study were metabolically active and inducible for CYP enzymes. TR-700, at any of the tested concentrations (0.1, 1, and 10 μM), did not show significant induction (fold-induction<2) on any of the tested CYP enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, and 3A4) for all three human hepatocyte donors except for CYP2C19 at 10 μM of TR-700 in human hepatocyte donor 1 (in duplicate), which demonstrated 2-fold induction response (Table 6). Per the Sponsor, the increase in CYP2C19 enzyme activity relative to the positive control was 21%, which was lower than the FDA inducer criteria 40%.

Reviewer Comment: The DDI guidance (dated September 2006) referred by the Sponsor is outdated; the inducer criteria of 40% increase in enzyme activity relative to the positive control is no longer used as a cut-off value in the current updated DDI guidance (dated 02/17/2012). Instead, the induction potential (R value) of new molecular entities on the enzyme activity and/or mRNA expression should be evaluated based on the "Decision Tree" in the current DDI guidance. However, the current DDI guidance states that "activation of the nuclear receptor, Pregnane X receptor (PXR), results in co-induction of CYP3A and CYP2C. Thus, a negative in vitro result for CYP3A induction eliminates the need for additional in vitro or in vivo induction studies for CYP3A and CYP2C enzymes." In this study, TR-700 did not show apparent induction effect on CYP3A4 enzyme activity at concentrations up to 10 μM for all three donors (Table 7). Of note, following administration of multiple doses of 200 mg TR-701 FA once daily in healthy human subjects, the mean C_{max} values of TR-700 were 2.2 and 3 μg/ml for oral and IV administration, respectively (equivalent to 5.9 and 8.1 μM, respectively). Taken together, the induction effect of TR-700 towards CYP2C19 is expected to be unlikely.

SPONSOR'S CONCLUSIONS: TR-700 is unlikely to be an inducer of the major CYP enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, and 3A4). TR-700 did not show significant damaging effects

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on cell viability at any tested concentrations, suggesting that TR-700 had no or very low cytotoxicity under the experimental conditions.

REVIEWER ASSESSMENT: The Sponsor's conclusions appear appropriate based on study results.

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STUDY NO.: CPH-13-0701-090

EVALUATION OF THE POTENTIAL OF TR-700 TO INDUCE CYP1A2, CYP2B6, AND CYP3A4 IN CULTURED HUMAN HEPATOCYTES

Date: 04/2013-05/2013
Laboratory Site: (b) (4)

OBJECTIVE: The purpose of this study was to investigate the potential of TR-700 to induce CYP1A2, CYP2B6, and CYP3A4 in sandwiched cultured human hepatocytes.

METHODS

Study Procedures: CYP induction was determined by measuring CYP messenger ribonucleic acid (mRNA) expression and in situ enzyme activity in cultured human hepatocytes following treatment with TR-700. Primary cultures of human hepatocytes from 3 individual donors were treated with vehicle alone (0.1% dimethyl sulfoxide [DMSO]), positive controls (50 µM omeprazole, 1 mM phenobarbital, and 10 µM rifampicin), negative control (25 µM flumazenil), or TR-700 at 0.3, 3, or 30 µM. All treatments were conducted in triplicate, with daily replacement of media containing test compound. After 48 hours of treatment, monolayers were lysed for RNA preparation and quantitative reverse transcriptase-polymerase chain reaction analysis using gene-specific probe sets for CYP1A2, CYP2B6, or CYP3A4. The mRNA levels were determined relative to an endogenous control (GAPDH) and normalized to the vehicle control. CYP enzyme activity levels were measured after 72 hours of treatment. Monolayers were incubated with a cocktail of CYP substrates (**Table 1**).

Table 1. CYP450 Probe Substrates and Metabolites

CYP	Substrate	Metabolite	Final Concentration (µM)
1A2	Phenacetin	Acetaminophen	100
2B6	Bupropion	Hydroxybupropion	50
3A4	Midazolam	1'-Hydroxymidazolam	10

Final concentration of DMSO = 0.1%

Reviewer Comment: According to the literature and FDA draft Guidance for DDI studies, the selection of probe substrates and inducers, as well as corresponding concentrations for each test CYP enzyme appears appropriate.

Analytical/Data Analysis: CYP enzyme activities were measured by determining the formation of CYP-specific probe substrate metabolites using LC-MS/MS. The criteria for positive CYP induction were increased mRNA levels ≥4-fold above vehicle (DMSO) control or increased enzyme activity of ≥40% of the relevant positive control induction.

RESULTS

The induction effects of TR-700 at 0.3, 3, and 30 µM, the selected inducers/non-inducers, and vehicle control on CYP enzyme activities in Sandwich-cultured human hepatocytes are

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summarized in Table 2 and Table 3 (CYP1A2), Table 4 and Table 5 (CYP2B6), and Table 6 and Table 7 (CYP3A4).

Table 2. CYP1A2 mRNA Changes in Human Hepatocytes Treated with DMSO (solvent control), Omeprazole or TR-700.

Isoform (Positive Control)	Donor #	Fold Change Over Control[1]				
		Positive Control	Negative Control (Flumazenil)	TR-700		
				0.3 µM	3.0 µM	30 µM
CYP1A2 (50 µM Omeprazole)	1	28 ± 4.49	0.85 ± 0.04	1.16 ± 0.04	1.38 ± 0.13	2.01 ± 0.67
	2	49.6 ± 1.60	1.14 ± 0.15	2.09 ± 1.55	1.18 ± 0.30	1.73 ± 0.22
	3	60.3 ± 4.64	1.23 ± 0.04	1.04 ± 0.03	1.07 ± 0.08	1.04 ± 0.18

[1] Data is mean ± standard deviation.

Table 3. Relative CYP1A2 Activity of Human Hepatocytes Treated with DMSO (solvent control), Omeprazole or TR-700.

Donor	Compound	Treatment	Acetaminophen formation (pmol/min/10^6 cells)[1]	Fold Induction[2]	% of Omeprazole Treated Cells[3]
1	Controls	0.1% DMSO	9.10 ± 1.87	1 ± 0.21	0 ± 0.67
		25 µM Flumazenil	13.1 ± 2.06	1.44 ± 0.23	1.44 ± 0.73
		50 µM Omeprazole	289 ± 62.9	31.78 ± 6.91	100 ± 22.5
	Test Article	0.3 µM TR-700	11.57 ± 0.22	1.27 ± 0.02	0.88 ± 0.08
		3 µM TR-700	12.6 ± 0.70	1.39 ± 0.08	1.26 ± 0.25
		30 µM TR-700	13.7 ± 2.97	1.51 ± 0.33	1.65 ± 1.06
2	Controls	0.1% DMSO	3.57 ± 0.92	1 ± 0.26	0 ± 0.57
		25 µM Flumazenil	4.56 ± 1.09	1.27 ± 0.31	0.61 ± 0.68
		50 µM Omeprazole	164 ± 21.7	46.0 ± 6.06	100 ± 13.5
	Test Article	0.3 µM TR-700	4.25 ± 0.53	1.19 ± 0.15	0.42 ± 0.33
		3 µM TR-700	4.90 ± .70	1.37 ± 0.20	0.83 ± 0.44
		30 µM TR-700	4.80 ± 0.61	1.34 ± 0.17	0.76 ± 0.38
3	Controls	0.1% DMSO	5.90 ± 0.84	1 ± 0.14	0 ± 0.26
		25 µM Flumazenil	7.91 ± 0.60	1.34 ± 0.10	0.62 ± 0.19
		50 µM Omeprazole	329 ± 22.0	55.9 ± 3.74	100 ± 6.81
	Test Article	0.3 µM TR-700	7.84 ± 1.20	1.33 ± 0.20	0.60 ± 0.37
		3 µM TR-700	8.42 ± 1.21	1.43 ± 0.21	0.78 ± 0.37
		30 µM TR-700	6.43 ± 2.71	1.09 ± 0.46	0.16 ± 0.84

[1] Data is mean ± standard deviation.
[2] Fold induction is calculated by dividing the specific activity of the treatment condition by the specific activity of the 0.1% DMSO control.
[3] Percent of positive control is calculated by subtracting the 0.1% DMSO control activity from the treatment condition activity and dividing by the 0.1% DMSO control corrected positive control activity and multiplying by 100.

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Table 4. CYP2B6 mRNA Changes in Human Hepatocytes Treated with DMSO (solvent control), Phenobarbital or TR-700.

Isoform (Positive Control)	Donor #	Fold Change Over Control[1]				
		Positive Control	Negative Control (Flumazenil)	TR-700		
				0.3 µM	3.0 µM	30 µM
CYP2B6 (1 mM Phenobarbital)	1	9.68 ± 0.84	1.07 ± 0.17	0.89 ± 0.01	1.33 ± 0.37	1.50 ± 0.70
	2	15.6 ± 1.26	1.32 ± 0.15	1.31 ± 0.12	1.41 ± 0.39	1.49 ± 0.16
	3	8.72 ± 0.91	1.17 ± 0.09	1.07 ± 0.13	1.16 ± 0.07	1.23 ± 0.14

[1] Data is mean ± standard deviation.

Table 5. Relative CYP2B6 Activity of Human Hepatocytes Treated with DMSO (solvent control), Phenobarbital or TR-700.

Donor	Compound	Treatment	Hydroxybupropion formation (pmol/min/10^6 cells)[1]	Fold Induction[2]	% of Phenobarbital Treated Cells[3]
1	Controls	0.1% DMSO	1.63 ± 0.51	1 ± 0.31	0 ± 3.52
		25 µM Flumazenil	2.57 ± 0.20	1.57 ± 0.12	6.45 ± 1.38
		1 mM Phenobarbital	16.1 ± 4.89	9.87 ± 3.00	100 ± 33.8
	Test Article	0.3 µM TR-700	2.21 ± 0.30	1.35 ± 0.18	3.98 ± 2.04
		3 µM TR-700	2.78 ± 0.10	1.70 ± 0.06	7.92 ± 0.68
		30 µM TR-700	2.45 ± 0.53	1.50 ± 0.32	5.65 ± 3.64
2	Controls	0.1% DMSO	0.31 ± 0.07	1 ± 0.24	0 ± 0.95
		25 µM Flumazenil	0.48 ± 0.14	1.54 ± 0.43	2.16 ± 1.74
		1 mM Phenobarbital	8.10 ± 1.00	25.9 ± 3.18	100 ± 12.8
	Test Article	0.3 µM TR-700	0.41 ± 0.06	1.31 ± 0.20	1.26 ± 0.81
		3 µM TR-700	0.56 ± 0.16	1.78 ± 0.51	3.15 ± 2.06
		30 µM TR-700	0.47 ± 0.10	1.51 ± 0.31	2.07 ± 1.23
3	Controls	0.1% DMSO	0.76 ± 0.17	1 ± 0.22	0 ± 0.86
		25 µM Flumazenil	0.96 ± 0.15	1.27 ± 0.20	1.05 ± 0.77
		1 mM Phenobarbital	20.1 ± 2.06	26.4 ± 2.71	100 ± 10.7
	Test Article	0.3 µM TR-700	1.05 ± 0.30	1.38 ± 0.39	1.49 ± 1.53
		3 µM TR-700	1.68 ± 0.46	2.21 ± 0.60	4.76 ± 2.38
		30 µM TR-700	1.24 ± 0.56	1.61 ± 0.74	2.50 ± 2.89

[1] Data is mean ± standard deviation.

[2] Fold induction is calculated by dividing the specific activity of the treatment condition by the specific activity of the 0.1% DMSO control.

[3] Percent of positive control is calculated by subtracting the 0.1% DMSO control activity from the treatment condition activity and dividing by the 0.1% DMSO control corrected positive control activity and multiplying by 100.

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Table 6. CYP3A4 mRNA Changes in Hepatocytes Treated with DMSO (solvent control), Rifampicin or TR-700.

Isoform (Positive Control)	Donor #	Fold Change Over Control[1]				
		Positive Control	Negative Control (Flumazenil)	TR-700		
				0.3 µM	3.0 µM	30 µM
CYP3A4 (10 µM Rifampicin)	1	31.9 ± 6.01	1.41 ± 0.35	1.05 ± 0.19	2.18 ± 1.07	2.58 ± 1.72
	2	40.5 ± 7.69	2.82 ± 0.65	1.76 ± 0.04	1.84 ± 0.13	2.92 ± 0.22
	3	36.1 ± 11.8	1.12 ± 0.05	0.85 ± 0.22	1.22 ± 0.17	1.95 ± 0.09

[1] Data is mean ± standard deviation.

Table 7. Relative CYP3A4 Activity of Human Hepatocytes Treated with DMSO (solvent control), Rifampicin or TR-700.

Donor	Compound	Treatment	1'-Hydroxymidazolam formation (pmol/min/10^6 cells)[1]	Fold Induction[2]	% of Rifampicin Treated Cells[3]
1	Controls	0.1% DMSO	15.2 ± 3.77	1.00 ± 0.25	0 ± 4.26
		25 µM Flumazenil	20.9 ± 0.85	1.38 ± 0.06	6.53 ± 0.96
		10 µM Rifampicin	104 ± 5.23	6.84 ± 0.35	100 ± 5.91
	Test Article	0.3 µM TR-700	18.0 ± 1.44	1.19 ± 0.10	3.25 ± 1.63
		3 µM TR-700	18.41 ± 1.03	1.21 ± 0.07	3.67 ± 1.16
		30 µM TR-700	18.2 ± 1.26	1.20 ± 0.08	3.49 ±1.42
2	Controls	0.1% DMSO	3.73 ± 0.61	1.00 ± 0.16	0 ± 1.74
		25 µM Flumazenil	6.18 ± 1.78	1.66 ± 0.48	6.96 ± 5.06
		10 µM Rifampicin	39.0 ± 5.65	10.45 ± 1.52	100 ± 16.04
	Test Article	0.3 µM TR-700	4.57 ± 0.58	1.23 ± 0.16	2.40 ± 1.66
		3 µM TR-700	5.76 ± 0.88	1.54 ± 0.24	5.76 ± 2.51
		30 µM TR-700	5.77 ± 0.52	1.55 ± 0.14	5.80 ± 1.49
3	Controls	0.1% DMSO	5.73 ± 0.67	1.00 ± 0.12	0 ± 1.26
		25 µM Flumazenil	7.86 ± 0.74	1.37 ± 0.13	3.97 ± 1.38
		10 µM Rifampicin	59.4 ± 0.15	10.4 ± 0.03	100 ± 0.27
	Test Article	0.3 µM TR-700	6.74 ± 1.25	1.18 ± 0.22	1.89 ± 2.33
		3 µM TR-700	8.23 ± 1.74	1.44 ± 0.30	4.65 ± 3.24
		30 µM TR-700	10.5 ± 5.00	1.83 ± 0.87	8.90 ± 9.31

[1] Data is mean ± standard deviation.
[2] Fold induction is calculated by dividing the specific activity of the treatment condition by the specific activity of the 0.1% DMSO control.
[3] Percent of positive control is calculated by subtracting the 0.1% DMSO control activity from the treatment condition activity and dividing by the 0.1% DMSO control corrected positive control activity and multiplying by 100.

Marked induction of CYP1A2, CYP2B6, and CYP3A4 mRNA expression and enzyme activity was observed with the positive controls omeprazole, phenobarbital, and rifampin, respectively, while no induction was observed with the negative control flumazenil. Three batches of hepatocytes treated with TR-700 showed no significant increases in mRNA levels of CYP1A2 (≤2.09-fold increase), CYP2B6 (≤1.5-fold increase), or CYP3A4 (≤2.92-fold increase). In assays of TR-700-induced enzyme activity, CYP1A2 activity increased 1.09- to 1.51-fold compared to vehicle (DMSO) control (≤1.65±1.06% of omeprazole-treated cells), CYP2B6 activity increased 1.31- to 2.21-fold compared to vehicle control (≤7.92±0.68% of phenobarbital-treated cells), and CYP3A4 activity increased 1.18- to 1.83-fold compared to vehicle control (≤8.90±9.31% of rifampicin-treated cells). None of these changes in enzyme activity met the criteria for positive induction.

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SPONSOR'S CONCLUSIONS: CYP induction was assessed by determining changes in mRNA levels and specific enzyme activities for 3 CYP isoforms. Under the conditions utilized in this study, TR-700 did not cause significant induction of CYP1A2, CYP2B6 or CYP3A4 up to the highest concentration tested, 30 μM, in the three human hepatocyte batches tested. Based on these results, TR-700 appears unlikely to be a CYP inducer.

REVIEWER ASSESSMENT: The study design is in compliance with the current FDA DDI guidance for induction studies. The Sponsor's conclusions appear appropriate based on study results.

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STUDY NO.: CPH-10-0701-013

BIDIRECTIONAL PERMEABILITY ASSESSMENT OF TR-700 THROUGH CACO-2 MONOLAYERS

Date: 10/2010-11/2010
Laboratory Site: (b) (4)

OBJECTIVE: To assess the permeability of TR-700 in the Caco-2 cell line.

METHODS
Study Procedures: The apparent apical-to-basolateral (A-B) permeability (P_{app} A-B) and apparent basolateral-to-apical (B-A) permeability (P_{app} B-A) of TR-700 were investigated with Caco-2 cell lines. The Caco-2 cells were seeded onto 12-well Costar Transwell® plates. The cells were grown for 20 days until formation of a confluent cell monolayer. The quality of the Caco-2 monolayer batch was verified using marker compounds on Day 20 prior to experimental use. The formation of tight junctions was assessed by a resistance measurement, transepithelial electric resistance (TEER), permeability and efflux activity utilizing several probe substrates. The TEER value was 541 Ω*cm^2. High and low permeability markers were propranolol and atenolol, in which the P_{app}(A-B) were 19.5 x10^{-6} cm/s and 0.20 x10^{-6} cm/s, respectively. Efflux activity assessed by the transport of digoxin indicated an efflux ratio (P_{app} (B-A) / P_{app} (A-B)) of 15.6. The integrity of the monolayer was assessed by the Lucifer yellow (200 µM) with a P_{app}(A-B) of 0.14 x10^{-6} cm/s. The Caco-2 cells utilized for the permeability and efflux ratio assessment of TR-700 met all the quality control criteria set forth (b) (4).

On day 24, bidirectional transport studies in Caco-2 cells were conducted in Hank's balanced salt solution containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and 15 mM glucose, pH 7.4. The receiver compartment was fortified with 1% bovine serum albumin. TR-700 at 1, 10 and 100 µM (organic content was ≤0.5% dimethyl sulfoxide were added to the apical side of the membrane to measure the apical-to-basolateral (A-B) transport or added to the basolateral side of the membrane to measure the basolateral-to-apical (B-A) transport across the monolayer at 37°C with 5% CO_2 in a humidified incubator. Following the 2 hour incubation, samples were taken from the donor and receiver chambers, 200 µL for receiver samples from apical dosing experiments, 50 µL for receiver samples from basolateral dosing experiments, and 50 µL for all donor samples. Each condition was evaluated in duplicate.

Analytical/Data Analysis: Samples were processed for quantification of TR700 by LC-MS/MS. TR-700 P_{app} values, recovery, and efflux ratio were determined using the equations below. Significant efflux is defined as efflux ratio > 3.0 and Papp (B-A) > 1.0 x 10^{-6} cm/s.

$Papp = (dC_r / dt) \times V_r/(A \times C_N)$ (1)
Percent Recovery = $100 \times ((V_r \times C_r^{final}) + (V_d \times C_d^{final}))/(V_d \times C_N)$ (2)
Efflux ratio= P_{app} (B-A) / P_{app} (A-B)

Where,
dC_r / dt is the slope of the cumulative concentration in the receiver compartment versus time in µM s^{-1};

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V_r is the volume of the receiver compartment in cm^3;
V_d is the volume of the donor compartment in cm^3;
A is the area of the insert (1.13 cm^2 for 12-well Transwell);
C_N is the nominal concentration of the dosing solution in µM;
C_r^{final} is the cumulative receiver concentration in µM at the end of the incubation period;
C_d^{final} is the concentration of the donor in µM at the end of the incubation period.

RESULTS

The bidirectional permeability and efflux ratio of TR700 in Caco-2 cells are shown below in **Table 1.** TR-700 exhibited high permeability as P_{app}(A-B) was greater than 1.0 x 10^{-6} cm/s at all three dosing concentrations, 1, 10, and 100 µM with no significant efflux. Recovery of TR-700 in the bi-directional transport was ≥70%.

Table 1. Permeability and Efflux Ratio for TR-700 in Caco-2 Cells

Compound	Average P_{app} (x10^{-6} cm/s)		Efflux Ratio
	A-B	A-B	
1 µM TR-700	21.3	40.2	1.9
10 µM TR-700	18.5	29.8	1.6
100 µM TR-700	29.1	24.6	0.8

Abbreviations: A=apical; B=basal; P_{app}=apparent permeability.
Note: Values represent mean of duplicate samples.

Reviewer Comments:

1) *The 3rd column in the above table should be B-A instead of A-B.*
2) *Given that efflux ratios are less than 2 at all concentrations tested, TR-700 is considered as poor or non-P-gp/BCRP substrate as instructed in the current FDA DDI guidance.*

SPONSOR'S CONCLUSIONS: TR-700 was classified as having a high permeability coefficient with no significant efflux at all three dosing concentrations, 1, 10, and 100 µM. Lack of significant efflux indicates TR-700 is not a substrate for endogenous efflux transporters (P-gp and BCRP)

REVIEWER ASSESSMENT: The Sponsor's conclusions appear appropriate based on study results.

Reference ID: 3476543

STUDY NO.: CPH-11-0701-022

P-GLYCOPROTEIN INHIBITION POTENTIAL OF TR-700 IN CACO-2 MONOLAYERS

Date: 12/2010-01/2011
Laboratory Site: (b) (4)

OBJECTIVE: To assess the potential of TR-700 to inhibit P-glycoprotein (P-gp), an efflux transporter, in the Caco-2 cell system.

METHODS

Study Procedures: The effect of TR-700 on the apparent apical-to-basolateral (A-B) permeability (Papp A-B) and apparent basolateral-to-apical (B-A) permeability (Papp B-A) of a P-gp probe substrate, digoxin (10 µM), across a Caco-2 cell monolayer was determined in vitro. The Caco-2 cells at passage 62 were seeded onto 12-well Costar Transwell® plates. The cells were grown for 20 days until formation of a confluent cell monolayer. The quality of the Caco-2 monolayer batch was verified using marker compounds on Day 20 prior to experimental use (Day 23). The formation of tight junctions was assessed by a resistance measurement, transepithelial electric resistance (TEER), permeability and efflux activity utilizing several probe substrates. The TEER value was 574 Ω*cm^2. High and low permeability markers were propranolol and atenolol, in which the Papp(A-B) were 17.5 $x10^{-6}$ cm/s and 0.21 $x10^{-6}$ cm/s, respectively. Efflux activity assessed by the transport of digoxin indicated an efflux ratio (Papp (B-A) / Papp (A-B)) of 16.2. The integrity of the monolayer was also assessed by the Lucifer yellow (200 µM) with a Papp(A-B) of 0.10 $x10^{-6}$ cm/s.

On day 23, bidirectional transport studies were conducted in Hank's buffered salt solution containing 10 mM HEPES and 15 mM glucose, pH 7.4. Cell monolayers were first pre-incubated for 30 minutes with either assay buffer or assay buffer containing TR-700 to pre-saturate P-gp binding sites. After 30 minutes, the buffer was removed and replaced with fresh dosing solution. Digoxin (10 µM), was added to the apical or basolateral side of the membrane to measure the A-B or B-A transport, respectively, in the absence or presence of TR-700 (10 µM). Directional transport across the monolayer was determined after incubation in a humidified incubator at 37°C with 5% CO_2 for 2 hours. The final organic content was 0.2% dimethyl sulfide in each well. Each condition was conducted in duplicate. Following the incubation, samples were taken from the donor and receiver chambers, 200 µL for receiver samples from apical dosing experiments, 50 µL for receiver samples from basolateral dosing experiments, and 50 µL for all donor samples.

Analytical/Data Analysis: Samples were processed for quantification of digoxin by LC-MS/MS. The flux of digoxin in the A-B and B-A directions and the apparent permeability values for digoxin P_{app} (A-B) and P_{app} (B-A) were determined in the absence and presence of TR-700 using the equations below. The net B-A flux was determined by subtracting the A-B flux from the B-A flux. The P_{app}(A-B) and P_{app} (B-A) values were determined by normalizing the A-B and B-A flux by digoxin concentration (10 µM).

$$Papp = (dC_r / dt) \times V_r/(A \times C_N) \quad (1)$$
$$\text{Percent Recovery} = 100 \times ((V_r \times C_r^{final}) + (V_d \times C_d^{final}))/(V_d \times C_N) \quad (2)$$

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Where,
dC_r/dt is the slope of the cumulative concentration in the receiver compartment versus time in $\mu M\ s^{-1}$;
V_r is the volume of the receiver compartment in cm^3;
V_d is the volume of the donor compartment in cm^3;
A is the area of the insert (1.13 cm^2 for 12-well Transwell);
C_N is the nominal concentration of the dosing solution in µM;
C_r^{final} is the cumulative receiver concentration in µM at the end of the incubation period;
C_d^{final} is the concentration of the donor in µM at the end of the incubation period.

RESULTS
A-B and B-A flux, Papp in both the A-B and B-A direction, efflux ratio, and net B-A flux of digoxin are shown in **Tables 1** and **2**. Significant inhibition of P-glycoprotein activity was defined as a >50% reduction in digoxin efflux in the presence of test compound. Recovery of digoxin in the bi-directional transport studies (A-B and B-A) ranged from 101-108%.

Table 1. A-B and B-A Flux, Papp Values and Efflux Ratio for P-gp-mediated Digoxin Transport in the Absence and Presence of TR-700 in Caco-2 cells (n=2).

Compound	Average Flux (pmol/hr/cm^2)		Average P_{app} (nm/sec)		Efflux Ratio*
	A-B	B-A	A-B	B-A	
10 µM Digoxin	0.47	8.16	0.78	13.6	17
10 µM Digoxin + 10 µM TR-700	0.56	8.40	0.93	14.0	15

Abbreviations: A=apical; B=basolateral; P_{app}=apparent permeability
*Efflux ratio = (P_{app} B-A) /(P_{app} A-B)

Table 2. Net B-A flux and Percent Inhibition for P-gp-mediated Digoxin in Absence and Presence of TR-700 in Caco-2 cells (n=2).

Compound	Net B-A Flux* (pmol/hr/cm^2)	% Inhibition
10 µM Digoxin	7.7	--
10 µM Digoxin + 10 µM TR-700	7.8	-2.0

Abbreviations: A=apical; B=basolateral
*Net B-A flux = B-A flux – A-B flux

SPONSOR'S CONCLUSIONS: TR-700 (10 µM) did not inhibit P-glycoprotein activity assessed by digoxin transport in the Caco-2 cell line.

REVIEWER ASSESSMENT: The Sponsor's conclusions appear appropriate based on study results.
STUDY NO.: CPH-12-0701-033

Reference ID: 3476543

ASSESSMENT OF TR-700 AS AN INHIBITOR OF HUMAN BCRP, OCT1, OCT2, OAT1, OAT3, OATP1B1 AND OATP1B3 MEDIATED TRANSPORT

Date: 11/2011-01/2012
Laboratory Site: (b) (4)

OBJECTIVE: To assess the potential of TR-700 as an inhibitor of human breast cancer resistance protein (BCRP), organic cation transporter 1 (OCT1), organic cation transporter 2 (OCT2), organic anion transporter 1 (OAT1), organic anion transporter 3 (OAT3), organic anion transporting polypeptide 1B1 (OATP1B1) and organic anion transporting polypeptide 1B3 (OATP1B3)-mediated transport.

METHODS

Study Procedures:

BCRP: TR-700 was evaluated as an inhibitor of the human efflux transporter, BCRP, utilizing polarized monolayer of Caco-2 cells grown on permeable support. Transport studies were carried out approximately 7 days after seeding. The permeability of 25 nM [^{3}H]-genistein across the monolayer in the apical-to-basal direction (A-B) and the basal-to-apical direction (B-A) was measured in the absence and presence of 30 µM TR-700 or 100 µM chrysin (BCRP positive control inhibitor) following a 2 hour incubation. Following the 2 hour incubation, 100 µL sample from the receiver well was removed and added to 700 µL scintillation fluid.

SLC Transporters: TR-700 was also evaluated as an inhibitor of various human solute carrier (SLC) transporters namely OCT1, OCT2, OAT1, OAT3, OATP1B1, and OATP1B3. Each transporter was expressed in the Madin-Darby canine kidney (MDCK-II) polarized cell line grown on permeable supports. MDCK-II cells were seeded 24 hours before transfection of the cell line with SLC transporters of interest. Uptake transport studies were conducted 24 hours after transfection. Identical transport studies were conducted using MDCK-II cells expressing the transporter of interest and control cells which do not express the transporter for TR-700 and positive control inhibitor. The activity of the SLC transporters was evaluated based on uptake of the probe substrate into MDCK-II cell line expressing a specific transporter in the absence and presence of 30 µM TR-700. The incubation time for the uptake studies was 5 minutes. The probe substrates and positive control inhibitors are listed in **Table 1**.

Table 1. Probe Substrate and Positive Control Inhibitor for SLC Uptake Inhibition Assay

SLC Transporter	Probe substrate	Positive Control Inhibitor
OCT1 & OCT2	10 µM [^{14}C]-metformin	100 µM quinidine
OAT1	2 µM [^{3}H]-p-aminohippurate	100 µM probenecid
OAT3	0.75 µM [^{3}H]-estrone -3-sulfate	100 µM probenecid
OAPT1B1	2 µM [^{3}H]-estradiol 17β-d-glucuronide	100 µM rifampicin
OATP1B3	10 µM [^{3}H]-CCK8	100 µM rifampicin

Reference ID: 3476543

Reviewer Comment: According to the literature and FDA draft Guidance for DDI studies, the selection of probe substrates and inhibitors, as well as corresponding concentrations for each test transporter appears appropriate.

Analytical/Data Analysis:
BCRP: The amount of genistein transported across the membrane was quantified by radiometric detection. The flux of genistein in the A-B and B-A directions and the apparent permeability (P_{app}) values for genistein, P_{app}(A-B) and P_{app} (B-A), were determined in the absence and presence of TR-700 or chrysin. The net B-A flux was determined by subtracting the A-B flux from the B-A flux. The P_{app} (A-B) and P_{app} (B-A) were determined by normalizing the A-B and B-A flux by genistein concentration (25 nM). Percent inhibition is calculated by dividing the net (B → A) flux in the presence of TR-700 or positive control inhibitor by the mean net (B → A) flux in the absence of inhibitor. Statistical Analysis was done using Student's t-test. A *p* value of <0.05 was considered statistically significant.

SLC Transporters: The uptake of substrates was determined by radiometric detection. The net transporter mediated uptake of the substrate by SLC transporter was determined by subtracting the mean uptake in the control MDCK-II cell line (n=3) from the uptake value of each replicate MDCK-II-SLC expression cell line (n=3). The percent inhibition was determined by the net transporter uptake in the presence of TR-700 or positive control inhibitor divided by the mean net transporter mediated substrate uptake in the absence of inhibitor. Statistical Analysis was done using Student's t-test. A *p* value of <0.05 was considered statistically significant.

RESULTS
BCRP: The flux of genistein in the A-B and B-A directions and the P_{app} values for genistein, P_{app}(A-B) and P_{app} (B-A), were determined in the absence and presence of TR-700 or chrysin **(Table 2)**. The net B-A flux BCRP mediated activity was modestly inhibited (27.9 %; p=0.0058) by 30 μM TR-700 **(Table 3)**.

Table 2. A-B and B-A Flux and Papp Values for BCRP-mediated Genistein Transport in the Absence and Presence of TR-700 or Chrysin Across Caco-2 cells (n=3)

Compound	Flux (pmol/hr/cm^2)		P_{app} (nm/sec)	
	A-B	B-A	A-B	B-A
Genistein	0.23±0.02	1.26±0.02	25.2±1.81	140±2.09
Genistein + 30 μM TR-700	0.41±0.01	1.15±0.09	45.2±2.29	128±10.1
Genistein + 100 μM Chrysin	0.74±0.03	0.84±0.04	82.5±2.85	93.7±4.75

Abbreviations: A=apical; B=basal; P_{app}=apparent permeability

Reference ID: 3476543

Table 3. Net B-A flux and Percent Inhibition for BCRP-mediated Genistein Transport in Absence and Presence of TR-700 or Chrysin Across Caco-2 cells (n=3)

Compound	Net B-A Flux* (pmol/hr/cm2)	Percent Inhibition
Genistein	1.04±0.02	NA
Genistein + 30 μM TR 700	0.75±0.09	27.9±8.82
Genistein + 100 μM Chrysin	0.10±0.04	90.3±4.13

Abbreviations: A=apical; B=basal
Net B-A flux = B-A flux – A-B flux); Percent inhibition = 100-[100(Net B-A flux)inhibitor/ (mean Net B-A flux) no inhibitor)]

SLC Transporters: As shown in **Table 4**, TR-700 significantly ($p<0.05$) inhibited OCT1, OAT3 and OATP1B1 mediated transport by 23.6% (p=0.0023), 25.1% (p=0.0327), 29.0% (p=0.0178), respectively, while OCT2, OAT1 and OATP1B3 mediated transport was not significantly inhibited.

Table 4. Inhibition of OCT1, OCT2, OAT1, OAT3, OATP1B1 and OATP1B3-mediated Transport by TR-700

Transporter	Condition	Inhibition (%)
OCT1	Metformin + TR-700	23.6±4.43*
OCT2	Metformin + TR-700	4.72±9.94
OAT1	p-Aminohippurate + TR-700	3.88±8.04
OAT3	Estrone -3-sulfate + TR-700	25.1±9.62*
OATP1B1	Estradiol 17β-d-glucuronide + TR-700	29.0±11.9*
OATP1B3	CCK-8 +TR-700	13.2±7.04

*Statistically different from substrate uptake in the absence of inhibitor using student t-test, $p<0.05$.
Inhibition for positive control inhibitors were OCT1 (81.2±4.47%); OCT2 (81.5±5.08%); OAT1 (74.4±2.08%); OAT3 (83.2±1.51%); OATP1B1 (92.1±2.68%); OATP1B3 (94.1±1.95%).

SPONSOR'S CONCLUSIONS: BCRP, OCT1, OAT3 and OATP1B1 were modestly inhibited (inhibition <30%) at 30 μM TR-700 while inhibition towards OCT2, OAT1 and OATP1B3 was minimal.

REVIEWER ASSESSMENT: The Sponsor's conclusions appear appropriate based on study results. However, additional studies on further evaluation of TR-700 as an inhibitor of BCRP, OCT1, OAT3 and OATP1B1using multiple concentrations of TR-700 is warranted.

Reference ID: 3476543

STUDY NO.: CPH-12-0701-046

ASSESSMENT OF TR-700 AS A POTENTIAL INHIBITOR OF HUMAN ORGANIC ANION TRANSPORTING POLYPEPTIDE, P-GLYCOPROTEIN, AND BREAST CANCER RESISTANCE PROTEIN-MEDIATED TRANSPORT

Date: 08/2012-08/2012
Laboratory Site: (b) (4)

OBJECTIVE: To determine the inhibitory potency of TR-700 towards P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and organic anion transporting polypeptide 1B1 (OATP1B1).

METHODS

Study Procedures: P-gp and OATP1B1 inhibition were measured in Multidrug Resistance Protein 1 (MDR1) and OATP1B1 transiently transfected over-expressed Madin-Darby canine kidney (MDCKII) polarized cell lines (MDCKII-MDR1 and MDCKII-OATP1B1), respectively, grown on permeable membranes. For BCRP, Caco-2 cells were maintained in DMEM with high glucose and 10% FBS. All transport studies (P-gp, BCRP and OATP1B1) were conducted in Hank's Balanced Salt Solution (HBSS), pH 7.4.

P-gp, BCRP, and OATP1B1 activities were assessed by the permeability of 100 nM [^{3}H]-digoxin, 25 nM [^{3}H]-genistein and 2 µM [^{3}H]-estradiol 17β-D-glucuronide, respectively, across the monolayer in the apical-to-basolateral (A-B) direction and the basolateral-to-apical (B-A) direction in the absence and presence of TR-700 at concentrations of 3, 10, 30, and 60 µM. TR-700 concentrations of 3, 10, and 30 µM were prepared in HBSS with 0.5% DMSO, whereas 60 µM was prepared in HBSS with 2% DMSO. 100 µM verapamil, 100 µM chrysin, and 100 µM rifampicin served as P-gp, BCRP, and OATP1B1 positive control inhibitors, respectively. Following the incubation period (5 min for OATP1B1; 120 min for BCRP and P-gp), 100 µL aliquot from the receiver well was removed and added to a vial and 700 µL of scintillation fluid was added.

Analytical/Data Analysis: The amount of [^{3}H]-digoxin, [^{3}H]-genistein and [^{3}H]-estradiol 17β-D-glucuronide transported across the membrane was quantified by radiometric detection. The P_{app}, net B-A flux and efflux ratio were determined for [^{3}H]-digoxin and [^{3}H]-genistein and net transporter cellular accumulation and percent inhibition were determined for [^{3}H]-estradiol 17β-D-glucuronide in the absence and presence of increasing concentrations of TR-700 or 100 µM positive control inhibitor. The net B-A flux was determined by subtracting the A-B flux from the B-A flux. The efflux ratio was determined by taking the ratio of P_{app} (B-A) and P_{app} (A-B). The net transporter cellular accumulation was determined by subtracting uptake in the wild type cell line from the uptake in OATP1B1 over-expressed MDCKII cell line.

RESULTS

Consistent to previous results (CPH-11-0701-022), TR-700 did not inhibit P-gp at concentrations up to 10 µM, and showed slight increase in the inhibition of P-gp mediated digoxin transport at 30 µM (by 8.4%; p=0.0022) and 60 µM (15.5%; p=0.0101) (**Table 1**).

Reference ID: 3476543

Table 1. P-gp-Mediated Digoxin Transport in the Absence and Presence of TR-700 or Positive Control Inhibitor

Compound	P_{app}($x10^{-6}$ cm/s)		Net B-A Flux (pmol/hr/cm^2)	Efflux Ratio	Percent Inhibition
	A-B	B-A			
Digoxin	1.05±0.11	21.9±0.34	7.51±0.12	21.0±0.33	NA
Digoxin[a]	1.14±0.10	22.2±0.94	7.56±0.34	19.5±0.82	NA
Digoxin +3 µM TR-700	1.07±0.11	23.9±0.86	8.23±0.31	22.5±0.80	-9.52±4.10
Digoxin + 10 µM TR-700	1.08±0.08	22.0±0.23	7.53±0.08	20.4±0.21	-0.24±1.11
Digoxin + 30 µM TR-700	1.37±0.16	20.5±0.27	6.88±0.10	14.9±0.20	8.42±1.29
Digoxin + 60 µM TR-700[a]	1.63±0.12	19.4±0.79	6.39±0.28	11.9±0.48	15.5±3.76
Digoxin + 100 µM Verapamil	5.86±NA	6.35±0.21	0.18±0.08	1.08±0.04	97.7±1.02
Digoxin + 100 µM Verapamil[a]	5.55±0.10	7.87±1.67	0.83±0.60	1.42±0.30	89.0±7.93

Source: Optivia Report OPT-2012-109 Table 1
Abbreviations: A-B=apical-to-basolateral; B-A=basolateral-to-apical; NA=not applicable
[a]Samples contain 2% DMSO

Reviewer Comment: Following administration of multiple doses of 200 mg TR-701 FA once daily in healthy human subjects, the mean C_{max} values of TR-700 were 2.2 and 3 µg/ml for oral and IV administration, respectively (equivalent to 5.9 and 8.1 µM, respectively). This C_{max} is 7.4-10-fold lower than the highest concentration of TR-700 tested (60 µM) in this study. Given that 1) IC_{50} is >60 µM; 2) protein binding of TR-700 in human plasma is high (70-90%). Therefore, the inhibitory potential of TR700 on P-gp mediated transport is expected to be very low in vivo in humans.

TR-700 did not show appreciable inhibition of OATP1B1 transport at concentrations up to 60 µM (**Table 2**).

Reference ID: 3476543

Table 2. OATP1B1-Mediated Estradiol 17β-D-Glucuronide Transport in the Absence and Presence of TR-700 or Positive Control Inhibitor

Compound	Net Transporter Cellular Accumulation (pmol/min/cm^2)	% Inhibition
Estradiol 17β-D-glucuronide	2.57 ±0.35	NA
Estradiol 17β-D-glucuronide[a]	2.33 ±0.09	NA
Estradiol 17β-D-glucuronide + 3 μM TR-700	2.86 ±0.03	-11.5 ±1.08
Estradiol 17β-D-glucuronide + 10 μM TR-700	3.14 ±0.40	-22.3 ±5.6
Estradiol 17β-D-glucuronide + 30 μM TR-700	2.69 ±0.40	-4.76 ±15.7
Estradiol 17β-D-glucuronide + 60 μM TR-700[a]	2.20 ±0.18	5.58 ±7.80
Estradiol 17β-D-glucuronide + 100 μM Rifampicin	0.0003 ±0.16	100 ±6.29
Estradiol 17β-D-glucuronide + 100 μM Rifampicin[a]	-0.027 ±0.014	101 ±0.58

Source: Optivia Report OPT-2012-109, Table 1
Abbreviations: NA=not applicable
[a]Samples contain 2% DMSO

Reviewer Comment: Based on the results showed in Table 2, it appeared that TR-700 is a poor/non-inhibitor of OATP1B1.

In contrast, TR-700 produced a concentration-dependent inhibition of BCRP-mediated transport, with 8%, 20%, and 31% and 58% inhibition observed at 3, 10, 30 and 60 μM, respectively (**Table** 3). An IC_{50} value for inhibition of BCRP-mediated transport (51.1 μM) was calculated using a 4 parameter non-linear regression model with top and bottom constrained to 100% and 0%, respectively (**Figure 1**).

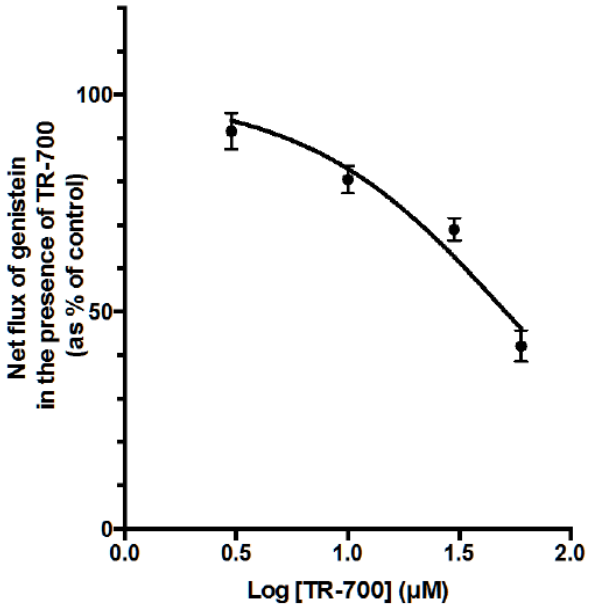
Reference ID: 3476543

Table 3. BCRP-Mediated Genistein Transport in the Absence and Presence of TR-700 or Positive Control Inhibitor

Compound	P_{app}($x10^{-6}$ cm/s)		Net B-A Flux (pmol/hr/cm^2)	Efflux Ratio	% Inhibition
	A-B	B-A			
Genistein	11.9±1.03	72.2±0.61	5.43±0.05	6.07±0.05	NA
Genistein[a]	9.99±0.57	67.2±2.48	5.15±0.22	6.72±0.25	NA
Genistein + 3 µM TR-700	13.9±1.42	69.2±2.49	4.97±0.22	4.97±0.18	8.39±4.13
Genistein + 10 µM TR-700	15.5±1.23	64.1±1.90	4.37±0.17	4.12±0.12	19.5±3.15
Genistein + 30 µM TR-700	20.6±2.89	62.2±1.56	3.75±0.14	3.02±0.08	31.0±2.58
Genistein + 60 µM TR-700[a]	29.5±0.24	53.6±2.03	2.17±0.18	1.82±0.07	57.9±3.56
Genistein + 100 µM Chrysin	40.2±3.86	45.6±3.11	0.48±0.28	1.13±0.08	91.1±5.16
Genistein + 100 µM Chrysin[a]	39.0±3.24	45.4±2.37	0.57±0.21	1.16±0.06	88.9±4.15

Source: Optivia Report OPT-2012-109, Table 2
Abbreviations: A-B=apical-to-basolateral; B-A=basolateral-to-apical; NA=not applicable
[a]Samples contain 2% DMSO

Figure 1. Determination of IC_{50} Value of TR-700 against BCRP-Mediated Transport of Genistein.



Reviewer Comment: *Per the Sponsor's Summary of Clinical Pharmacology Studies, "It should be noted that TR-700 concentrations in this study exceeded the thermodynamic solubility of approximately 8 to 10 µM in Hank's Balanced Salt solution containing 0.5 or 2% DMSO, respectively (BAN-12-0701-059). Inhibition by TR-700 could only be fully characterized by using supersaturated solutions of TR-700 with unknown clinical relevance."*

Reference ID: 3476543

Summary of Study BAN-12-0701-059: *"Kinetic solubility experiments of TR-700 were conducted in Hank's Buffered Salt Solution (HBSS) with 0.5% or 2% dimethyl sulfoxide (DMSO) cosolvent to determine if 30 µM and 60 µM in HBSS, with 0.5% DMSO, remains in solution under standard in vitro testing conditions. The kinetically prepared solutions were also compared to the thermodynamic solubility of TR-700 in HBSS in the presence of 0.5% and 2% DMSO. Quantitation was by chromatographic peak area comparison with a standard curve, prepared by injection of standards at 245, 24.5, and 2.45 µg/mL TR-700 concentrations. This study demonstrated that the kinetic solubility limit of TR-700 in HBSS is ≥30 µM with 0.5% DMSO and is ≥60 µM with 2% DMSO added. The thermodynamic solubility of TR-700 is approximately 3 fold lower in the 0.5% DMSO buffer system. Superstaturated TR-700 solutions were demonstrated to be stable for at least 3 hours and can be prepared for use in in vitro studies by preparing from a concentrated DMSO stock solution."*

Reviewer Comment: *Following administration of multiple doses of 200 mg TR-701 FA once daily in healthy human subjects, the mean steady-state C_{max} values of TR-700 were 2.2 and 3 µg/ml for oral and IV administration, respectively (equivalent to 5.9 and 8.1 µM, respectively). Assuming the IC_{50} derived in this study is correct, $C_{max,ss}/IC_{50}$ is slightly greater than 0.1 (0.12-0.16), which is the cutoff value indicated in the FDA current DDI draft guidance. This is unlikely to be of clinical significance. Of note, TR-701 FA is not intended for chronic administration (i.e. treatment duration not to exceed 6 days).*

SPONSOR'S CONCLUSIONS: TR-700 did not inhibit P-gp and OATP1B1 but did inhibit BCRP with an IC50 of 51.1 µM.

REVIEWER ASSESSMENT: The Sponsor's conclusions appear appropriate based on study results. However, per the FDA current DDI draft guidance, an in vivo drug interaction study with a BCRP substrate is recommended as a Post-Marketing Requirement (PMR).

Additional Reviewer Comments

1. *Of a total of 7 transporter-related in vitro DDI study reports, four study reports related to TR-700 were reviewed; the other three study reports (cph-12-0701-045, cph-12-0701-064, and cph-12-0701-065) related to TR-701 were not reviewed, because 1) partial conversion from TR-701 to TR-700 was observed in in vitro transport systems (i.e., Caco-2 cells; BAN-13-0701-089) and 2) clinical studies showed that TR-701 is rapidly and extensively converted by phosphatases to TR-700 and TR-701 plasma concentrations were undetectable in almost all subjects.*
2.
    a) *The current FDA DDI draft guidance states that "Investigational drugs should be evaluated in vitro to determine whether they are a substrate of hepatic uptake transporters OATP1B1/OATP1B3 when their hepatic pathway is significant (e.g., clearance through hepatic or biliary secretion is more than or equal to 25% of the total clearance)."*
    b) *Based on the results from study TR701-106, hepatic (biliary) excretion appears to be the major elimination pathway for TR-701/TR-700 and its derivatives (TR-700 sulfate conjugate) and renal excretion was a minor pathway.*

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c) *No studies on the investigation of TR-700 as a substrate of OATP1B1/OATP1B3 were conducted by the Sponsor.*
d) *The decision tree in the DDI guidance indicates that, to determine whether an investigational drug is a substrate for OATP1B1 or OATP1B3 and when an in vivo clinical study is needed, the following question needs to be answered first to proceed along the decision tree: "Does the compound have active hepatocyte uptake, do the drug's physiological properties (e.g., low passive membrane permeability, high hepatic concentrations relative to other tissues, organic anion/charged at physiological pH) support importance of active uptake into liver? "*
e) *As shown in the in vitro study (CPH-10-0701-013), TR-700 has high permeability across Caco-2 monolayers, which suggests that TR-700 may enter into hepatocytes primarily via passive diffusion, and the importance of active uptake of via hepatic uptake transporters OATP1B1/OATP1B3, if any, is considered to be low.*
f) *Taken together, it may be appropriate to exempt TR-701 from the in vitro/in vivo evaluation with a substrate of OATPs.*

Reference ID: 3476543

STUDY NO.: TR701-101

A Double-Blind, Placebo- and Active-Controlled, Single- and Multiple-Ascending Dose, Safety, Tolerability, and Pharmacokinetic Study of TR-701 FA in Normal Healthy Adults

Date(s): 06 January 2008– 06 June 2008
Sponsor: Trius Therapeutics, Inc., San Diego, CA
Clinical Site: Covance Clinical Pharmacology Inc., Madison, WI
Analytical Site: (b) (4)

OBJECTIVE(S):

Part A: Single Ascending Dose

- To determine the safety and tolerability of TR-701 in healthy adult subjects when administered as a single oral dose
- To evaluate the PK of single rising doses of TR-701 and its microbiologically active moiety, TR-700, in healthy adult subjects

Part B: 21-Day Multiple Ascending Dose

- To determine the safety and tolerability of TR-701 in healthy adult subjects when administered as multiple oral doses for 21 days
- To characterize the plasma PK and rate and extent of urinary excretion of TR-701 and its microbiologically active moiety, TR-700, in healthy adult subjects
- To determine the threshold for biological evidence of oxazolidinone-induced myelosuppression
- To compare the biological evidence of oxazolidinone-induced myelosuppression for cohorts receiving TR-701 and the cohort receiving a therapeutic dose of linezolid

METHODS

Study Design: Study TR701-101 was a double-blind, placebo- and active-controlled, randomized, single and multiple ascending dose study to assess the safety, tolerance, and PK of oral TR-701 in 80 healthy male and female subjects. Subjects in the single dose portion (Part A) were randomized to receive a single 200 mg, 400 mg, 600 mg, 800 mg, or 1200 mg oral dose of TR-701 or placebo (active:placebo=6:2). Subjects in the multiple dose portion were randomized to receive once-daily oral doses of 200 mg, 300 mg, or 400 mg of TR-701 or 600 mg twice-daily doses of linezolid or placebo for 21 days (**Table 1**). Safety and PK were monitored through 72 hours following the final dose of TR-701. A follow-up phone call for safety evaluation was performed on Day 28±2.

Each dose (TR-701/linezolid/placebo; **Table 2**) was administered orally with 240 mL of room temperature tap water. Doses were followed by a 4-hour fast in Part A and a 1-hour fast in Part B. In Part B, on Days 1, 15, and 21 (or final dosing day), morning doses were preceded by a 10-hour fast and followed by a 4-hour fast. Before the next cohort was dosed, there was at least a 7-day interval following the last dose of TR-701 to evaluate safety and tolerability.

Reference ID: 3476543

Table 1. Dose Cohorts

	Cohort (1-8 for TR701 10 for Linezolid)	TR-701 or Linezolid (n)	Placebo (n)	Dose of TR701 or Linezolid
Part A (n=40)	1	6	2	200 mg
	2	6	2	400 mg
	3	6	2	600 mg
	4	6	2	800 mg
	5	6	2	1200 mg
Part B (n=40)	6	8	2	200 mg QD for 21 days
	7	8	2	300 mg QD for 21 days
	8	8	2	400 mg QD for 21 days
	10 Linezolid	8	2	600 mg BID for 21 days

Table 2. Drug Products

Drug Name	**TR-701**		**Placebo**		**Linezolid (Zyvox®)**
Formulation	capsule		capsule to match 50 mg	capsule to match 200 mg	tablet
Strength	50 mg	200 mg	0 mg		600 mg
Lot Number	07-0111	07-0110	07-0113	07-0112	C071068
Supplier	(b) (4)				Clinic obtained from commercial source (b) (4)
Manufacturer					Pfizer, Inc.

Inclusion Criteria: Males and females of non-childbearing potential; 18-50 years of age (inclusive); 20-29.9 kg/m^2 (inclusive) in body mass index; and in good health as confirmed by medical history, physical exam, and laboratory evaluations were enrolled.

Subjects were required to abstain from any medications (including over-the-counter remedies) from 7 days prior to first dose administration until after completion of follow-up. Subjects were also required to abstain from alcohol-, grapefruit-, or caffeine-containing foods or beverages or foods or beverages with high levels of tyramine from 72 hours before Study Day 1 until after completion of follow-up. Use of selective serotonin re-uptake inhibitors (SSRIs), monoamine oxidase (MAO) inhibitors, and other serotonergic medications were not allowed within 14 days prior to first dose administration until after completion of follow-up.

Reference ID: 3476543

PK Sample Collection: Plasma and urine samples were collected (**Table 3**) and analyzed for pharmacokinetic purposes.

Table 3. Pharmacokinetic sampling scheme for Part A (single-dose) and Part B (multiple-dose)

PART A	
DAY 1	**Plasma** • Pre-dose • 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 18, 24, 48, and 72 hours postdose **Urine** • -24 to 0, 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours postdose and at 24-hour intervals until Clinic Discharge
PART B	
DAY 1	***Groups 1-8 (TR-701)*** **Plasma** • Pre-dose • 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 18, and 24 hours after the first dose **Urine** • -24 to 0, 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours postdose and at 24-hour intervals until Clinic Discharge ***Groups 10 (Linezolid)*** **Plasma** • Pre-dose • 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, and12 hours after first dose (12 hour blood sample was collected prior to second dose) **No urine samples were collected for linezolid.**
DAY 15	**Plasma (TR-701 and Linezolid)** • Pre-dose • 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, and12 hours after first dose (12 hour blood sample was collected prior to second BID dose of linezolid)
DAYS 3-14 **DAYS 16-20**	**Plasma (Trough)** • Pre-dose (0 hour)
DAY 21	***Groups 1-8 (TR-701)*** **Plasma** • Pre-dose (0 hour prior to the last dose) • 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 18, 24, 48, and 72 hours postdose. **Urine** • -24 to 0, 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours postdose and at 24-hour intervals until Clinic Discharge ***Groups 10 (Linezolid)*** **Plasma** • Pre-dose (0 hour prior to the morning dose) • 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, and12 hours after first dose (12 hour blood sample was collected prior to the second dose in BID dosing) **No urine samples were collected for linezolid.**

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Analytical Methods: Pharmacokinetic samples were analyzed for TR-701, TR-700, and linezolid in plasma/urine by validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) (**Table 4**). For the calculation of summary statistics, all concentrations below the limit of quantification (BLQ) were excluded from the PK analysis.

Table 4. Bioanalytical results of TR-701, TR-700, and linezolid in plasma /urine

Criterion	TR-701	TR-700	Linezolid	Comments
PLASMA				
Calibration Range	5-1000 ng/mL	5-1000 ng/mL	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	5 ng/mL	Satisfactory
Linearity, mean R^2	0.9977	0.9985	0.9976	Satisfactory
Accuracy	Within ±7%	Within ±4.8%	Within ±7.2%	Satisfactory
Precision, CV	≤5.2 %	≤4.7 %	≤4.3	Satisfactory
Quality Control*	15, 150, 750, 750(5x) ng/mL	15, 150, 750, 750(5x), 5000 (10x), 5000(50x) ng/mL	15, 150, 800, 5000(10x), 5000(20x), 5000(25x), 5000(50x) ng/mL	Satisfactory
Accuracy	Within ±4%	Within ±4.3%	Within ±6.6%	Satisfactory
Precision, CV	≤10.5 %	≤8.5%	≤6.9 %	Satisfactory
Stability	• Maximum duration from sample collection to end of analysis: 133 days • Stability: 156 days at -60 to -80 ºC		• Maximum duration from sample collection to end of analysis: 96 days • Stability: 369 days at -60 to -80 ºC	Satisfactory
URINE				
Calibration Range	5-1000 ng/mL	5-1000 ng/mL	N/A	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	N/A	Satisfactory
Linearity, mean R^2	0.9983	0.9991	N/A	Satisfactory
Accuracy	Within ±7%	Within ±2.7%	N/A	Satisfactory
Precision	≤5.2 %	≤4.3%	N/A	Satisfactory
Quality Control	15, 150, 750, 5000(10x) ng/mL	15, 150, 750, 5000(10x) ng/mL	N/A	Satisfactory
Accuracy	Within ±6%	Within ±4.7%	N/A	Satisfactory
Precision, CV	≤9.1 %	≤8.2 %	N/A	
Stability	• Maximum duration from sample collection to end of analysis: 179 days • Stability: 378 days at -60 to -80 ºC		N/A	

**Numbers in the parentheses indicate the dilution factor for QC samples.*

Pharmacokinetic Assessment: Pharmacokinetic parameters for TR-701, TR-700, and linezolid were determined using single- and multiple-dose data with non-compartmental methods. Parameters included the following:

- C_{max}, peak observed plasma concentration
- $AUC_{0\text{-}inf}$, area under the curve from time 0 to infinity
- $AUC_{0\text{-}\tau}$, area under the curve during a dosing interval

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- T_{max}, corresponding time of C_{max}
- C_{min}, pre-dose trough plasma concentration
- λ_Z, apparent terminal phase elimination rate constant
- $t_{1/2}$, elimination half-life
- CL/F, apparent oral clearance
- CL_r, renal clearance
- Ae, plasma and amount of drug excreted in urine
- Fe, percent of dose excreted in urine
- V_{ss}, steady-state volume of distribution
- V_z/F, apparent volume of distribution of terminal phase
- Accumulation Ratio, ratio of last dose AUC_τ to first dose AUC_τ

Statistical Methods:

Statistical Analysis of Pharmacokinetic Data

Descriptive statistics were calculated where appropriate for PK parameters, including C_{max}, T_{max}, AUC_{0-12}, AUC_{0-24}, $AUC_{0-\tau}$, and AUC_{0-inf} (Part A and B) including the mean, standard deviation, median, minimum, and maximum. Dose-proportionality was assessed by a linear regression model on dose normalized (DN) PK parameters which were C_{max} and AUC_{0-inf} for Part A, C_{max} and AUC_{0-inf} on the first dosing day, and C_{max} and AUC_{0-24} on last dosing day for Part B. The model was the function of continuous variable dose. Data for DN PK parameters were natural log-transformed prior to analysis.

Statistical Analysis of Hematology Data

A repeated-measure analysis of covariance was performed for selected hematology data to assess the trends across day between active treatments in Part B and pooled placebo. The linear model of hematology parameters was built on data from Baseline (defined as Day 0 in model) to Day 21 and also in 3 time periods. To further examine the relationship between the drug exposure and observed maximum hematology parameter change, a correlation analysis was performed between maximum change from baseline in hematology value and C_{max} and AUC of TR-700 and linezolid on the last dosing day. The hematology parameters included platelet, red blood cell (RBC), white blood cell (WBC), absolute neutrophil counts (ANCs), and absolute reticulocyte counts.

Safety Assessment: Safety and tolerability were assessed by physical examinations, medical history, clinical laboratory evaluations (chemistry panel including 20 analytes, complete blood count, and urinalysis [UA]), 12-lead ECGs, adverse events (AEs), and vital signs at protocol-specified timepoints. In Part B, safety and tolerability assessments also included ophthalmologic and neurologic physical examinations and additional clinical laboratory evaluations (plasma L-lactate and reticulocyte count).

RESULTS

Study Population: In total, 80 healthy adults were enrolled (n=40 for both Part A and Part B). All 40 of the subjects enrolled in Part A of the study completed the study. In Part B, 4 of the 40 subjects enrolled discontinued due to AEs (please refer to the Safety section below for details).

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There were no protocol violations during Part A (Single Ascending Dose); however, protocol violations were noted for 21 subjects during Part B (Multiple Ascending Dose). For the 10 subjects in Cohort 7 (200 mg TR-701), baseline reticulocyte counts were not measured. This omission confounded analysis of potential effects of TR-701 on this parameter at this dose level. In Cohort 10 (600 mg linezolid BID), 9 subjects received a second dose of linezolid or placebo on Day 21 in violation of the protocol. As a result, the Day 21 linezolid PK parameter $AUC_{0-\infty}$ was not reported for Day 21.

Protocol violations were also observed for 2 individual subjects. Subject No. 048 (Cohort 6; 400 mg TR-701) withdrew early from the study and therefore had unscheduled collections on Day 10, the last day dosed, from 0 to 72 hours postdose to define the PK profile, and no samples were collected on Day 15 or on Day 21. As steady state was attained by Day 3, Day 10 data were used in place of Day 21 data for the TR-700 PK analysis for Subject No. 048. Additionally, the 3 hour sample on Day 10 was not processed due to unknown reasons. Day 21 urine samples were missing and as a result, fe and amount of drug excreted in urine were not reported for Day 21 for this subject. Similarly, the 2 hours postdose PK sample was not collected for Subject No. 064 (Cohort 8; 300 mg TR-701) and this missing sample was also adjacent to the subject's C_{max} value, this timepoint was excluded from the PK analysis. The remaining issues are mainly involved with missing single blood or urine sampling timepoints, which is considered not to significantly affect the analysis because these timepoints were not necessary for determination of PK parameters.

Reviewer Comment: These deviations documented above do not appear to adversely affect the results of the study.

Demographics:
A summary of demographic and baseline characteristics for the study population is presented in **Table 5**. There were no substantial differences in demographic characteristics between the study groups.

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Table 5. Demographic Characteristics

Demographic Variable	Part A Single Ascending Dose N = 40	Part B Multiple Ascending Dose N = 40
Mean age in years (range)	25 (18 to 41)	33 (18 to 49)
Mean weight in kg (range)	73.1 (52.2 to 92.9)	80.3 (60.3 to 100.9)
Mean height in cm (range)	173.7 (158.2 to 189.1)	176.0 (159.0 to 190.0)
BMI in kg/m^2 (range)	24.2 (20.1 to 30.0)	25.8 (22.2 to 29.5)
Gender (n[%])		
Male	23 (57.5%)	31 (77.5%)
Female	17 (42.5%)	9 (22.5%)
Ethnicity (n[%])		
Hispanic or Latino	6 (15.0%)	3 (7.5%)
Not Hispanic or Latino	34 (85.0%)	37 (92.5%)
Race (n[%])		
White	36 (90.0%)	28 (70.0%)
Black	3 (7.5%)	8 (20.0%)
Other: Filipino	1 (2.5%)	---
Other: Biracial	---	1 (2.5%)
Other: Hispanic	---	1 (2.5%)
Other: Japanese/American	---	1 (2.5%)
Other: Puerto Rican	---	1 (2.5%)

Pharmacokinetics:
Pharmacokinetic parameters of TR-700 and linezolid following single and multiple dose administration are listed in **Table 6** and **Table 7**, respectively. Concentration-time profiles of TR-700 and linezolid are displayed in **Figures 1-2** (single- and multiple-dose) and **Figure 3**, respectively. Dose proportionality on mean TR-700 PK parameters for single- and multiple-dose studies are summarized in **Table 8** and **Table 9**, respectively.

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Table 6. Mean (SD) Plasma and Urinary PK Parameters for TR-700 after Single Ascending Dose Administration under Fasted Conditions

PK Parameter	200 mg TR-701	400 mg TR-701	600 mg TR-701	800 mg TR-701	1200 mg TR-701
C_{max} (μg/mL)	1.99 (0.43)	3.77 (0.96)	5.21 (0.73)	5.52 (1.19)	9.49 (1.92)
t_{max} (hr)[a]	3.01 (1.0, 4.0)	3.50 (2.0, 4.0)	2.50 (2.0, 4.0)	4.00 (2.0, 8.0)	4.00 (2.0, 4.0)
$AUC_{0-\infty}$ (μg•hr/mL)	25.35 (4.6)	56.10 (13.20)	79.26 (31.34)	91.80 (12.87)	123.10 (31.20)
$t_{½}$ (hr)	11.2 (3.59)	10.8 (0.80)	11.4 (2.57)	10.6 (1.29)	10.4 (1.43)
CL/F (L/hr)	6.08 (1.08)	5.58 (1.23)	6.58 (3.00)	6.65 (0.94)	7.77 (2.24)
V_z/F (L)	95.7 (23.5)	87.0 (18.0)	101 (23.5)	101 (14.0)	116 (29.2)
$f_{e\,(0-72)}$ (%)	0.638 (0.291)	0.876 (0.462)	0.664 (0.347)	0.518 (0.222)	0.660 (0.254)
CL_R (mL/hr)	41.5 (13.0)	54.4 (30.6)	41.0 (22.4)	36.7 (13.2)	53.8 (13.3)

[a]*Median (minimum, maximum) presented for T_{max}*

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Table 7. Summary of the Mean (SD) Plasma Pharmacokinetic Parameters for TR- 700 and Linezolid after Multiple Dose Administrations under Fasted Conditions

	TR-701 Dose						Linezolid Dose	
	200 mg QD[a]		300 mg QD		400 mg QD		600 mg BID	
PK Parameter	**Day 1**	**Day 15[b]**	**Day 1**	**Day 21**	**Day 1**	**Day 21**	**Day 1**	**Day 21**
C_{max} (µg/mL)	1.8 (1.2)	1.8 (0.4)	2.1 (0.5)	2.7 (0.5)	4.2 (0.8)	4.7 (0.5)	12.2 (3.0)	16.5 (4.6)
C_{min} (µg/mL)	NA	0.41 (0.17)	NA	0.51 (0.14)	NA	0.85 (0.16)	NA	5.1 (2.6)
t_{max} (hr)[c]	3 (1.5, 4)	3 (2, 4)	2 (1.5, 4)	3 (1.6, 8)	4 (1, 4)	2.5 (1.5, 4)	1 (0.35, 1.5)	1.5 (0.5, 4)
$AUC_{0-\tau}$ (µg•hr/mL)	16.7 (3.8)	22.5 (6.5)	24.1 (5.4)	31.2 (6.6)	46.0 (6.4)	52.0 (5.1)	65.9 (19.2)	114.6 (38.2)
$AUC_{0-\infty}$ (µg•hr/mL)	21.6 (6.5)	NC	29.6 (7.5)	NC	54.0 (8.2)	NC	78.1 (31.6)	NC
$t_{½}$ (hr)	11.1 (1.20)	10.2 (2.05)	10.1 (1.36)	11.9 (1.67)	8.01 (1.17)	12.8 (1.42)	3.80 (1.67)	5.75 (1.15)
CL/F (L/hr)	7.48 (2.12)	7.16 (1.99)	7.98 (1.80)	7.51 (1.60)	5.66 (0.84)	5.82 (0.61)	8.73 (3.09)	5.76 (1.89)
V_z/F (L)	117 (21.9)	108 (38.1)	116 (27.8)	127 (22.0)	64.8 (9.96)	108 (20.40)	42.9 (11.2)	46.1 (12.7)
Accumulation Ratio								
Observed[d]	1.31 (0.11)		1.31 (1.18)		1.18 (0.13)		1.72 (0.22)	
Predicted[e]	1.31 (0.08)		1.24 (0.05)		1.17 (0.06)		1.16 (0.14)	
Linearity Factor[f]	1.00 (0.08)		1.02 (0.10)		1.01 (0.10)		1.55 (0.32)	

[a]*Therapeutic dose.*
[b]*Steady state Day 15 and Day 21 PK results were comparable for most individuals; Day 15 shown for 200 mg dose due to one subject suspected to have missed the Day 21 administration).*
[c]*Median (minimum, maximum) presented for* T_{max}.
[d]*Observed Accumulation Ratio=Day 15/21 AUC0-τ / Day 1 AUC0-τ.*
[e]*Predicted Accumulation Ratio=Day 1 AUC0-∞, / Day 1 AUC0-τ*
[f]*Linearity Factor=Day 15/21 AUC0-τ / Day 1 AUC0-∞.*

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Figure 1. Mean plasma concentration-time profiles for TR-700 in healthy human subjects following single ascending oral doses

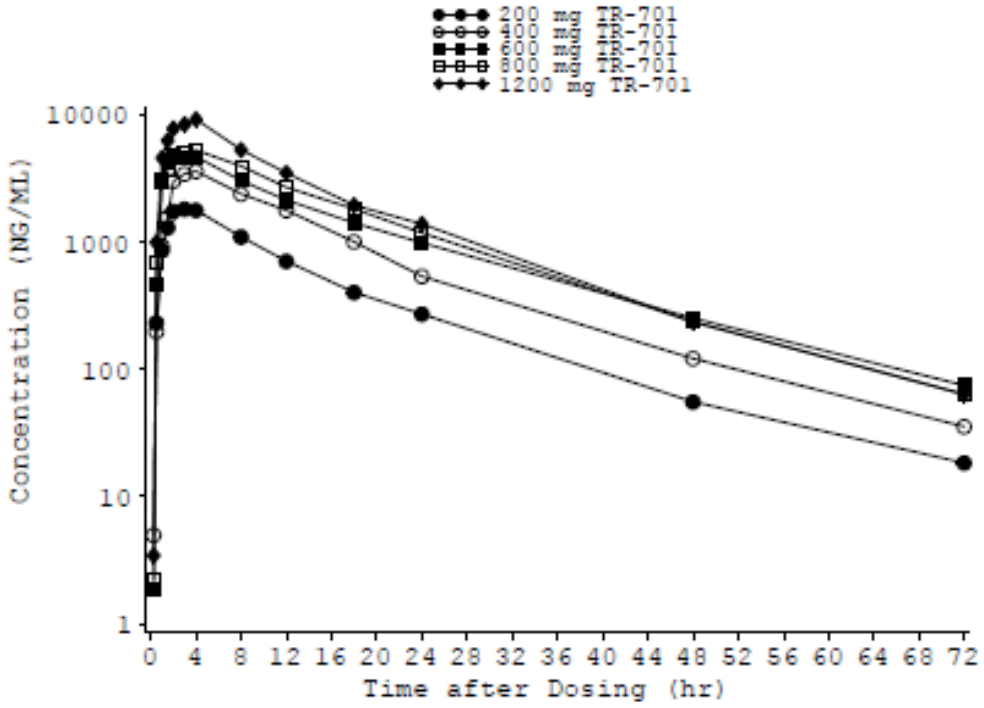
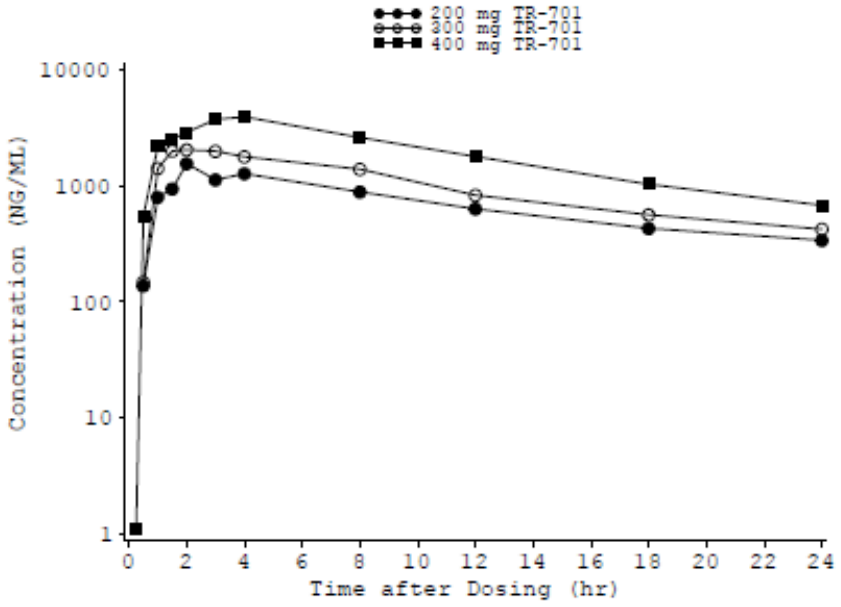


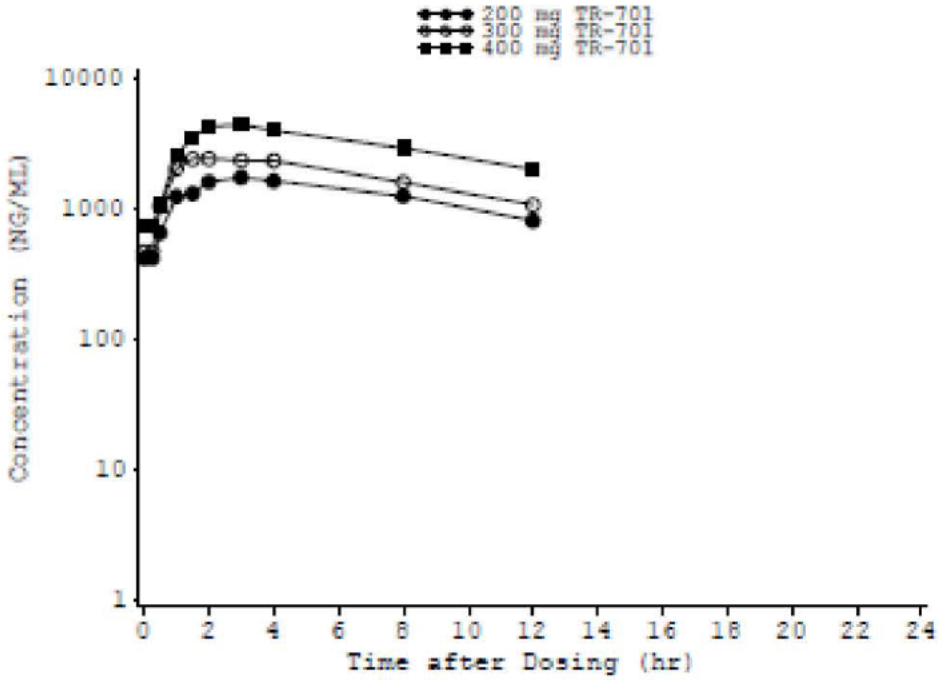
Figure 2. Mean Plasma Concentration-Time Profiles for TR-700 on Day 1 (A), Day 15 (B), and Day 21 (C) in healthy human subjects following multiple ascending oral doses of 200, 300, and 400 mg QD for 21 days

A)



Reference ID: 3476543

B)



C)

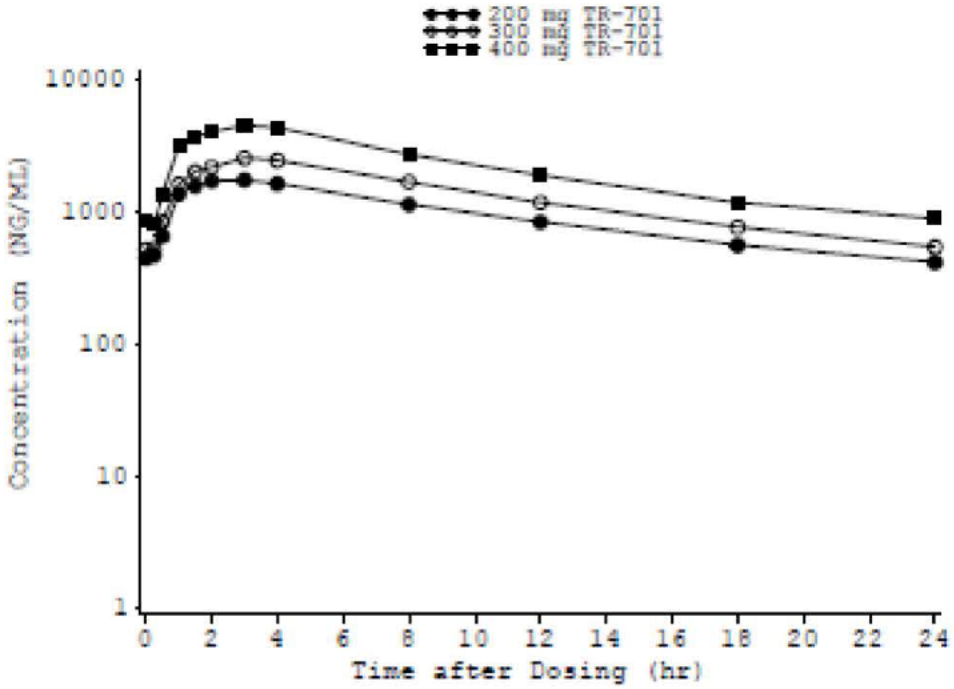


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Figure 3. Mean Plasma Concentration-Time Profiles for linezolid on Day 1 and Day 21 in healthy human subjects following oral doses of 600 mg BID for 21 days

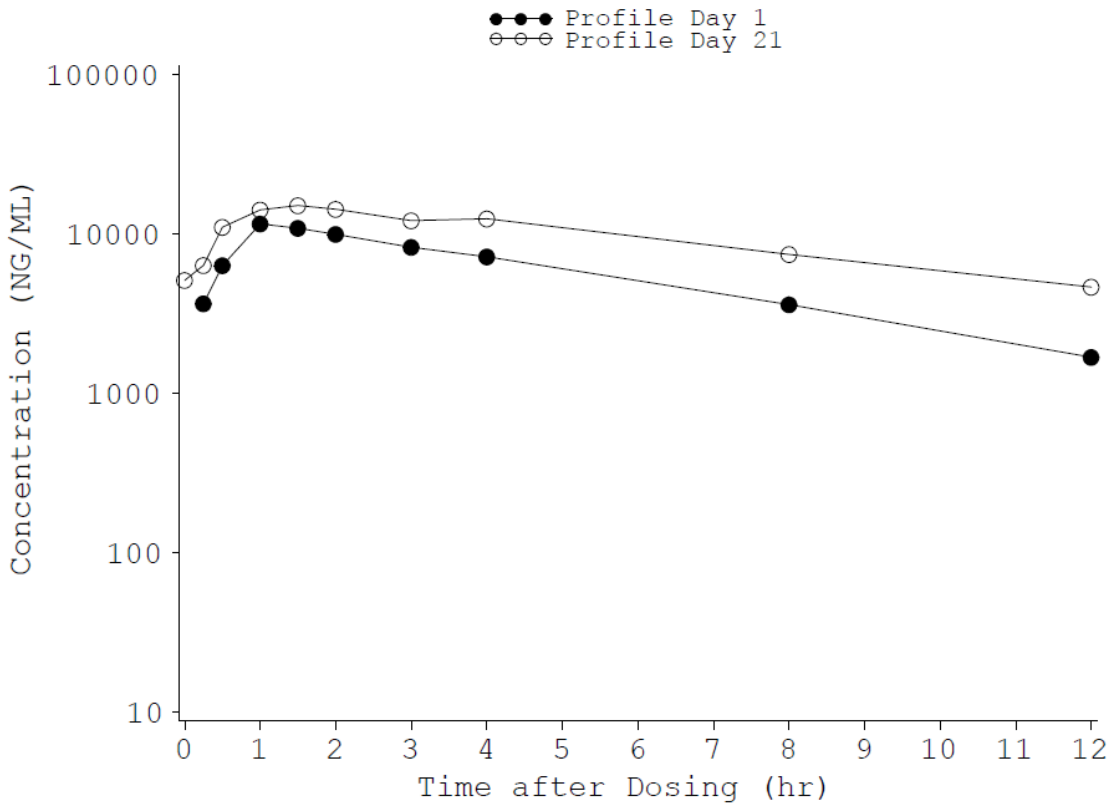

Table 8. Dose (DN) and Weight (WN) Normalized Pharmacokinetics and Statistical Analysis of Dose-Proportionality on Mean (SD) TR-700 PK Parameters for Part A (Single Ascending Dose)

PK Parameter	200 mg TR-701	400 mg TR-701	600 mg TR-701	800 mg TR-701	1200 mg TR-701
DN-C_{max}(ng/mL/mg)	13.3 (2.86)	12.6 (3.20)	11.6 (1.61)	9.20 (1.98)	10.5 (2.14)
DN-$AUC_{0\text{-}inf}$ (ng*hr/mL/mg)	169 (30.7)	187 (44.0)	176 (69.7)	153 (21.4)	137 (34.7)
WN-C_{max} ([ng/mL]/[mg/kg])	914 (132)	908 (245)	805 (155)	742 (112)	746 (90.2)
WN-$AUC_{0\text{-}inf}$ ([ng*hr/mL]/[mg/kg])	11858 (2736)	13543 (3500)	12653 (6388)	12558 (2373)	9754 (2463)

Part	Parameters	N[a]	Slope[b]	P-value[c]
Part A	DN-C_{max}	30	-.000283	0.0197*
	DN-$AUC_{0\text{-}inf}$	30	-.000277	0.0524

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Table 9. Dose (DN) and Weight (WN) Normalized Pharmacokinetics and Statistical Analysis of Dose-Proportionality on Mean (SD) TR-700 PK Parameters for Part B (Multiple Ascending Dose)

PK Parameter		200 mg QD TR-701		300 mg QD TR-701		400 mg QD TR-701	
		Day 1	Day 21	Day 1	Day 21	Day 1	Day 21
DN-C_{max} (ng/mL/mg)	Mean	12.0	12.8	9.49	11.9	13.8	15.6
	SD	8.30	5.70	2.26	2.37	2.63	1.63
	CV%	69	44	24	20	19	10
DN-AUC[a] (ng*hr/mL/mg)	Mean	144	148	132	139	180	173
	SD	43.6	67.3	33.2	29.3	27.3	17.1
	CV%	30	46	25	21	15	10
WN-C_{max} ([ng/mL]/[mg/kg])	Mean	1103	1126	786	993	1029	1172
	SD	874	455	139	189	164	150
	CV%	79	40	18	19	16	13
WN-AUC[a] ([ng*hr/mL]/[mg/kg])	Mean	12726	12966	10799	11629	13525	13066
	SD	3167	5462	2143	2662	2310	2051
	CV%	25	42	20	23	17	16

Part	Parameters	N[b]	Slope[c]	P-value[d]
Part B (first day)	DN-C_{max}	24	0.001293	0.1538
	DN-$AUC_{0\text{-}inf}$	22	0.001301	0.0554
Part B (last dosing day)	DN-C_{max}	24	0.001689	0.1086
	DN-$AUC_{0\text{-}24}$	24	0.001501	0.1441

Single- and Multiple-dose PK of TR-701 (prodrug)

Plasma:

Following a single oral dose administration of TR-701 capsules at 200, 400, 600, 800, and 1200 mg doses, only one subject at the highest dose (Subject 040, Cohort 5, 1200 mg TR-701) had quantifiable concentration results for TR-701 in plasma. For this subject, TR-701 plasma concentrations were quantifiable for 3 hours allowing for the determination of T_{max}, C_{max}, and $AUC_{0\text{-}t}$ values of 1.00 hours, 16.9 ng/mL, and 24.1 ng·hr/mL, respectively. TR-701 was not detected in any subject following QD oral administrations of 200, 300, or 400 mg TR-701 through 21 days.

Urine:

Following single oral dose administrations of TR-701 capsules, mean recovery of TR-701 in urine was undetectable in most patients at the lower doses and overall was low with less than 0.001% of the dose recovered through 72 hours for all dose levels. In Part B, TR-701 was generally not detected in the urine following multiple QD oral administrations of 200, 300, or 400 mg TR-701 regardless of days for urine sample collection.

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Reviewer Comment: Sponsor's PK summary of TR701 in plasma and urine are supported by the individual PK data.

Single- and Multiple-dose PK of TR-700 (active metabolite)

Plasma:

After a single oral administration of TR-701, TR-700 rapidly reached the systemic circulation and remained quantifiable for the duration of the blood sampling period up to72 hours (**Figure 1**). A summary of the mean (standard deviation [SD]) plasma PK parameters for TR-700 (Part A: Single Ascending Dose) is presented in **Table 6**.

Following oral administration of TR-701 capsules at 200, 300, and 400 mg QD for 21 days, TR-700 peak concentrations were observed rapidly, with median T_{max} values of 2.02 to 4.00 hours across Days 1, 15, and 21 (**Table 7** and **Figure 2**). Mean TR-700 C_{max} and $AUC_{0\text{-}inf}$ values following 200 to 400 mg doses of TR-701 were generally comparable to those results observed in Part A of this study and increased in a dose proportional manner. TR-700 was eliminated with mean TR-700 $t_{1/2}$ values ranging overall from 8.01 to 14.7 hours (**Table 7**). Large fluctuations were observed in the trough concentration levels collected from each subject; however, mean trough concentration values were relatively flat after a few days of dosing (Day 3 onwards), suggesting steady state had been achieved. Based on comparison of $AUC_{0\text{-}24}$ on Day 21 versus Day 1, moderate accumulation was observed with mean values ranging from 1.18 to 1.31 across all dose levels.

Reviewer Comment: Based on PK results shown in Table 7, the mean TR-700 $t_{1/2}$ values ranged from 8.01 to 12.8 hours, instead of 8.01 to 14.7 hours as reported by the Sponsor.

Dose normalization analysis (**Tables 8 and 9**) showed that the PK of TR-700 following both single administration from 200 to 1200 mg doses and QD dosing of 200 to 400 mg, resulted in dose proportional systemic exposure, as measured by AUC ($p>0.05$). However, TR-700 C_{max} was significantly less than dose proportional following single ascending doses of 200-1200 mg ($p<0.05$); TR-700 C_{max} was proportional following the QD 200 to 400 mg dosing regimen of TR-701 ($p>0.05$). There appears to be a general numeric decrease in both weight-normalized parameters, as was observed with dose-normalizing. The sponsor explained that the size of the study probably precludes the determination of a significant effect on TR-700 exposure due to weight.

Reviewer Comment: The effect of weight on TR-700 exposure was further analyzed via population PK analysis (CLN-13-0701-072). We defer to the Pharmacometric reviewer for evaluation of weight as a potential covariate of TR-700 PK.

Urine:

The TR-700 urine PK results were similar between single- and multiple-dose administrations and renal excretion of TR-700 was less than 1% of dose administered (**Table 6**).

Multiple-dose PK of Linezolid (active control)

Following multiple oral administration of 600 mg linezolid capsules BID, median linezolid T_{max} values were 1.00 and 1.50 hours on Days 1 and 21, respectively (**Table 7**). Steady state

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concentrations of linezolid were reached after 5 days of BID administration. Linezolid was readily absorbed and eliminated in a log monoexponential manner with mean $t_{1/2}$ values increasing from 3.80 and 5.75 hours on Days 1 and 21, respectively (**Table 7**). Likewise, mean linezolid C_{max} and AUC values increased with repeat BID dosing as a function of the duration of dosing, with the highest values observed on Day 21. Linezolid mean CL/F values decreased approximately 34% from Day 1 to Day 21, with values on Day 1 of 8.73 L/hr and decreasing to 5.76 L/hr on Day 21. Mean linezolid Vz/F values were comparable on Days 1 and 21 with observed values of 42.9 and 46.1 L, respectively (**Table 7**). Low inter-subject variability was observed for mean linezolid PK values. The mean estimated accumulation ratios of linezolid for Cohort 10 on Day 15 and Day 21 were 1.61 and 1.72, respectively. The mean predicted accumulation ratio of linezolid for Cohort 10 was 1.16. The linearity ratio of linezolid for Cohort 10 ranged from 1.40 to 1.55 indicating the observed accumulation was greater than predicted.

Reviewer Comment: The observed PK of linezolid following 600 mg BID dosing in this study are consistent with those reported for linezolid in the literature.

Safety:
Overall, 39 of the 80 subjects reported at least 1 TEAE and 33 of these subjects experienced TEAEs that were judged treatment-related. The majority of TEAEs were mild and no severe TEAEs were reported. There were no deaths or SAEs during the study; however, 4 subjects discontinued from the study due to TEAEs.

Part A (Single Ascending Dose)
Thirteen (43.3%) of the 30 subjects receiving TR-701 in Part A reported at least 1 TEAE and none of the 10 subjects receiving placebo reported any TEAE. Ten of the 30 subjects receiving TR-701 experienced TEAEs that were judged treatment-related. Although only subjects receiving TR-701 reported TEAEs, there was no apparent dose-related trend in the number of subjects reporting TEAEs. The greatest number of subjects (4 subjects [66.7%]) reported TEAEs following administration of 200 mg TR-701 and the fewest (1 subject [16.7%]) reported TEAEs following administration of 800 mg TR-701.

The most common TEAEs reported following single doses of TR-701 were nausea (3 subjects [10.0%]), dizziness (2 subjects [6.7%]), diarrhea (2 subjects [6.7%]), and nasal congestion (2 subjects [6.7%]).

Part B (Multiple Ascending Dose)
Over the 21-day dosing period in Part B, 17 (70.8%) of the 24 subjects receiving TR-701, 4 (50.0%) of the 8 subjects receiving linezolid, and 5 (62.5%) of the 8 subjects receiving placebo reported at least 1 TEAE. Fifteen (62.5%) of the subjects receiving TR-701, 4 (50.0%) of the subjects receiving linezolid, and 4 (50.0%) of the subjects receiving placebo experienced TEAEs that were judged treatment-related. Similar numbers of subjects reported TEAEs in each treatment; although, the greatest number of subjects reported TEAEs following administration of 400 mg TR-701 (7 subjects [87.5%]), the highest dose administered in Part B. The numbers of subjects reporting treatment-related TEAEs were also similar for each treatment.

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All TEAEs were mild or moderate in severity; no severe TEAEs were reported. There were no apparent trends in TEAE severity. The greatest number of subjects reported moderate TEAEs following administration of 400 mg TR-701 (3 subjects [37.5%]), but 1 subject (12.5%) receiving placebo reported a moderate TEAE, and no subjects receiving 200 mg TR-701 reported a moderate TEAE. The most common TEAEs reported following multiple dose administration of TR-701 were nausea (5 subjects [20.8%]), headache (5 subjects [20.8%]), stomach discomfort (4 subjects [16.7%]), dysgeusia (3 subjects [12.5%]), and pharyngolaryngeal pain (3 subjects [12.5%]). With the exception of pharyngolaryngeal pain, each of these TEAEs was reported by a greater number of subjects receiving the highest dose of TR-701 than any of the other treatments administered.
There were no deaths or SAEs during Part B; however, 4 subjects reported Significant AEs resulting discontinuation from the study. Subject Nos. 050 and 084 discontinued due to reticulocyte counts <75% of LLN, Subject No. 058 discontinued due to ALT values >2 x ULN and increased gamma glutamyl transferase (GGT) values that were reported as an AE, and Subject No. 048 discontinued due to an AE of abnormal laboratory test which was a white blood cell (WBC) count <75% of LLN.

Hematology Parameters
The frequency of subjects with significant decreases from baseline for selected hematologic parameters in the multiple dose study is summarized in **Table 10**.

Table 10. Frequency Count of Subjects (%) with Significant Decreases from Baseline for Selected Hematologic Parameters for Part B (Multiple Ascending Dose)

Treatment	Number of Subjects Receiving Treatment	Number of Subjects (%) with Significant Decrease Compared to Baseline Value		
		Platelet Count >20%	ANC >40%	Reticulocyte Count >50%
Placebo	8	1 (12.5%)	1 (12.5%)	---
200 mg TR-701	8	4 (50.0%)	2 (25.0%)	---[a]
300 mg TR-701	8	6 (75.0%)	1 (12.5%)	1 (12.5%)
400 mg TR-701	8	8 (100%)	3 (37.5%)	5 (62.5%)
600 mg Linezolid BID	8	5 (62.5%)	5 (62.5%)	3 (37.5%)

Platelet Count
There was a trend toward decreased platelet counts in subjects receiving multiple doses of TR-701 (**Figure 4**). In the first interval (Baseline through Day 6) there were no treatments with a statistically significant trend across days. In Interval 2 (Day 6 through 15), however, all treatments including placebo demonstrated significant decreases in platelet count. The decline in the 400 mg TR-701 treatment was statistically different than placebo, while the other treatments were not. By the third time interval (Days 15 through 21), only the 600 mg linezolid BID treatment had a statistically significant slope across days, and this slope was positive (indicating the values were increasing and returning toward baseline).

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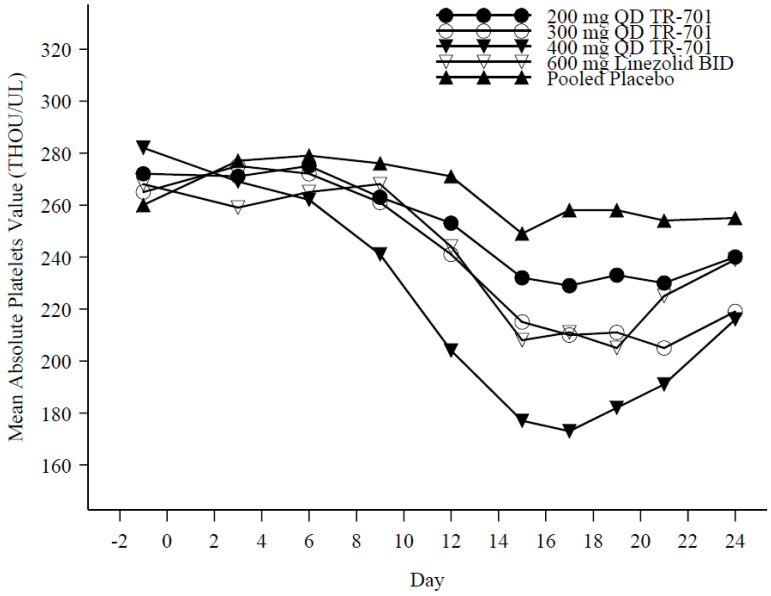
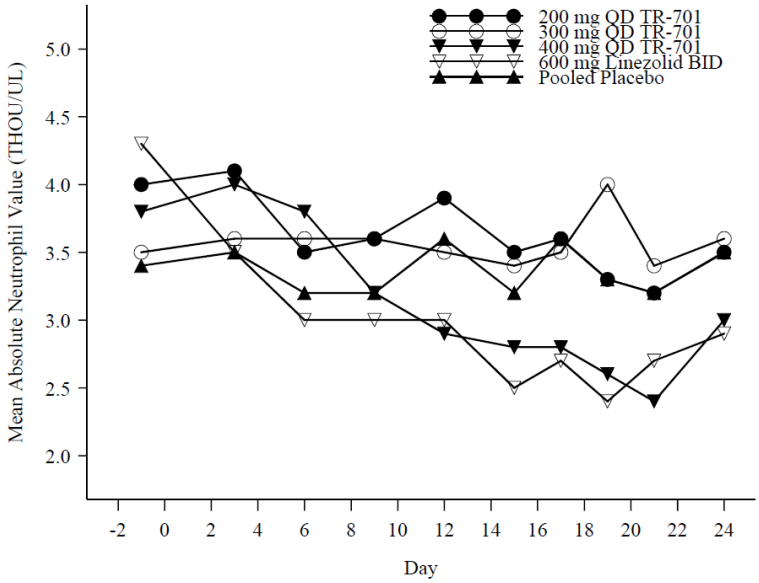
Figure 4. Mean Platelet Counts over Time for Part B (Multiple Ascending Dose)



Absolute Neutrophil Count (ANC)

There was a trend toward decreased ANCs in subjects receiving the highest multiple dose of TR-701 (400 mg) or 600 mg linezolid BID (**Figure 5**). Beginning on Day 6 through 15, and continuing through the interval from Day 15 through 21, the 400 mg TR-701 group demonstrated a statistically significant downward trend, which during the interval from Day 6 to 15 was also statistically different than the trend seen in the placebo group. The maximum mean percent change from baseline in TR-701 (400 mg QD) or linezolid (600 mg BID) groups was 37% and 38%, (respectively, compared with 2%, 18%, and 4% for subjects receiving placebo, 200 mg QD, and 300 mg QD TR-701, respectively.

Figure 5. Mean Absolute Neutrophil Count (ANC) Values over Time for Part B (Multiple Ascending Dose)



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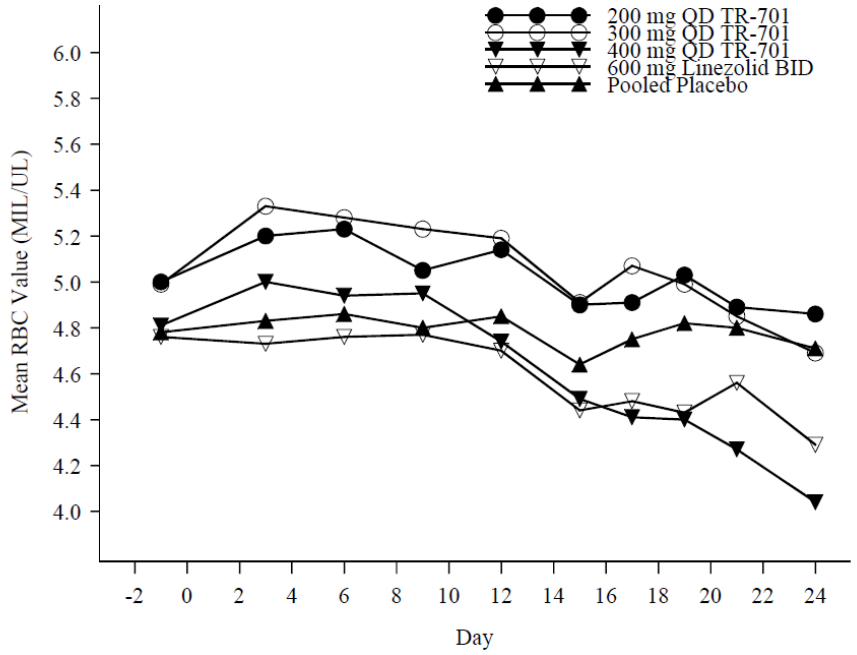
Red Blood Cell (RBC) Count
Similar to the trend seen with platelets, there was a general downward trend in all treatment groups over the course of the study. The decrease appeared greater in subjects receiving 400 mg TR-701 or 600 mg linezolid BID. The results of statistical analysis for RBC count were generally consistent with these observations. From Day 6 through 15, all treatments (including placebo), demonstrated downward trends in RBC count. Only the 400 mg TR-701 treatment group had a significantly greater slope than placebo. During the interval from Day 15 through 21, the 400 mg TR-701 treatment group was significantly less than zero and different from placebo.

Figure 6. Mean Red Blood Cell (RBC) Counts over Time for Part B (Multiple Ascending Dose)



Reticulocyte Count
A greater number of subjects had significant decreases from baseline in reticulocyte count (>50% decrease) in the 400 mg TR-701 and 600 mg linezolid BID treatment groups (5 subjects receiving 400 mg TR-701 and 3 subjects receiving 600 mg linezolid BID compared with 1 subject receiving 300 mg TR-701 and no subjects receiving placebo; change from baseline could not be assessed for subjects receiving 200 mg TR-701 since samples for baseline reticulocyte counts were not collected). This decrease met the protocol-specified criteria for potential early signals of myelosuppression (<75% LLN) in 1 subject receiving 400 mg TR-701 and 1 subject receiving 600 mg linezolid. All values returned to within the normal range.

White Blood Cell Count
There appeared to be a gradual decrease in WBC count in subjects receiving 200 mg TR-701, 400 mg TR-701, and 600 mg linezolid BID. The greatest mean percent decreases from baseline were observed in subjects receiving 400 mg TR-701 and 600 mg linezolid BID and these decreases were similar between the two treatments.

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Correlation between Pharmacokinetic Parameters and Hematology Data
The correlation analysis of PK (C_{max} and AUC) and hematologic parameters was performed within each dose group. Results showed there is little or no significant association between hematological parameter values and log plasma concentration of study drug. However, as demonstrated in the repeated measures analysis of hematology data, there is a clear dose response relationship with decreases in hematological values.

Reviewer Comment: The exposure-response relationship of TR-700 for hematologic parameters was further analyzed via population PK analysis (CLN-13-0701-072). We defer to the Pharmacometric reviewer for evaluation of the correlation between TR-700 exposure and hematologic safety response.

SPONSOR'S CONCLUSIONS

- No SAEs were reported with single- or multiple-dose exposures
- Single doses of 200, 400, 600, 800, and 1200 mg TR-701 were generally well-tolerated in this group of healthy male and female subjects
- Multiple doses of 200 and 300 mg TR-701 for 21 days were generally well tolerated; however, there was a higher incidence of mild to moderate TEAEs with multiple doses of 400 mg TR-701 for 21 days in this group of healthy subjects confined to a CRU for 25 days
- There was a more pronounced decrease in platelet and ANCs in subjects receiving the highest multiple dose of TR-701 (400 mg QD). This effect appeared approximately 7 days after dosing and stabilized after approximately 2 weeks
- For the 7-day exposure anticipated for the treatment of patients with complicated skin and skin structure infections, all TR-701 doses were generally well-tolerated
- The correlation analysis of hematology and log PK parameters demonstrates that within any dose group, there is little or no association between hematological parameter values and plasma concentration of study drug in log transformation. However, as demonstrated in the repeated measures analysis of hematology data, there is a clear dose response relationship with decreases in hematological values
- TR-701 was rapidly and extensively converted to the active moiety TR-700, and <1% of the dose was excreted in urine as either TR-701 or TR-700
- After single (200, 400, 600, 800, and 1200 mg) administration of TR-701, dose proportional increases in AUC, and less than proportional increases in C_{max}, were observed for TR-700
- After repeat (200, 300, and 400 mg) administration of TR-701, dose proportional increases in C_{max} and AUC were observed for TR-700
- The CL/F values for TR-700 were generally independent of dose, whereas linezolid showed decreased CL/F values following multiple BID dosing
- Steady state TR-700 PK was predicted by single dose kinetics across the 3 doses as observed and predicted accumulation ratios were similar, and the resulting linearity factor was close to 1
- Greater than predicted accumulation (approximately 72%) after repeated BID administration over 21 days was observed for linezolid, resulting in a linearity factor of approximately 1.6

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- Approximately 2-fold increases in mean TR-700 $t_{1/2}$ and Vz/F (Day 21) values were observed compared to linezolid

REVIEWER ASSESSMENT: The Sponsor's conclusions are appropriate based on study results. For labeling, only steady-state pharmacokinetic data of TR-700 for the proposed therapeutic 200 mg QD regimen will be used; pharmacokinetic data of prodrug TR-701 will not be included.

Reference ID: 3476543

STUDY NO.: TR701-102

An Open-Label, Single Dose, Microdialysis and Pharmacokinetic Study of TR-701 in Normal, Healthy Adults

Date(s): 17 April 2008 – 12 August 2008
Investigator(s): Hartmut Derendorf, Ph D.
Clinical Site(s): General Clinical Research Center, University of Florida, Gainesville, FL, US
Analytical Site(s): Plasma samples - (b) (4)
Dialysate samples – (b) (4)

OBJECTIVE(S):

- Pilot study: To investigate the feasibility of using the microdialysis technique to measure the recovery of a calibration solution of TR-700 (the biologically active moiety of TR-701), when administered via a subcutaneous microdialysis probe. Another objective of the Pilot Study was to determine the necessary washout period needed to ensure all of the calibration solution is cleared from the tissues and microdialysis probes before oral dosing.
- Main study: 1) To measure the penetration of TR-700 into subcutaneous (s.c.) adipose tissue and skeletal muscle by determining the concentration-time profiles of the TR-700 in plasma and in tissues using the microdialysis technique after administration of a single 600 mg oral dose of TR-701 days; 2) To further assess the safety and tolerability of TR-701 after a 600 mg single oral dose.

METHODS

Study Design: A preliminary Pilot Study was conducted in 3 subjects, administered a TR-700 solution directly into the subcutaneous microdialysis probe. An additional 12 subjects were enrolled in the Main Study, an open-label, nonrandomized, plasma and tissue PK study following a single 600 mg oral dose of TR-701. In the Main Study, after washout of the calibration solution, each subject received a single oral dose of 600mg TR-701. Subjects were admitted to the General Clinical Research Center (GCRC) the day before dosing and were confined to the GCRC until all post study procedures were completed approximately 24 hours postdose.

Inclusion/Exclusion Criteria: Healthy subjects from 18 to 50 years of age.

Treatment:
Doses of 0.36 µg TR-700 (0.18 µg per probe; 2 µg/mL at a flow rate of 1.5 µL/min for 60 min, solution administered, Lot Number M009-08) were administered as s.c. infusions through the microdialysis probe for 60 minutes in both the Pilot Study and the Main Study. In the Main Study, TR-701 600mg (200 mg per oral capsule, Lot Number 07-0110) was administered orally with 240 mL of water approximately 4 to 5 hours following the completion of the infusion.

Sample Collection:
PK blood samples collected during the main study at the following time points: predose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 18, and 24 hours postdose. Dialysate samples (30 µL) were taken every 30 minutes for 3 hours during the Washout period in the Pilot Study. In the Main Study, one cumulative dialysate sample was taken from 3.5 to 4 hours at the end of the Washout period.

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Dialysate samples were taken every 20 minutes for 12 hours after a single oral dose of 600 mg TR-701 in the Main Study.

Plasma protein binding of TR-700 was assessed for each subject with blood taken at 2 and 12 hours post dose.

Analytical Methods: Plasma samples were analyzed for TR-700 by validated high-performance liquid chromatography (HPLC) with tandem mass spectrometric (MS/MS) detection. Dialysate samples were analyzed for TR-700 by validated high-performance liquid chromatography (HPLC) with UV detection. TR-700 protein binding was determined based on plasma ultrafiltrate concentrations using a validated analytical method (**Table 1**).

Table 1. Bioanalytical results of TR-700 and TR-701 in human plasma

Criterion	TR-700 plasma	TR-700 plasma ultrafiltrate	TR-700 dialysate	Comments
Range	5.00-1000 ng/mL	2.00-400 ng/mL	50-2400 ng/mL	Satisfactory
LLOQ	5 ng/mL	2 ng/mL	50 ng/mL	Satisfactory
Linearity	linear	linear	linear	Satisfactory
Accuracy	98.5% - 103.3% [a] 98.9% - 102.7 [b]	97.3% - 100.7% [b]	84.3%-131% [b]	Satisfactory
Precision	1.1%-5.8% [a]	1.6%-4.1% [b]	0.98%-12.66% [b]	Satisfactory
Recovery over range	86.2-87.7%	80.9%-85%	79.06%-98.79%	Satisfactory
Selectivity	No interference			Satisfactory
Stability	Bench/freeze-thaw/long term			Satisfactory

[a] Intra run; [b] Inter run

Assessment:

PK:

For the Main Study, based on the individual plasma concentration-time data, given actual sampling times, the following PK parameters of TR-700 in plasma and dialysate were estimated: Cmax, Tmax, AUClast, AUC∞, apparent terminal phase elimination rate constant (λz), apparent terminal half-life (t½), oral clearance (CL/F; for plasma only), and volume of distribution during the terminal phase (Vz/F; for plasma only). As a measure of tissue penetration, unbound tissue and plasma concentration ratios (AUCtissue/*f*AUCplasma) were determined for s.c. adipose and skeletal muscle tissues. The plasma concentration was corrected to account for plasma protein binding.

PK/PD:

The PK/PD parameter area under the concentration-time curve to minimum inhibitory concentration ratios (AUC/MIC) in plasma corrected for protein binding, in s.c. adipose tissue and skeletal muscle were determined.

Safety:

Adverse event (AE) assessments, 12-lead ECGs, vital signs, physical examinations, and laboratory assessments.

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Statistical Methods:
PK parameters, PK/PD estimates, and protein binding information (% bound and unbound) were summarized with mean, median, geometric mean, minimum, maximum, standard deviation (SD), and coefficient of variation. No formal statistical analyses were planned.

Data Analysis:
In Vivo Recovery and Concentration Measurements
The *in vivo* recovery was calculated as follows:

$$\text{Recovery [\%]} = 100 - (100 \times C[\text{dialysate}] \times C[\text{perfusate}]^{-1})$$

Observed concentrations were then adjusted for recovery using the following formula:

$$C(\text{final}) = C(\text{observed}) \times \text{Recovery}^{-1}$$

Free plasma concentrations were calculated by multiplying each individual's fraction unbound with the observed total plasma concentration.

RESULTS
Study Population: Three subjects were enrolled in the Pilot Study and 12 subjects were enrolled in the Main Study. All subjects completed the study per protocol.

Table 2. Demographic characteristics of enrolled subjects

Demographic Variable	Pilot Study N = 3	Main Study N = 12
Mean age in years (range)	21 (19 to 22 years)	24 (19 to 29 years)
Mean weight in kg (range)	65.9 (63.0 to 70.3 kg)	71.7 (52.5 to 95.9 kg)
Mean height in cm (range)	170.5 (163.8 to 175.0 cm)	171.5 (160.0 to 191.9 cm)
BMI in kg/m^2 (range)	22.7 (21.1 to 24.0 kg/m^2)	24.1 (19.7 to 28.7 kg/m^2)
Gender (n[%])		
Male	---	5 (41.7%)
Female	3 (100.0%)	7 (58.3%)
Ethnicity (n[%])		
Hispanic or Latino	---	1 (8.3%)
Not Hispanic or Latino	3 (100.0%)	11 (91.7%)
Race (n[%])		
White	3 (100.0%)	10 (83.3%)
Black or African American	---	2 (16.7%)

Pilot Study:
The results of the pilot study indicated that TR-700 recovery from the microdialysis probe was approximately 90%. The originally estimated 3-hour washout period had to be extended to 4 hours because levels of TR-700 found in the washout dialysate were still close to the lower level of quantitation after 3 hours.

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Main Study:
Pharmacokinetics:
TR-700 mean plasma protein binding was 87% with a mean unbound fraction of 13%. Free plasma concentrations of TR-700 were calculated by multiplying each individual's respective fraction unbound with the assayed total plasma values. TR-700 plasma, adipose and muscle tissue concentration-time profiles and PK parameter summary are presented in **Figures 1&2** and **Table 3**.

Figure 1: Mean (SD) TR-700 Plasma Concentration-Time Profiles (Main Study)

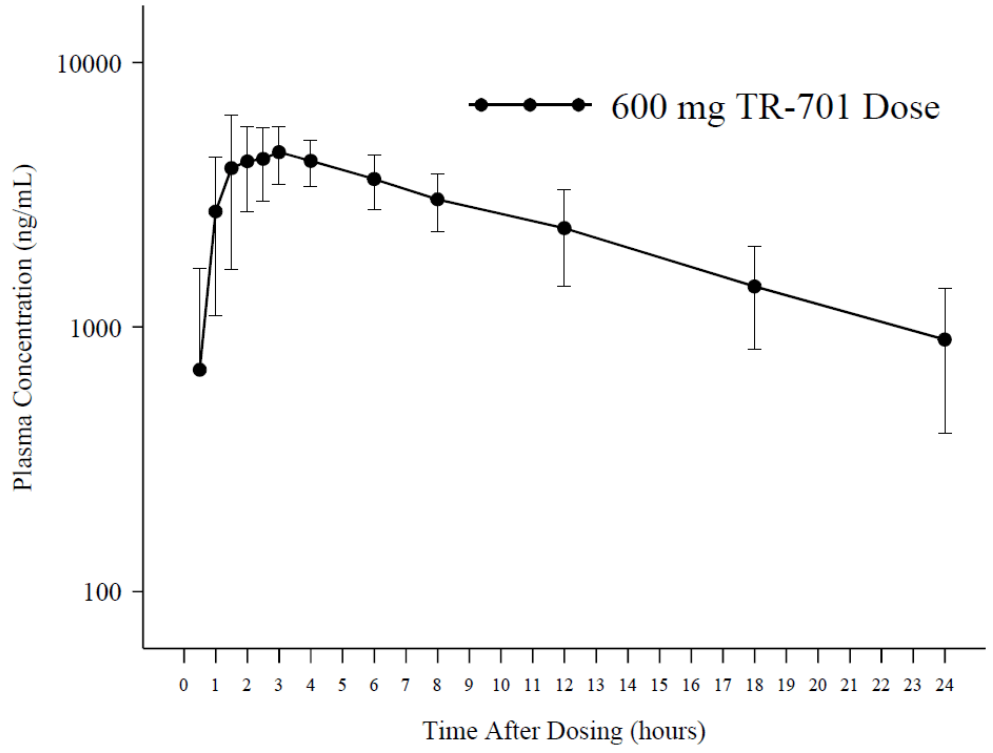

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Figure 2: Mean (SD) TR-700 Adipose Tissue and Muscle Tissue Interstitial Fluid (ISF) Concentration Time Profiles (Main Study)

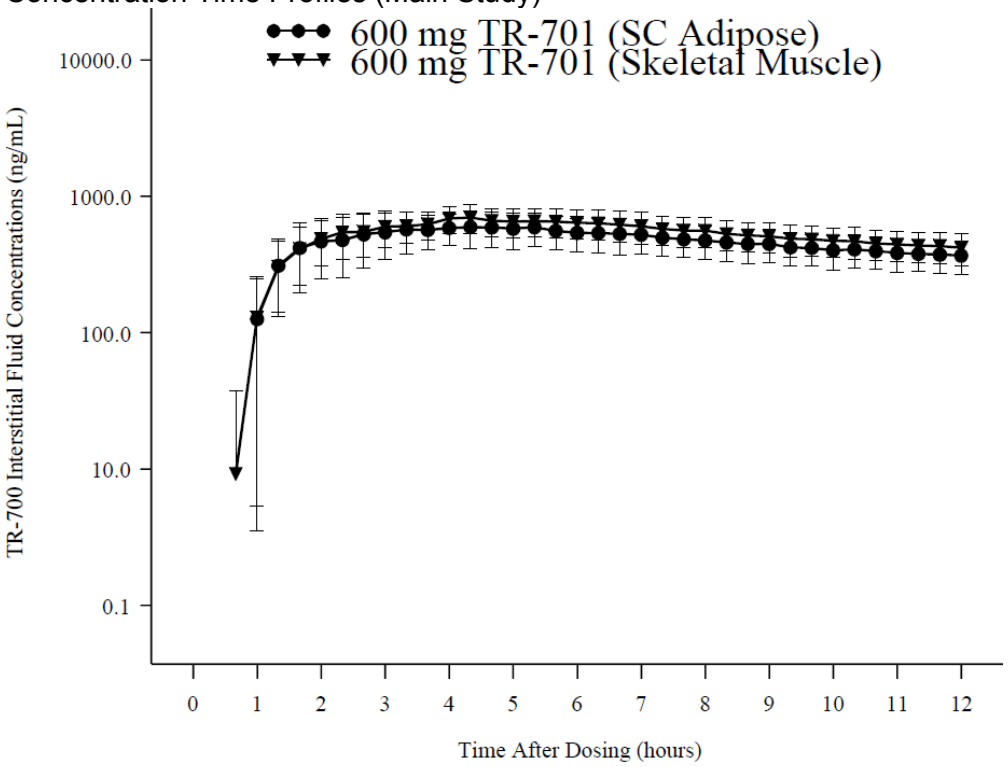

Table 3: Mean (SD) TR-700 PK Parameter Data for Plasma, Adipose Tissue, and Muscle Tissue (Main Study)

PK Parameter	Plasma	Adipose Tissue[a]	Muscle Tissue[a]
C_{max} (µg/mL)	5.37 (1.51)	0.66 (0.16)	0.74 (0.16)
t_{max} (hr)[b]	2 (1-4)	3.33 (2.33-10.3)	3.67 (1.67-7)
AUC_{0-12} (µg•hr/mL)	38.81 (7.55)	5.27 (1.28)	5.95 (1.11)
$t_{½}$ (hr)[b]	8.13 (5.94-12.8)	9.22 (5.98-85.9)	9.59 (6.15-48.2)
$AUC_{tissue}/fAUC_{plasma}$[c]	NA	1.08 (0.22)	1.22 (0.18)

Abbreviations: AUC0-12=area under the plasma concentration-time curve from Hour 0 to Hour 24; Cmax=maximum observed concentration; NA=not applicable; PK=pharmacokinetic; SD=standard deviation; t½=apparent terminal elimination half-life

[a] In tissues, all concentrations are considered as free, unbound to proteins.

[b] Median (minimum, maximum) presented for tmax.

[c] Ratio of AUC0-12 was determined after adjusting total plasma concentration for ~87% protein binding.

Pharmacodynamics:

Mean fAUC∞/MIC values were calculated and shown in **Table 4**.

Table 4: Summary of Mean (SD) Unbound Plasma, Adipose Tissue, and Muscle Tissue AUC∞/MIC (Main Study)

	Plasma	Adipose Tissue	Muscle Tissue
MIC (µg/mL)			
0.25	35.6 (12.2)	66.5 (63.2)	58.3 (42.0)
0.5	17.8 (6.12)	33.3 (31.6)	29.2 (21.0)
4	2.22 (0.76)	4.16 (3.95)	3.65 (2.62)

Safety: Each of the 3 subjects in the Pilot Study and the 12 subjects in the Main Study reported at least 1 AE after study drug administration. All AEs were mild and only 3 subjects reported AEs that were judged by the Investigator to be treatment-related. There were no deaths or SAEs during the study and no subjects discontinued from the study due to AEs**.**

SPONSOR'S CONCLUSIONS: The Main Study demonstrated that TR-700 penetrated rapidly into the interstitial space fluid of adipose and skeletal muscle tissue. Furthermore, TR-700 concentrations in interstitial space fluid of subcutaneous adipose and skeletal muscle tissue were comparable to free plasma concentrations of TR-700. Thus, free plasma concentrations could be used as surrogate for subcutaneous adipose and muscle TR-700 concentrations in future studies.

REVIEWER ASSESSMENT: Results from Study TR701-102 adequately described the PK and TR-700 penetration into subcutaneous adipose tissue and skeletal muscle interstitial fluids in 12 healthy volunteers via *in vivo* microdialysis. The results demonstrate that TR-700 concentrations in interstitial space fluid of subcutaneous adipose and skeletal muscle tissue were comparable to TR-700 unbound concentration in plasma.

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STUDY NO.: TR701-103

An Open-Label, Single-Dose, Two-Treatment, Randomized, Two-Way Crossover, Safety, Tolerance, and Pharmacokinetic Study of TR-701 in Normal Healthy Adults in the Fed and Fasted State

Date(s): 18 March 2008 to 15 May 2008
Sponsor: Trius Therapeutics, Inc., San Diego, CA
Clinical Site: Covance Clinical Research Unit Inc., San Diego, CA
Analytical Site: (b) (4)

OBJECTIVE(S):

1. To determine the safety and tolerability of TR-701 in healthy adult volunteers when administered as a single 600 mg oral dose.
2. To evaluate the single-dose pharmacokinetics (PK) of TR-701 and TR-700 in healthy adult volunteers in the fed and fasted state.

METHODS

Study Design: This was an open-label, randomized, 2-sequence, 2-treatment, 2-period, crossover design investigating the safety, tolerance, and PK food-effect of TR-701 in 12 healthy male and female subjects. Subjects were confined to the clinical research unit from approximately 24 hours predose to approximately 72 hours postdose. The planned study conduct duration was approximately 7 weeks.

In Treatment A, doses were preceded by an overnight fast (i.e., at least 10 hours) from food (not including water). In Treatment B, subjects fasted overnight (i.e., at least 10 hours) and were then served a standardized FDA high-fat breakfast. Subjects consumed the entire meal in 30 minutes and the TR-701 dose was administered with 240 mL of water within 10 minutes of completion of the standardized breakfast. All doses were followed by a 4-hour fast from food (not including water). Except as part of the standardized meal and dose administration, subjects restricted their consumption of water for 1 hour prior to dose and for 1 hour post-dose. At all other times during the study, subjects could consume water on an ad libitum basis. The study scheme is shown in **Table 1**.

Table 1. Study Scheme

N	Screening	Period 1	Washout	Period 2	End of Study
6		**Treatment A:** 600 mg TR-701 in the fasted state		**Treatment B:** 600 mg TR-701 in the fed state	
6		**Treatment B:** 600 mg TR-701 in the fed state		**Treatment A:** 600 mg TR-701 in the fasted state	
DAY -28 →→→ DAY -1 →→ DAY 4 →			≥7 days	→ DAY -1 →→ DAY 4 →→ DAY 8±2	

Reviewer Comments:

1. *The study design meets the criteria outlined in the FDA Food-Effect guidance.*

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2. *Per the Sponsor, the dose chosen for this study, 600 mg, was considered as a potential therapeutic dose to be tested in a Phase 2 study. No significant AEs or laboratory abnormalities were observed with single doses of 200 to 1200 mg in a single ascending dose study; therefore, a single dose of 600 mg was considered well tolerated and appropriate for further studies.*
3. *Results from study TR701-101 showed that single doses of 200 to 1200 mg were well tolerated in healthy human subjects; PK were linear at single TR-701 doses up to 600 mg. Therefore, the results at the 600 mg TR-701 dose used in this food-effect study appear to be appropriate to predict those at the final proposed clinical dose 200 mg.*

Drug Product: Tedizolid phosphate disodium salt, also known as TR-701 (200 mg oral capsules).

Inclusion Criteria: Males and females of non-childbearing potential; 18-50 years of age (inclusive); 20-29.9 kg/m^2 (inclusive) in body mass index; nonsmokers, and in good health as confirmed by medical history, physical exam, 12-lead electrocardiogram (ECG; within normal limits), and laboratory evaluations were enrolled.

Subjects were required to abstain from any medications (including over-the-counter remedies) from 7 days prior to Check-in and during the entire study. Subjects were also required to abstain from alcohol-, grapefruit-, or caffeine-containing foods or beverages or foods or beverages with high levels of tyramine from 72 hours prior to Check-in and during the entire study. Use of selective serotonin re-uptake inhibitors (SSRIs), monoamine oxidase (MAO) inhibitors, and other serotonergic medications were not allowed within 14 days prior to Check-in and during the entire study.

PK Sample Collection: Blood samples were collected at the following timepoints for each study period: 0 Hour (pre-dose), 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 18, 24, 48, and 72 hours post-dose.

Analytical Methods: PK samples were analyzed for TR-701 and TR-700 by validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) (**Table 2**). For the calculation of summary statistics, all concentrations below the limit of quantification (BLQ) were to be set to zero.

Table 2. Bioanalytical results of TR-701 and TR-700 in plasma

Criterion	TR-701	TR-700	Comments
PLASMA			
Calibration Range	5-1000 ng/mL	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	Satisfactory
Linearity, R^2	≥0.9924	≥0.9960	Satisfactory
Accuracy	Within ±4%	Within ±3.9%	Satisfactory
Precision, CV	≤7.1 %	≤4.4 %	Satisfactory
Quality Control*	15, 150, 750, 750(50x), 5000(10x) ng/mL	15, 150, 750, 750(10x), 750(50x), 5000(10x) ng/mL	Satisfactory
Accuracy	Within ±7.5%	Within ±7.3%	Satisfactory

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Precision, CV	≤6.8 %	24.8% for 15 ng/ml; ≤6% for the rest QC samples	Satisfactory
Stability	• Maximum duration from sample collection to end of analysis: 86 days • Stability: 156 days at -60 to -80 ºC		Satisfactory

**Numbers in the parentheses indicate the dilution factor for QC samples.*

Pharmacokinetic Assessment: Analysis included measurements of the prodrug (TR-701), if applicable, and the active moiety (TR-700) C_{max}, T_{max}, AUC_{0-12}, AUC_{0-24}, AUC_{0-t}, AUC_{0-inf}, λ_Z, and $t_{½}$ for plasma.

Statistical Methods: Where data were available, food effect was examined between test (fed state) and reference (fasted state) groups. An analysis of variance (ANOVA) was performed. Data for C_{max} and AUC_{0-inf} (or AUC_{0-t} as appropriate) were natural log (ln)-transformed prior to analysis using the SAS® Mixed Procedure. The 90% confidence intervals (CIs) of the test group means relative to the reference group means were obtained by taking the antilog of the corresponding 90% CIs for the differences between the means on the log scale. Food effect was assessed by examining the 90% CIs for the ratios of the test group means relative to the reference group means.

Safety Assessment: Safety and tolerance were assessed at specified timepoints by physical examinations, medical history, clinical laboratory evaluations (Chem-20, complete blood count [CBC], and urinalysis [UA]), 12-lead ECGs, adverse events (AEs), and vital signs.

RESULTS

Study Population: In total, 12 subjects were enrolled in the study and completed the study. A summary of demographic and baseline characteristics for the study population is presented in **Table 3**.

Table 3. Demographic Characteristics

Demographic Variable	
Mean age in years (range)	35 (21 to 50)
Mean weight in kg (range)	73.1 (51.4 to 104.0)
Mean height in cm (range)	171.5 (154.3 to 187.0)
BMI in kg/m^2 (range)	24.6 (20.2 to 29.7)
Gender (n [%])	
Male	8 (66.7%)
Female	4 (33.3%)
Ethnicity (n [%])	
Hispanic or Latino	2 (16.7%)
Not Hispanic or Latino	10 (83.3%)
Race (n [%])	
White	9 (75.0%)
Black or African American	2 (16.7%)
Other	1 (8.3%)

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Some protocol deviations were identified as follows: the 18 hours post-dose PK blood sample was processed 40 hours late for 1 subject; other minor protocol deviations (e.g., late PK blood draws) occurred.

Reviewer Comment: These deviations do not appear to affect the validity of PK data.

Pharmacokinetics:

TR-701

One TR-701 concentration value of 5.38 ng/mL was observed for Subject No. 004 (Period 2, fasted) at the 2-hour collection time, as well as one TR-701 concentration value of 6.76 ng/mL for Subject No. 011 (Period 1, fasted) at the 1-hour collection time. Due to limited data, these subjects were not included in the PK analysis. All other subjects and timepoints were BLQ for TR-701 and were also excluded from the PK analysis.

TR-700

Following a single oral administration of three 200 mg capsules (600 mg dose) of TR-701 in the fed and fasted states (Treatments B and A, respectively), TR-701 was rapidly and extensively converted to TR-700 (**Figure 1**). TR-700 T_{max} was delayed by 6 hours following administration of TR-701 in the fed state compared to fasted state (8 vs. 2 hours) (**Table 4**). Mean $t_{1/2}$ values of TR-700 were not changed at the fed and fasted states (10.4 vs. 10.9 hours) (**Table 4**).

In the fed state, mean TR-700 C_{max} (4746 ng/mL) was 26% lower than that observed in the fasted state (6438 ng/mL), and the 90% CIs associated with the ratio of C_{max} (67.81%, 80.12%) were not entirely contained within the 80% to 125% equivalence interval, indicating significant food effect on TR-700 C_{max} (**Table 5**). Mean TR-700 AUC_{0-t} and AUC_{0-inf} values after administration of TR-701 in the fed state (81026 ng*hr/mL and 81822 ng*hr/mL, respectively) were similar to the corresponding values after administration in the fasted state (79189 and 79912 ng*hr/mL, respectively) and the 90% CIs associated with the ratio of AUC_{0-t} (98.09%, 106.74%) and AUC_{0-inf} (98.18%, 106.79%) were contained within the 80% to 125% equivalence interval, indicating no significant food effect on the AUC_{0-t} and AUC_{0-inf} of TR-700 (**Table 5**).

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Figure 1. Mean (SD) Plasma Concentration-Time Profiles for TR-700.

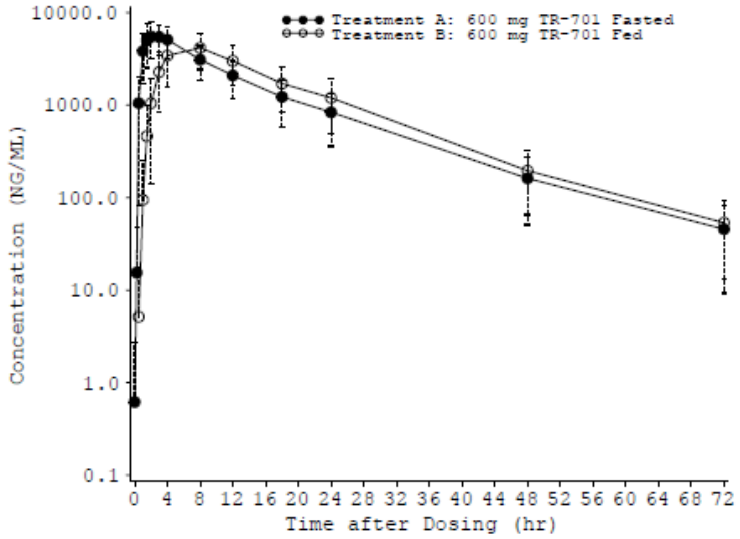

Table 4. Summary of Arithmetic Mean (SD) TR-700 Pharmacokinetic Parameter Data

PK Parameter	Units	N	600 mg TR-701, fasted Treatment A (Reference)	N	600 mg TR-701, fed Treatment B (Test)
C_{max}	(ng/mL)	11	6502 (1362)	11	4786 (1117)
T_{max}[a]	(hr)	11	2.00 (1.50, 3.00)	11	8.00 (4.00, 12.0)
AUC_{0-12}	(ng*hr/mL)	11	47763 (10147)	11	37703 (9972)
AUC_{0-24}	(ng*hr/mL)	11	65397 (15546)	11	62656 (17471)
AUC_{0-t}	(ng*hr/mL)	11	81176 (22575)	11	84139 (27141)
AUC_{0-inf}	(ng*hr/mL)	11	81992 (23111)	11	85043 (27690)
λ_z	(1/hr)	11	0.0641 (0.00519)	11	0.0673 (0.00584)
$t_{½}$	(hr)	11	10.9 (0.901)	11	10.4 (0.883)

Table 5. Statistical Analysis of Pharmacokinetic Data (Food Effect of TR-700)

Analyte[a]	Parameter[b] (Units)	LS Means[c] N	Fed (Test)	N	Fasted (Reference)	Test/Reference (%)[d]	90% Confidence Interval[e]
TR-700	C_{max} (ng/mL)	11	4746	11	6438	73.7	(67.81 , 80.12)
	AUC_{0-t} (ng*hr/mL)	11	81026	11	79189	102	(98.09 , 106.74)
	AUC_{0-inf} (ng*hr/mL)	11	81822	11	79912	102	(98.18 , 106.79)

Safety: Overall, 8 AEs were reported, with 5 AEs reported following administration of 600 mg TR-701 in the fed state and 3 AEs reported following administration of TR-701 in the fasted state. There were no apparent trends in AEs and no AE was reported more than once.

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All of the 8 reported AEs were mild in severity. The relationship to study drug of the reported AEs was: 6 unrelated AEs and 2 possibly related AEs. The 2 treatment-related AEs were gastroesophageal reflux disease and insomnia. No clinically significant changes or findings were noted from clinical laboratory evaluations, vital sign measurements, physical examinations, or 12-lead ECGs for this study.

SPONSOR'S CONCLUSIONS

- Based on AEs, clinical laboratory evaluations, vital signs measurements, physical examinations, and 12-lead ECGs, single doses of 600 mg TR-701 administered in the fed or fasted state were generally well tolerated in this group of healthy males and females.
- Following oral administration of TR-701 in the fed and fasted states, TR-701 was rapidly and extensively converted to TR-700.
- After oral administration of TR-701 in the fed or fasted state, no significant food effect was observed on $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ of TR-700.
- In the fed state, TR-700 C_{max} was significantly (26%) lower than that observed in the fasted state, and a 6-hour delay in TR-700 median T_{max} was observed in the fed compared to the fasted state.

REVIEWER ASSESSMENT: The Sponsor's conclusions are appropriate based on study results. The prodrug formulation used in this study is tedizolid phosphate disodium salt TR-701 200 mg capsule, which is different from the formulation used in the Phase 3 trials (to be marketed formulation). Per the FDA food-effect guidance, bioequivalence should be demonstrated between the to-be-marketed formulation and the clinical trial formulation. There are no direct in vitro or in vivo comparison between the final TR-701 FA tablet drug product and TR-701 or TR-701 FA capsules. The sponsor provided the following rationale to bridge the results using TR-701 capsule in this study and those expected with the to-be-marketed TR-701 FA tablet (adapted from the Sponsor's Summary of Biopharmaceutics).

1. [redacted] (b) (4)
2. The findings from the TR-701 food effect study are highly likely to reflect the in vivo performance of TR-701 FA. [redacted] (b) (4)
3. TR-701 FA is presumably converted to TR-700 by phosphatase enzymes, which are ubiquitously distributed in the human body. Phosphatase enzymes line the apical surface of the small intestine and are an important marker for differentiation of small intestinal cells. As established in studies TR701-107 and Bayer 16101, TR-700 is rapidly and

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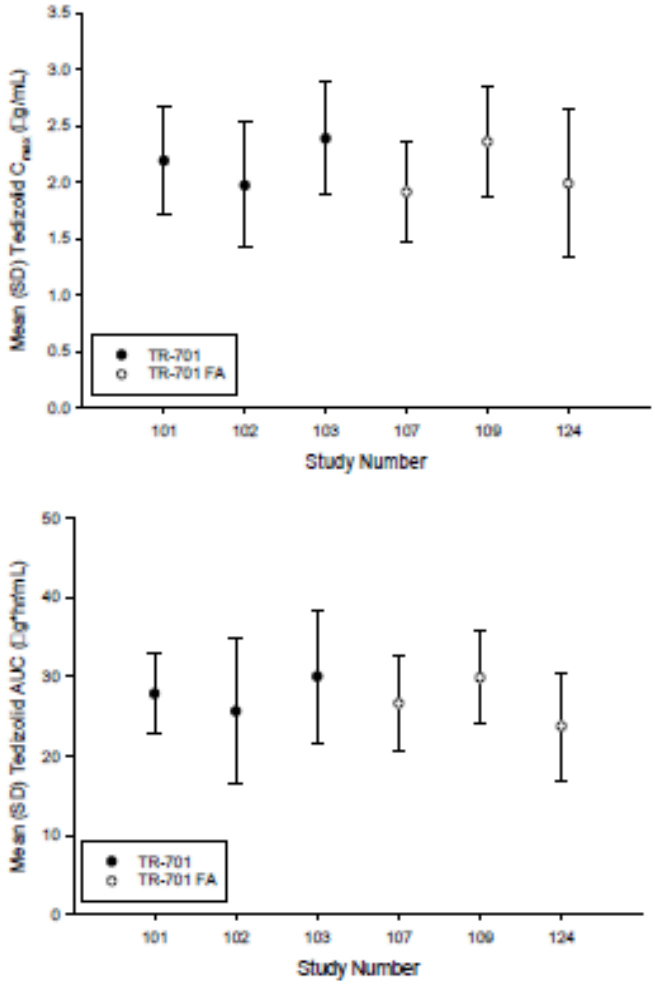
extensively absorbed, with high oral bioavailability of >80% after administration of 200-mg therapeutic doses of TR-701 FA.

4. The nominal dose level of TR-701 FA 200 mg (164 mg TR-700 equivalents) was retained for simplicity for the Phase 3 dose, which resulted in a minor (<10%) increase in the dose of the active moiety compared to a 200 mg dose of TR-701 (150 mg TR-700 equivalents). Given this small change and demonstrated lack of PK differences (Figure 2), the TR-700 PK exposures from 200 mg of TR-701 FA were expected to be similar to those from 200 mg of TR-701, and well within the range of doses evaluated clinically in Phase 2.

Figure 2. Cross Study Comparison of Mean (SD) TR-700 C_{max} (top) and AUC (bottom) for Oral TR-701 and TR-701 FA



Note: All TR-701 FA results are for 200 mg Tablets. Exposure parameters for TR-701 were normalized to a 200 mg dose of TR-701 FA from 200 mg TR-701 (TR701-101) or 600 mg TR-701 (TR701-102 and TR701-103).

Reviewer Comment: The Sponsor's bridging rationale appears reasonable. The results from this food-effect study using TR-701 capsule appear to reflect those expected with the to-be-marketed TR701 FA tablet formulation.

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STUDY NO.: TR701-105

A Phase 1, blinded, placebo-controlled, crossover TR-701FA study of blood pressure response post-tyramine challenge

Date(s): 27 February 2012 – 11 May 2012
Investigator(s): Charles Crockett, M.D.
Clinical Site(s): Covance Clinical Research Unit Inc.; Evansville, IN, US
Analytical Site(s): (b) (4)

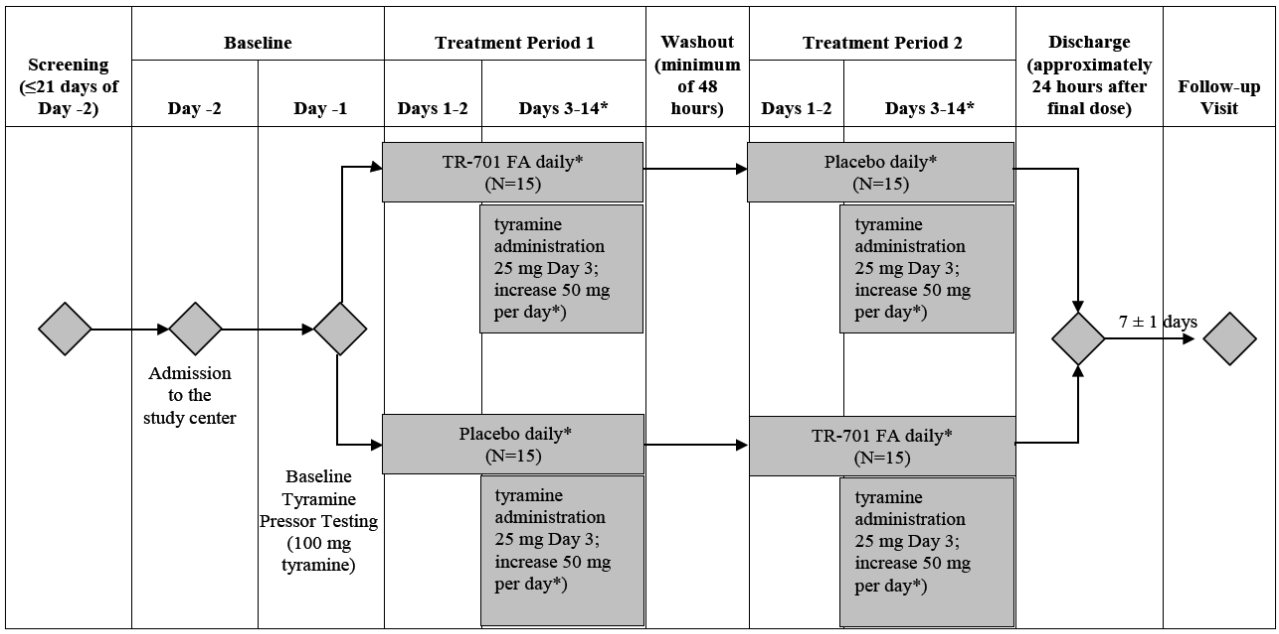
OBJECTIVE(S):

- Primary: To determine the dose of tyramine required to cause a 30 mmHg increase in systolic blood pressure (SBP) when administered in combination with TR-701 FA or placebo.
- Secondary: To assess the safety and tolerability of administration of 200 mg oral TR-701 FA when given in combination with tyramine for up to 14 days.

METHODS

Study Design: This was a Phase 1, randomized, double-blind, placebo-controlled, 2-way crossover study to determine the effect of 200 mg oral TR-701 FA on SBP response to open-label tyramine in healthy volunteers. Study schedule is depicted in Table 1. The disposition of subjects enrolled are presented in Table 2.

Table 1. Study Schedule



*Study drug and tyramine administration were to continue until TYR_{30} was reached or through Day 14, whichever occurred first.

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Table 2. Disposition of subjects (modified ITT analysis set)

	Number (%) of Subjects			
	Treatment Sequence			
	TR-701 FA/ Placebo (N = 15)	Placebo/ TR-701 FA (N = 15)	Not Randomized[a] (N = 9)	Overall (N = 39)
Received Tyramine on Day -1	15 (100.0)	15 (100.0)	9 (100.0)	39 (100.0)
Randomized	15 (100.0)	15 (100.0)	0	30 (76.9)
Completed Study	12 (80.0)	11 (73.3)	0	23 (59.0)
Withdrawal	3 (20.0)	4 (26.7)	9 (100.0)	16 (41.0)
Adverse Event	2 (13.3)	1 (6.7)	0	3 (7.7)
Withdrawal by Subject	0	2 (13.3)	0	2 (5.1)
Physician's Decision	0	1 (6.7)	0	1 (2.6)
Lost to Follow-up	1 (6.7)	0	0	1 (2.6)
Study Terminated by Sponsor	0	0	0	0
Death	0	0	0	0
Protocol Violation	0	0	0	0
Other	0	0	9 (100.0)	9 (23.1)

Inclusion/Exclusion Criteria: Healthy subjects from 18 to 45 years of age, without clinically significant abnormalities or medical history were eligible for enrollment. Subjects were required to have resting SBP 90 to 130 mmHg, diastolic blood pressure 50 to 90 mmHg, heart rate (HR) 45 to 90 beats per minute (bpm), and QT interval corrected for HR (QTc) ≤500 msec. A high tyramine diet and exposure to certain drugs, foods, and beverages, or any investigational drug were reasons for exclusion from enrollment.

Treatment: 200 mg TR-701 FA or placebo daily (fasted condition) for up to 14 days. Beginning on Day 3 of each treatment period, tyramine was administered daily beginning with 25 mg and increasing by 50 mg each day up to a possible maximum dose of 575 mg on Day 14. Dosing continued until TYR30, defined as the dose of tyramine required to cause an increase in SBP of ≥30 mmHg within 2 hours following tyramine administration on any day (Days 3-14) from the pre-administration SBP on Day 3 of the same treatment period, was reached or through Day 14, whichever occurred first. Lot numbers for the treatments administered Study TR701-105 are as follows:

- TR-701 FA tablets, 200 mg (lot number B090282)
- Placebo for TR-701 FA tablets (lot number B110409)
- Tyramine hydrochloride powder (lot number 01TRI01C-01-49)

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Sample Collection: TR-700 and TR-701 plasma concentrations were determined from blood drawn 15 minutes prior to TR-701FA administration on Days 1 through 14.

Analytical Methods: Pharmacokinetic samples were analyzed for TR-700 and TR-701 by validated high-performance liquid chromatography (HPLC) with tandem mass spectrometric (MS/MS) detection in plasma (**Table 2**).

Table 2. Bioanalytical results of TR-700 and TR-701 in human plasma

Criterion	TR-700	TR-701	Comments
Range	5-1000 ng/mL	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	Satisfactory
Linearity	≥0.998	≥0.991	Satisfactory
Accuracy	95.3% - 98%[a] 98.7%-101.6%[b]	87.2% - 98%[a] 93.7%-106%[b]	Satisfactory
Precision (RSD)	2.7%-3.3%[a] 1.4% - 3.3%[b]	6.7%-9.6%[a] 2.2% - 8.5%[b]	Satisfactory
Selectivity	No interfering peak in the presence of tyramine 0.5 mcg/mL		Satisfactory
Stability	Stored at -60 to -80 ºC	Stored at -60 to -80 ºC	Satisfactory

[a] QC samples (15.0, 150, and 750 ng/mL); [b] Standards

PK/PD Assessment:

- PK: PK parameters are the trough concentration of TR-700 and TR-701.
- PD: The primary PD parameter was TYR30, defined as the dose of tyramine required to cause an increase in SBP of ≥30 mmHg within 2 hours following tyramine administration on any day (Days 3-14) from the pre-administration SBP on Day 3 of the same treatment period. TYR30 was determined for each treatment period.

Statistical Methods:
Trough concentrations of TR-700 and TR-701 were listed by subject and TR-700 concentration was summarized by study day using descriptive statistics. Plots of TR-700 trough concentration versus study day were prepared for the mean value and for individual subjects.
The primary PD analysis applied an analysis of variance (ANOVA) model using the natural logarithm of TYR30 as the dependent variable and period, treatment, and sequence as fixed effects, with subject within sequence as a random effect. The placebo to TR-701 FA geometric mean ratio and 90% confidence interval (CI) are reported for the PD Analysis Set. A secondary analysis repeated the primary analysis but used the PP Analysis Set. Another secondary analysis used ANOVA to compare the ratio of greatest SBP to HR (GSBP/HR) between treatment groups in the PD and PP Analysis Sets.

RESULTS

Study Population: In total, 39 subjects were enrolled (**Tables 3&4**). Nine subjects were not randomized because the target sample size (i.e. 30) had been reached. Among the randomized, 23 subjects completed the study.

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Table 3. Demographic characteristics of enrolled subjects

Baseline Characteristic	Statistic	Treatment Sequence: TR-701 FA/ Placebo (N=15)	Treatment Sequence: Placebo/ TR-701 FA (N=15)	Not Randomized[a] (N=9)	Overall (N=39)
Age (years)	Mean	31.7	33.8	33.0	32.8
	Standard Deviation	7.09	6.73	7.18	6.85
	Minimum	20	23	26	20
	Median	29.0	32.0	29.0	31.0
	Maximum	43	45	43	45
BMI (kg/m^2)	Mean	25.99	26.21	27.41	26.40
	Standard Deviation	2.038	3.136	1.795	2.481
	Minimum	22.3	21.4	24.4	21.4
	Median	26.00	24.90	27.70	26.10
	Maximum	29.8	30.7	29.9	30.7
Demographics		**Number (%) of Subjects**			
Sex					
Male		8 (53.3)	7 (46.7)	7 (77.8)	22 (56.4)
Female		7 (46.7)	8 (53.3)	2 (22.2)	17 (43.6)
Ethnicity					
Hispanic or Latino		3 (20.0)	2 (13.3)	3 (33.3)	8 (20.5)
Not Hispanic or Latino		12 (80.0)	13 (86.7)	6 (66.7)	31 (79.5)
Race					
White		12 (80.0)	8 (53.3)	5 (55.6)	25 (64.1)
Black or African American		2 (13.3)	6 (40.0)	2 (22.2)	10 (25.6)
American Indian/Alaskan Native		0	0	1 (11.1)	1 (2.6)
Other		1 (6.7)	1 (6.7)	1 (11.1)	3 (7.7)

Table 4: Summary of data analysis sets

Analysis Set	Number (%) of Subjects: Treatment Sequence: TR-701 FA/ Placebo (N=15)	Treatment Sequence: Placebo/ TR-701 FA (N=15)	Not randomized[a] (N=9)	Overall (N=39)
ITT	15 (100.0)	15 (100.0)	9 (100.0)	39 (100.0)
PD	13 (86.7)	11 (73.3)	0	24 (61.5)
PP	5 (33.3)	2 (13.3)	0	7 (17.9)
Modified ITT	15 (100.0)	15 (100.0)	9 (100.0)	39 (100.0)
Safety	15 (100.0)	15 (100.0)	0	30 (76.9)

The Intent to Treat (ITT) analysis set includes data from subjects who signed the informed consent form and who were admitted to the Study Center on Day 2.
The Pharmacodynamic (PD) Analysis Set includes data from subjects who were randomized and for whom a TYR30 was determined during at least 1 treatment period.
The Per Protocol (PP) Analysis Set includes data from subjects in the PD Analysis Set for whom a TYR30 was determined in both treatment periods.
The Modified ITT Analysis Set includes data from subjects who received at least 1 dose of study drug (TR-701 FA or placebo) or tyramine. Includes subjects who received only the initial 100-mg administration of tyramine on Day -1.
The Safety Analysis Set includes data from subjects who received at least 1 dose of study drug (TR-701 FA or placebo).
[a] Not Randomized subjects received the initial 100-mg administration of tyramine on Day -1.

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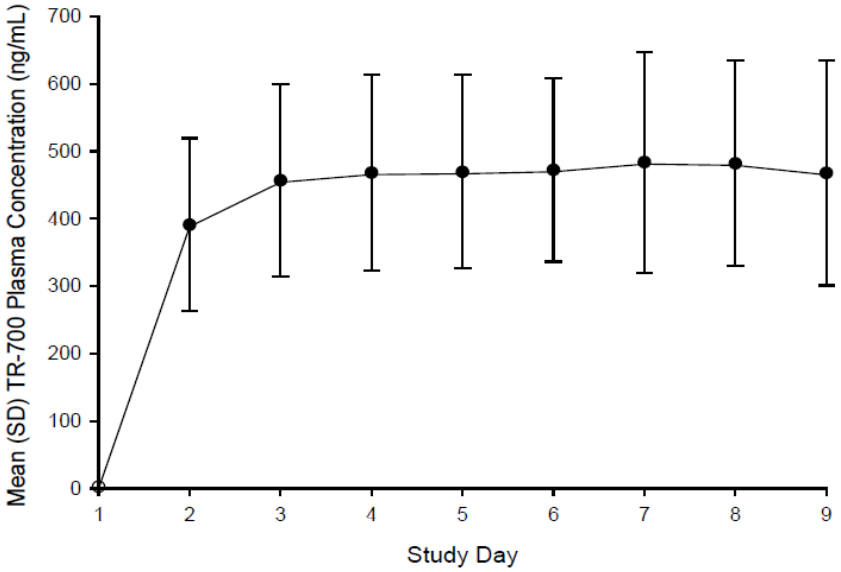
Pharmacokinetics:
Trough plasma concentrations of TR-700 on Days 2 through 14 of the TR-701 FA treatment period varied among individual subjects from 162 ng/mL to 984 ng/mL. Mean trough concentrations were relatively stable from Day 3 through Day 9, indicative of steady state (**Figure 1**). Trough plasma concentrations of TR-701 were all below the LLOQ of 5 ng/mL.



Note: Mean trough concentrations after Day 9 are not shown because values were available for less than half (n≤12) of the subjects who had trough concentrations available at Day 1 (n=29).

Figure 1: TR-700 Mean (± SD) Trough Concentration versus Study Day (Safety Analysis Set)

Pharmacodynamics:
Of 24 subjects in the PD Analysis Set who received placebo, 7 subjects reached TYR30 on or before Day 14, 2 subjects did not complete the treatment period and did not reach TYR30, and 15 subjects received all doses of tyramine, including the maximum 575 mg on Day 14, without reaching TYR30. During TR-701 FA treatment, all 24 subjects had a ≥30 mmHg response on or before Day 14. The PD analysis set (as the primary PD analysis) includes data from 7 subjects who reached TYR30 in the placebo treatment period, and 24 subjects who reached TYR30 in the TR-701 treatment period (**Table 5**). The mean TYR30 was 94 mg higher with placebo (418 mg) than with TR-701FA (324 mg). However, the geometric mean ratio (placebo:tedizolid phosphate) of 1.29 is below the predetermined TSF value of 2 (TSF: Tyramine Sensitivity Factor calculated from the individual ratios between TYR30 values for placebo and active oral treatment; A geometric mean TSF of ≥2 was considered to be clinically significant).

Table 5: Primary analysis of TYR30 (PD analysis set)

	Placebo		TR-701 FA		Placebo/	90% Confidence
Parameter (Units)	n	Geometric Mean[a]	n	Geometric Mean[a]	TR-701 FA[b]	Interval[c]
TYR_{30} (mg)	7	418	24	324	1.290	(0.943, 1.764)

Notes: TYR30 is the Tyramine dose required to cause an increase in systolic blood pressure (SBP) of ≥30 mmHg from predose baseline. An increase in SBP of ≥30 mmHg from predose baseline must be confirmed as sustained on 3 separate consecutive measurements, 5 minutes apart. TYR30 was derived as the midpoint between the log-transformed dose of Tyramine at which a ≥30 mmHg increase in SBP was observed and the previous dose of Tyramine.

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[a]Least squares mean from analysis of variance (ANOVA) model, calculated by transforming the natural log mean back to the linear scale (ie, geometric mean). The ANOVA model included the natural log of TYR30 as the response, period, treatment, and sequence as fixed effects, and subject nested within sequence as a random effect.
[b]Geometric mean ratio.
[c]90% confidence interval for the geometric mean ratio

The PP Analysis Set includes data from 7 subjects who reached TYR30 during both treatment periods; these are subjects who were most sensitive to the pressor effects of tyramine alone, and represent the vulnerable population in this study (**Table 6**). The median TYR30 was 100 mg higher with placebo (425 mg) than with TR-701FA (325 mg). The geometric mean ratio was 1.33 (90% CI, 1.05-1.69). Individual tyramine sensitivity factor values were >2 in 1 of the 7 subjects, (i.e. 2.09), while the other 6 subjects had TSF < 2.

Table 6: Individual TYR30 Values and Tyramine Sensitivity Ratios (PP analysis set)

	TYR_{30} (mg)		
Subject	**Placebo**	**TR-701 FA**	**TSF**
001-005	275	275	1.00
001-008	325	425	0.76
001-014	425	425	1.00
001-016	575	275	2.09
001-021	375	275	1.36
001-024	475	325	1.46
001-027	475	375	1.27
Median (range)	425.0 (275, 575)	325.0 (275, 425)	1.27 (0.76, 2.09)
Geometric Mean	439	330	1.33 (90% CI 1.05, 1.69)

Safety: Overall, 96.7% of subjects in the Safety Analysis Set had TEAEs, 96.6% in the TR-701 FA treatment group and 89.3% in the placebo treatment group. Palpitations were experienced by 25 subjects (83.3%) with 21 subjects having palpitations when tyramine was given with TR-701 FA (72.4%) and 13 subjects with placebo (46.5%); in many instances, the lowest tyramine dose at which palpitations occurred was the same as TYR30 during the same treatment period. All subjects in the PP Analysis Set (N=7) experienced palpitations; 3 had palpitations during TR-701 FA treatment only, 2 during placebo treatment only, and 2 during both treatment periods.

Other common AEs in both treatment groups were GI disorders (nausea, abdominal pain, vomiting, and diarrhea), headache, dizziness, and chest pain. There were no severe TEAEs, and moderate TEAEs were more common during the TR-701 FA treatment period (27.6% of subjects) than during placebo treatment (7.1% of subjects). No serious TEAEs or deaths occurred in this study. TEAEs led to withdrawal of study drug from 4 subjects (3 with palpitations and 1 with chest pain). Of these, 3 subjects were withdrawn from the study and 1 subject completed the study having reached TYR30 on the same day as experiencing palpitations**.**

SPONSOR'S CONCLUSIONS: Results of all analyses indicate clinically insignificant increases in tyramine sensitivity with TR-701 FA (measured by changes in SBP after oral tyramine administration) when compared with placebo; therefore, dietary tyramine restrictions are not warranted during TR-701 FA therapy at the clinically recommended dose.

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REVIEWER ASSESSMENT: The Sponsor's conclusions are appropriate based on study results. In this tyramine challenge study, the coadministration of TR-701FA (200 mg daily) did not lead into clinically significant increase in tyramine sensitivity in healthy subjects.

Reference ID: 3476543

STUDY NO.: TR701-106

An Open-Label Study to Evaluate the Absorption, Metabolism, and Excretion of Orally Administered [^{14}C]-TR-701 in Healthy Adult Male Subjects

Date(s): 29 September 2009– 29 January 2010
Sponsor: Trius Therapeutics, Inc., San Diego, CA
Clinical Site: Covance Clinical Research Unit Inc., 3402 Kinsman Boulevard, Madison, WI
Analytical Site: (b) (4)

OBJECTIVE(S):

- To describe the single dose pharmacokinetics (PK) of total radioactivity in plasma and whole blood following oral (PO) administration of [^{14}C]-TR-701 FA in healthy adult male subjects;
- To describe the single dose plasma PK of TR-700, the active metabolite of TR-701 FA, following PO administration of [^{14}C]-TR-701 FA in healthy adult male subjects;
- To determine the total radioactivity recovery and the routes/rates of excretion of a single dose of [^{14}C]-TR-701 FA administered orally in healthy adult male subjects;
- To characterize the metabolic profile of TR-700 in plasma, urine, and feces and PK of major metabolites of TR-700 in plasma (where possible) following a single oral dose of [^{14}C]-TR-701 FA in healthy adult male subjects; and
- To further assess the safety and tolerability of single PO doses of TR-701 FA in healthy adult male subjects.

METHODS

Study Design: This study was an open-label, single-dose study of the absorption, metabolism, and excretion of [^{14}C]-TR-701 FA administered orally at a dose of 204 mg* (100 µCi; *the planned dose was 200 mg TR-701, the actual calculated dose was 204 mg TR-701 to provide a 100 µCi dose in normal healthy male subjects. Subjects received a single dose of 204 mg [^{14}C]-TR-701 FA (100 µCi) administered as an oral solution. The dose was administered following at least an 8-hour fast and followed by a 4-hour fast from food, not including water. Except as part of dose administration, subjects restricted their consumption of water for 1 hour prior to dose and for 1 hour postdose; at all other times during the study, subjects were able to consume water on an ad libitum basis.

Subjects were admitted to the clinical research unit (CRU) approximately 24 hours prior to dose administration and were to be discharged from the CRU as early as Day 5 (96 hours postdose) based on satisfying each of the following Discharge Criteria:

- Plasma radioactivity reached levels below the limit of quantitation (BLQ);
- ≥90% of the dose was recovered;
- Urine total radioactivity reached ≤1% of the administered dose for 2 consecutive collections;
- Fecal total radioactivity reached ≤1% of the administered dose for 2 consecutive collections.

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If the Discharge Criteria were not met, subjects were to have been discharged no later than Day 14 (312 hours postdose). The study scheme is shown in **Table 1**.

Table 1. Study Scheme

	Screening Ch	eck-in	Dose	PK Sampling	Clinic Discharge
Oral Treatment	Days -28 to -2	Day -1	Day 1: 200 mg [^{14}C]-TR-701 (approximately 100 µCi) as a PO solution	Days 1 through 14[a] (0 Hour through 312 hours postdose)	Days 5 to 14

[a] Subjects may be discharged as early as Day 5 (96 hours postdose) or as late as Day 14 (312 hours postdose) based on satisfying Discharge Criteria.

Drug Product: A dose of 204 mg [^{14}C]-TR-701 FA with 100 µCi was administered to each subject orally. The [^{14}C]-TR-701 FA was from Lot No. 03-05-055. The unlabeled TR-701 FA was from Lot No. 02090118.

Inclusion Criteria: Males and females of non-childbearing potential; 18-50 years of age (inclusive); 20-29.9 kg/m^2 (inclusive) in body mass index; nonsmokers, and in good health as confirmed by medical history, physical exam, 12-lead electrocardiogram (ECG; within normal limits), and laboratory evaluations were enrolled.

Subjects were required to abstain from any medications (including over-the-counter remedies) from 7 days prior to Check-in and during the entire study. Subjects were also required to abstain from alcohol-, grapefruit-, or caffeine-containing foods or beverages or foods or beverages with high levels of tyramine from 72 hours prior to Check-in and during the entire study. Use of selective serotonin re-uptake inhibitors (SSRIs), monoamine oxidase (MAO) inhibitors, and other serotonergic medications were not allowed within 14 days prior to Check-in and during the entire study.

PK Sample Collection: Plasma and urine samples were collected (**Table 2**) and analyzed for pharmacokinetic purposes.

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Table 2. Pharmacokinetic sampling scheme

Whole Blood and Plasma Radioanalysis	• predose (0 Hour) • 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, and 312 hours postdose or until Discharge Criteria were met
Plasma PK	• predose (0 Hour) • 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 hours postdose
Plasma Metabolic Profiling	• predose (0 Hour)2, 4, 6, 12, 24, 48, 72, 96, and 120 hours postdose
Urine Collection	• predose (single void collected at approximately 0 Hour) • 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, 168 to 192, 192 to 216, 216 to 240, 240 to 264, 264 to 288, and 288 to 312 hours postdose or until Discharge Criteria were met.
Feces Collection	• Pre-dose • 0 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, 168 to 192, 192 to 216, 216 to 240, 240 to 264, 264 to 288, and 288 to 312 hours postdose or until Discharge Criteria were met.

Analytical Methods: Plasma PK: PK samples were analyzed for TR-700 in plasma by validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) (**Table 3**). For the calculation of summary statistics, all concentrations below the limit of quantification (BLQ) were to be set to zero.

Table 3. Bioanalytical results of TR-700 in plasma

Criterion	TR-700	Comments
	PLASMA	
Calibration Range	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	Satisfactory
Linearity, R^2	≥0.9976	Satisfactory
Accuracy	Within ±4.3%	Satisfactory
Precision, CV	≤5.1 %	Satisfactory
Quality Control*	15, 150, 750, 5000(5x) ng/mL	Satisfactory
Accuracy	Within ±3.3%	Satisfactory
Precision, CV	≤3.2%	Satisfactory
Stability	• Maximum duration from sample collection to end of analysis: 48 days • Stability: 156 days at -60 to -80 ºC	Satisfactory

**Numbers in the parentheses indicate the dilution factor for QC samples.*

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Radioanalysis: Concentrations of radioactivity in blood, plasma, urine, and feces samples were analyzed by Model 2900TR liquid scintillation counters (LSC). For the calculation of summary statistics, all concentrations below the limit of quantification (BLQ) were to be set to zero.

Metabolic Profiling: Plasma samples obtained from 6 male human subjects at 2, 4, 6, 12, and 24 hours postdose were pooled to generate single AUC-representative pooled samples; urine samples collected from 6 male human subjects at 0-4, 4-8, 8-12, 12-24, and 24-48 hours postdose were pooled by subject to generate single 0- to 12-hour and 12- to 48-hour pooled; feces samples collected from 6 male human subjects at 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours postdose were pooled by subject, including 0.5 to 2% (equivalent percent by interval) of each sample. Aliquots of each pooled sample (plasma/urine/feces) were combusted and analyzed by LSC to determine the concentrations of radioactivity; and the reconstituted samples were analyzed by HPLC to determine the metabolite profiles.

Pharmacokinetic Assessment: For each subject, the following PK parameters were calculated, whenever possible, based on the plasma concentrations of TR-700, the active moiety of TR-701 FA, and total radioactivity in whole blood and plasma, according to the model independent approach:

C_{max}: Maximum observed concentration.
T_{max}: Time to maximum concentration.
AUC_{0-t}: Area under the concentration-time curve from Hour 0 to the last measurable concentration, calculated by the linear trapezoidal rule.
$AUC_{0-\infty}$: Area under the concentration-time curve extrapolated to infinity, calculated using the formula: $AUC_{0-\infty} = AUC_{0-t} + Ct / \lambda_Z$. where Ct is the last measurable concentration and λ_Z is the apparent terminal elimination rate constant.
λ_Z: Apparent terminal elimination rate constant, where λ_Z is the magnitude of the slope of the linear regression of the log concentration versus time profile during the terminal phase.
$t_{1/2}$: Apparent terminal elimination half-life, where $t_{1/2} = \ln(2) / \lambda z$.
CL/F: Oral clearance, calculated for unlabeled analyte TR-700 only. Calculated as Dose/$AUC_{0-\infty}$.
The TR-700 dose was estimated for a given dose of TR-701 free acid (FA) as: TR-700 Dose = TR-701 FA Dose of 204 mg× (TR-700 MW of 370.34/TR-701 FA MW of 450.32).
Vz/F: Volume of distribution during the terminal phase after oral administration, for unlabeled analyte TR-700 only, calculated as (CL/F) / λ_Z.

In addition, the following PK parameter was calculated, when possible, for each subject based on urine total radioactivity concentrations:
CL_R: Renal clearance, where CL_R = Cum $A_{e(urine)}/AUC_{0-\infty}$, where Cum $A_{e(urine)}$ is the amount excreted in urine.

The following urinary parameters are included in the Radioanalysis Report:
C_{urine}: Urine concentration.
$A_{e(urine)}$: Amount excreted in urine during each collection interval, calculated as C_{urine} × urine volume.
Cum $A_{e(urine)}$: Cumulative amount excreted in urine.
% $A_{e(urine)}$: Percentage of dose excreted in urine over the collection interval.

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Cum% $A_{e(urine)}$: Cumulative percentage of dose excreted in the urine.

The following fecal parameters are included in the Radioanalysis report:
C_{feces}: Fecal concentration.
$A_{e(feces)}$: Amount excreted in urine during each collection interval, calculated as $C_{feces} \times$ feces volume.
Cum $A_{e(feces)}$: Cumulative amount excreted in feces.
% $A_{e(feces)}$: Percentage of dose excreted in feces over the collection interval.
Cum% $A_{e(feces)}$: Cumulative percentage of dose excreted in the feces.

Pharmacokinetic calculations were performed, if appropriate, using WinNonlin (Pharsight Corporation, Version 5.2). If pharmacokinetic analysis used actual times were missing, nominal times were used. Concentration values were not rounded prior to the calculation of PK parameters; the concentrations were used as supplied by the analytical laboratory for PK analysis.

Statistical Methods:
General:
Data listings are provided for PK and safety data. Summary statistics were provided for data if applicable. Subject eligibility data collected only at Screening or Check-in might not have been summarized in tables but is available in the database. Data analysis was performed using Statistical Analysis Software (SAS®) Version 9.1.

Pharmacokinetics:
Standard descriptive statistics were calculated for all PK parameters determined with the exception of major metabolite PK parameters. For major metabolites of TR-700, standard descriptive statistics were calculated only for primary PK parameters determined (C_{max}, T_{max}, AUC_{0-t}, and $AUC_{0-\infty}$). No formal statistical analyses were planned or performed.

Safety:
Summary statistical analyses were planned and changes from baseline provided.

Safety Assessment: Safety procedures included adverse event (AE) assessments, 12-lead ECGs, vital signs, physical examinations, and laboratory assessments.

RESULTS
Study Population: In total, 6 subjects were enrolled in the study and completed the study. There were no protocol deviations in the conduct of this study. A summary of demographic and baseline characteristics for the study population is presented in **Table 4**.

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Table 4. Demographic Characteristics

Demographic Variable	Result
Mean age (minimum, median, maximum years)	34 (20, 36, 46)
Mean weight (minimum, median, maximum kg)	78.4 (66.5, 72.3, 96.4)
Mean height (minimum, median, maximum cm)	175.9 (170.3, 175.7, 181.7)
BMI (minimum, median, maximum kg/m^2)	25.3 (20.8, 24.4, 29.8)
Gender n (%)	
Male	6 (100%)
Ethnicity n (%)	
Not Hispanic or Latino	6 (100%)
Race n (%)	
White	5 (83.3%)
Black or African American	1 (16.7%)

Pharmacokinetics:

Metabolite Profiling and Identification

Analyses of plasma, urine, and feces identified 8 metabolites and characterized six including the following: M3 (MS436; desmethyl TR-700 sulfate), M4 (desmethyl TR-700), M5 (MS328; dealkyl TR-701 carbamic acid), M6 (carboxy TR-700), M7 (TR-700 sulfate), and M8 (TR-700). Metabolites M1 and M2 were present in low concentrations and were not detected by LC-MS.

Plasma

Radiochemical profiles in pooled plasma samples from [^{14}C]-TR-701-FA-treated subjects showed 1 major and 1 minor metabolite. TR-701-FA underwent extensive hydrolysis of the phosphate moiety into TR-700. The hydrolyzed alcohol product, TR-700 (M8), accounted for 94.54% to 98.23% of sample radioactivity. Metabolite M7, the sulfate analog of TR-700, was a minor metabolite detected in Subject 002 only, and accounted for a mean of < 1% of sample radioactivity. [^{14}C]-TR-701 FA was not detected in plasma from any subject.

Urine

HPLC analysis of urine samples after an oral dose of [^{14}C]-TR-701 FA showed 1 major, 3 minor, and 4 trace metabolites. Metabolite M7 (TR-700 sulfate) was the most abundant urine metabolite and accounted for 56.81% to 79.49% of sample radioactivity and for 7.17% to 14.2% of administered dose through 48 hours postdose for all subjects. Desmethyl TR-700 (M4), carboxy TR-700 (M6), and TR-700 (M8) were minor metabolites that accounted for 0.530% to 2.38%, 2.54% to 4.68%, and 0.438% to 1.62% of the administered dose through 48 hours postdose. Trace metabolites M1, M2, M3, and M5 were variable between subjects and accounted for less than 1% of the dose through 48 hours postdose. TR-701 FA was not detected in urine from any subject.

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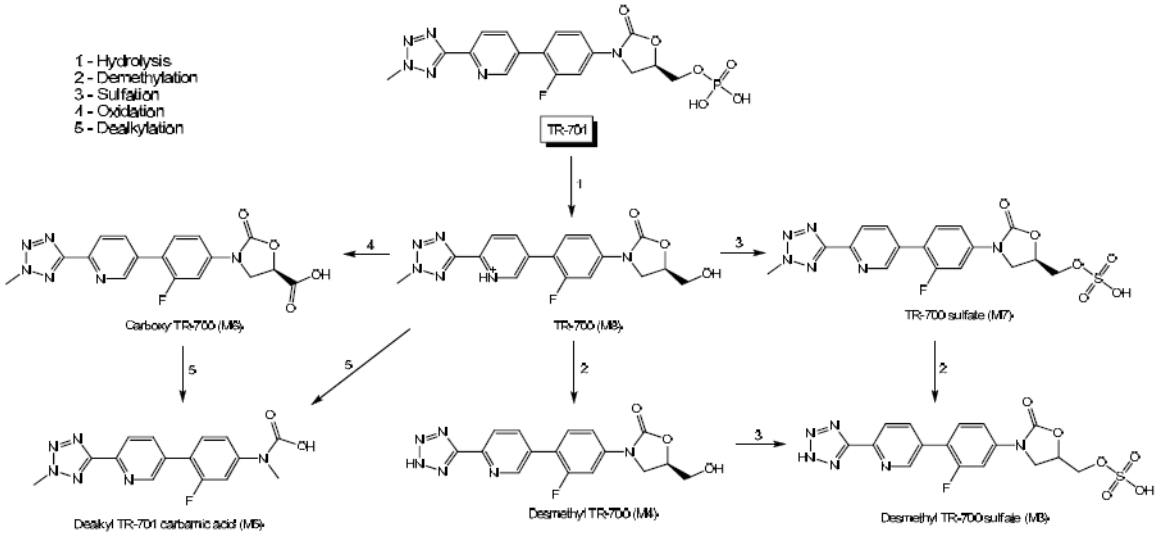
Feces

Radiochemical profiles of radioactivity in fecal samples from [^{14}C]-TR-701-FA-treated subjects showed 1 major and 2 minor metabolites. Metabolite M7, the sulfate analog of TR-700, accounted for 84.80% to 95.64% of sample radioactivity and for 63.3% to 79.3% of the administered dose across all subjects. Minor metabolites M6 and M8 accounted for less than 6% and less than 3% of administered dose, respectively across subjects. TR-701 was not detected in fecal samples from any subject.

Proposed Biotransformation Pathway

TR-701 underwent extensive metabolism to one primary metabolite; three secondary metabolites; one tertiary metabolite; two unidentified, low-abundance metabolites; and one carbamic acid metabolite. Initially, TR-701 was hydrolyzed to its alcohol metabolite TR-700 (M8). Subsequent oxidation, *N*-demethylation, or sulfation produced secondary metabolites carboxy TR-700 (M6), desmethyl TR-700 (M4), or TR-700 sulfate (M7), respectively. Sulfation of M4 or *N*-demethylation of M7 produced the tertiary metabolite M3. The biotransformation pathway of TR-701 to the carbamic acid metabolite M5 is not known. No substantive differences in the biotransformation of TR-701 were observed among human subjects. A proposed biotransformation pathway is presented in **Figure 1**.

Figure 1. Proposed biotransformation pathway of [^{14}C]-TR-701 in humans



TR-700 in Plasma and Total Radioactivity in Plasma and Whole Blood

Following a single oral dose of [^{14}C]-TR-701 FA (~100 µCi, 204 mg), TR-700 and total radioactivity readily appeared in plasma, with median T_{max} values of 1.28, 1.50, and 1.25 hours observed for TR-700 in plasma and total radioactivity in plasma and whole blood, respectively (**Table 5**). After reaching C_{max}, plasma and whole blood concentrations steadily declined with mean $t_{1/2}$ values of 10.6, 11.5, and 10.3 hours observed for TR-700 in plasma and total radioactivity in plasma and whole blood, respectively (**Table 5**).

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The mean C_{max} for total radioactivity in plasma (2558 ng eq./g) was approximately 22% higher than the observed mean C_{max} for TR-700 in plasma (1993 ng/mL). The mean AUC_{0-t} for total radioactivity in plasma (31361 ng eq.•hr/g) was approximately 21% higher than the observed mean AUC_{0-t} for TR-700 in plasma (24743 ng•hr/mL) (**Table 5**). The mean $AUC_{0-\infty}$ for total radioactivity in plasma (33207 ng eq. •hr/g) was approximately 22% higher than the observed mean $AUC_{0-\infty}$ for TR-700 in plasma (25838 ng•hr/mL) (Table 11-4). These results indicate that TR-700 constituted approximately 78% of the total radioactivity.

The mean C_{max} for total radioactivity in plasma (2558 ng eq./g) was comparable to the mean C_{max} for total radioactivity observed in whole blood (2430 ng eq./g) as shown in **Table 5**. The mean AUC_{0-t} for total radioactivity in plasma (31361 ng eq.•hr/g) was also comparable to the mean AUC_{0-t} for total radioactivity in whole blood (28187 ng eq.•hr/g) as shown in **Table 5**. Similar results were observed for $AUC_{0-\infty}$ values. The mean whole blood to plasma total radioactivity $AUC_{0-\infty}$ ratio (AUC_b:AUC_p) was approximately 0.925.

Table 5. Summary of Mean (SD) Pharmacokinetic Parameter Data for TR-700 in Plasma and Total Radioactivity in Plasma and Whole Blood

	Treatment: [^{14}C]-TR-701 (~100 µCi, 204[a] mg)					
		TR-700		Total Radioactivity		
PK Parameter (units)[b]	**N**	**Plasma**	**N**	**Plasma**	**N**	**Whole Blood**
C_{max} (ng/mL)	6	1993 (334)	6	2558 (467)	6	2430 (294)
t_{max} (hr)[c]	6	1.28 (1.00, 3.98)	6	1.50 (1.00, 2.00)	6	1.25 (1.00, 1.50)
AUC_{0-t} (ng•hr/mL)	6	24743 (4958)	6	31361 (6623)	6	28187 (5937)
$AUC_{0-\infty}$ (ng•hr/mL)	6	25838 (5455)	6	33207 (7020)	6	30721 (6236)
λ_z (1/hr)	6	0.0672 (0.0115)	6	0.0617 (0.00909)	6	0.0730 (0.0226)
$t_{½}$ (hr)	6	10.6 (1.79)	6	11.5 (1.85)	6	10.3 (3.04)
CL/F (L/hr)	6	6.81 (1.86)	0	NA	0	NA
V_z/F (L)	6	101 (15.1)	0	NA	0	NA

a The TR-701 equivalent dose of TR-700 (167.7681649 mg) was used in the CL/F and Vz/F calculations for TR-700 (unlabeled) in plasma.
b Units of ng eq./g and ng eq.·hr/g are presented for total radioactivity for C_{max} and AUC, respectively.
c Median (minimum, maximum) presented for T_{max}.

Total Radioactivity in Urine and Feces

Mean recovery of the radioactive dose in urine and feces was 18.0 and 81.5%, respectively (**Table 6**). Total recovery in urine and feces combined was approximately 99.5% by 288 hours postdose. The majority (87.6%) of the radioactivity was recovered by 96 hours postdose. Excretion through feces appeared to be the major elimination pathway for total radioactivity after [^{14}C]-TR-701 FA administration (**Table 6 and Figure 1**).

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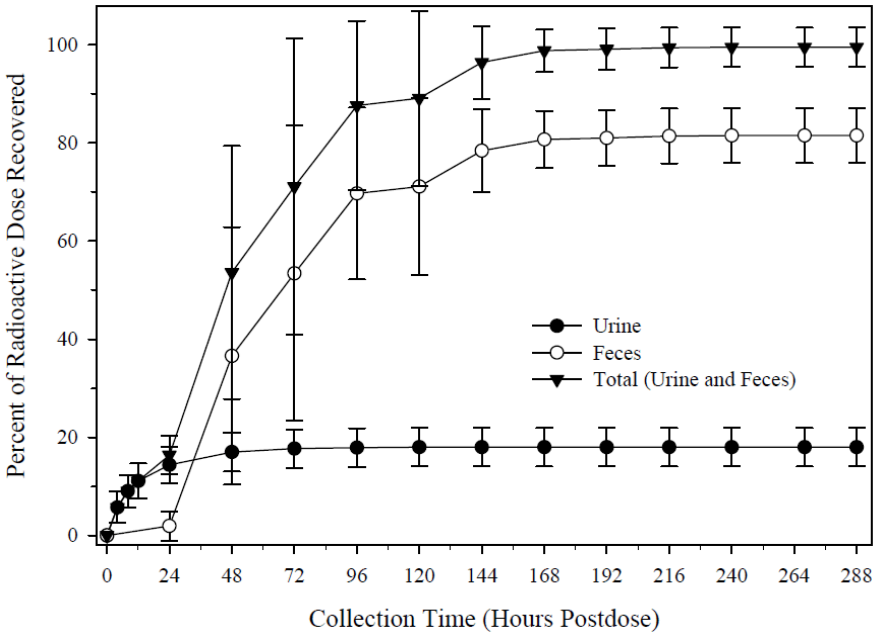
Table 6. Summary of Mean (SD) Pharmacokinetic Parameter Data for Total Radioactivity in Urine and Feces

			Treatment: [^{14}C]-TR-701 (100 µCi, 204 mg)		
			Total Radioactivity		
PK Parameter	**Units**	**N**	**Urine**	**N**	**Feces**
Cum A_e	µg equivalents	6	36800 (8050)	6	167000 (11500)
Cum %A_e	%	6	18.0 (3.94)	6	81.5 (5.57)
CL_R	L/hr	6	1.16 (0.398)	0	NA

Figure 1. Mean Cumulative Percent of Radioactive Dose Recovered in Urine and Feces at Specified Intervals After a Single 204-mg (100-µCi) Oral Dose of [^{14}C]-TR-701 FA to Healthy Male Subjects



Safety: Single doses of oral 204 mg of [^{14}C]-TR-701 FA were well-tolerated, with no discontinuations due to AEs and no SAEs reported. The 3 AEs observed were mild and resolved without treatment. There were no clinically significant changes noted in laboratory parameters, vital signs, physical examinations, or 12-lead ECGs.

SPONSOR'S CONCLUSIONS

- TR 701 underwent rapid and extensive hydrolysis of the phosphate moiety into TR-700.
- TR-700 constituted approximately 78% of the total radioactivity circulating in plasma based on AUC.

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- Eight metabolites were identified in plasma, urine, and feces. The only significant circulating metabolite found in plasma was the hydrolyzed alcohol product, TR-700. The main metabolite found in feces and urine was a sulfate analog of TR-700.
- Feces appeared to be the major elimination pathway for total radioactivity. The mean recovery of the radioactive dose in urine and feces was approximately 99.5% by 288 hours postdose, with 18.0% recovered in urine and 81.5% recovered in feces.
- Single oral doses of 204 mg TR-701 FA were safe and well tolerated in this group of healthy adult male subjects.

REVIEWER ASSESSMENT: The Sponsor's conclusions are appropriate based on study results.

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STUDY NO.: TR701-107

A Double-Blind, Placebo-Controlled, Single- and Multiple-Ascending Dose, Safety, Tolerability, and Pharmacokinetic Study of an Intravenous Formulation of TR-701 FA and an Open-Label, Crossover Absolute Bioavailability Determination of a TR-701 FA Tablet in Normal Healthy Adults

Date(s): 29 September 2009– 29 January 2010
Sponsor: Trius Therapeutics, Inc., San Diego, CA
Clinical Site: Covance Clinical Research Unit Inc., 3402 Kinsman Boulevard, Madison, WI
Analytical Site: (b) (4)

OBJECTIVE(S):

Part A: Single Ascending Dose

- To determine the safety and tolerability of TR-701 FA in healthy adult subjects when administered as a single intravenous (IV) dose.
- To evaluate the plasma pharmacokinetics (PK) of single rising IV doses of TR-701 FA and its microbiologically active moiety, TR-700, in healthy adult subjects.
- To evaluate the venous tolerability of a pilot IV dose and 3 IV administration regimens for each single rising dose of TR-701 FA in healthy adult subjects.

Part B: Multiple Ascending Dose

- To determine the safety and tolerability of TR-701 FA in healthy adult subjects when administered as multiple IV doses for 7 days.
- To evaluate the plasma PK and extent of urinary excretion of multiple rising IV doses of TR-701 FA and its microbiologically active moiety, TR-700, in healthy adult subjects.

Part C: Absolute Bioavailability

- To determine the absolute bioavailability of oral TR-701 FA (measured as TR-700) following single oral and IV dose administrations in healthy adult subjects.

Part D: Venous Tolerability

- To determine the venous tolerability of 200 mg TR-701 FA in healthy adult subjects when administered as multiple IV doses for 3 days.
- To determine the safety of 200 mg TR-701 FA in healthy adult subjects when administered as multiple IV doses for 3 days.

METHODS

Study Design: This study consisted of 4 parts: a double-blind, placebo-controlled, single- (Part A) and multiple-ascending (Part B) dose study of the safety, tolerability, and PK of 100 to 400 mg IV TR-701 FA; an open-label study of the absolute bioavailability of 200-mg TR-701 FA tablets (Part C); and a venous tolerability study of 200-mg TR-701 FA IV (Part D). The single ascending dose portion of the study included an open-label 50-mg pilot cohort to establish a baseline reference level of venous tolerability of IV TR-701 FA. Note that 50 mg TR-701 FA is equivalent to 41 mg TR-700; 100 mg TR-701 FA to 82 mg TR-700; 200 mg TR-701 FA to 164 mg TR-700; 300 mg TR-701 FA to 246 mg TR-700; and 400 mg TR-701 FA to 329 mg TR-700.

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Doses of TR-701 FA in 250 or 500 mL (two 250-mL bags) USP sterile saline were administered as 60-, 120-, or 180-minute IV infusions. For the oral administration in Part C, TR-701 FA tablets, 200 mg was to be administered orally with 240 mL of room temperature tap water. The drug products used in this study were listed in **Table 2**. In Parts A (single ascending dose) and C (bioavailability) and on Days 1 and 7 in Part B (multiple ascending dose), doses were to be administered following an overnight fast (at least 8 hours) from food and were followed by a fast from food for at least 4 hours post-dose (relative to the end of infusion).

Treatments were administered as shown in **Table 1**. In Part A, 4 subjects received 50 mg TR-701 FA as a 250-mL, 180-minute IV infusion in the Pilot Cohort to establish a baseline reference level of venous tolerability of IV TR-701 FA. Subjects in Cohorts 1, 2, 3, and 7 were assigned randomly to Treatment A, B, or C with subjects receiving either TR-701 FA (N=3) or placebo (N=1) in each treatment. In Cohorts 1, 2, 3 and 7, 4 subjects received Treatment A on Day 1. Once the safety of Treatment A had been established through review of available safety/PK data, 4 subjects received Treatment B on the following day and the same pattern was used for Treatment C. The study team could also have reduced the dose or modified infusion rate based on the available safety and PK data. Cohorts were dosed no sooner than 5 days following the last dosing of the preceding dose level. Once the safety and tolerability of the single 200-mg TR-701 FA IV dose (Cohort 3) had been established, Part C (absolute bioavailability; Cohort 6) could have proceeded. In Part C, there was to be at least a 7-day washout period between doses, which was chosen based on the half-life (10-11 hours at 200 mg TR-701) from study TR701-101.

In Part D (Venous Tolerability), 10 subjects received both 200 mg TR-701 FA and placebo as a 250-mL, 60-minute IV infusion in a crossover design to characterize venous tolerability to IV TR-701 FA using a standardized scale of phlebitis scoring. Subjects were randomly assigned to either Treatment A (200-mg IV TR-701 FA) followed by B (placebo; n=5) or B followed by A (n=5). Each treatment was administered via a 22 gauge polyetherurethane catheter (hand or wrist placement) on one side of the body (right side first), followed by the alternate treatment in a separately placed catheter (hand or wrist) on the opposite side of the body. Transparent adhesive dressing was to be used (no gauze). The IV could have been moved to a more proximal location if patency was compromised. The catheter was to be removed if a Visual Infusion Phlebitis score of 2 (early stage phlebitis) was met. Catheters were removed approximately 2 to 4 hours after the third dose of each treatment regimen. Catheters would have been in place for approximately 50 to 54 hours per catheter if all 3 doses were administered for each treatment.

Subjects were confined to the CRU from the time of Check-in (Day -1) until Clinic Discharge on Day 2 (Part A, Pilot Cohort), Day 4 (Parts A and C [in each of the 2 periods for the crossover dosing]), Day 8 (Part D), or Day 10 (Part B) of study. PK and safety were measured during the study.

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Table 1. Dose Cohorts

Cohort	Subject Numbers	Number of Subjects		Dose of Placebo or TR-701 FA
		TR-701 FA	Placebo	
Part A: Single Ascending Dose				
Pilot	001-004	4	0	50 mg as 250-mL, 180-minute infusion in (b) (4) bag
1	005-008	3	1	0 or 100 mg as 500-mL, 120-minute infusion in (b) (4) (b) (4) bags (Treatment A)
	009-012	3	1	0 or 100 mg as 250-mL, 120-minute infusion in (b) (4) (b) (4) bags (Treatment B)
	013-016	3	1	0 or 100 mg as 250-mL, 60-minute infusion in (b) (4) bags (Treatment C)
2	017-020	3	1	0 or 100 mg as 500-mL, 120-minute infusion in (b) (4) bags (Treatment A)
	021-024	3	1	0 or 100 mg as 250-mL, 120-minute infusion in (b) (4) bags (Treatment B)
	025-028	3	1	0 or 100 mg as 250-mL, 60-minute infusion in (b) (4) bags (Treatment C)
3	029-032	3	1	0 or 200 mg as 500-mL, 120-minute infusion in (b) (4) bags (Treatment A)
	033-036	3	1	0 or 200 mg as 250-mL, 120-minute infusion in (b) (4) bags (Treatment B)
	037-040	3	1	0 or 200 mg as 250-mL, 60-minute infusion in (b) (4) bags (Treatment C)
7	069-072	3	1	0 or 400 mg as 500-mL, 120-minute infusion in (b) (4) bags (Treatment A)
	073-076	3	1	0 or 400 mg as 250-mL, 120-minute infusion in (b) (4) bags (Treatment B)
	077-080	3	1	0 or 400 mg as 250-mL, 60-minute infusion in (b) (4) bags (Treatment C)
Part B: Multiple Ascending Dose				
4	041-050	8	2	0 or 200 mg as 250-mL, 60-minute infusion in (b) (4) bags x 7 days
5	051-060	8	2	0 or 400 mg as 250-mL, 60-minute infusion in (b) (4) bags x 7 days
Part C: Absolute Bioavailability (Crossover Design)				
6	061-068	4	0	Period 1: 200 mg as oral tablet Period 2: 200 mg as 250-mL, 60-minute infusion in (b) (4) bags
		4	0	Period 1: 200 mg as 250-mL, 60-minute infusion in (b) (4) bags Period 2: 200 mg as oral tablet
Part D: Venous Tolerability (Crossover Design)				
8	081-090	Crossover design 10		0 mg (Treatment B) and 200 mg (Treatment A) as 250-mL, 60-minute infusion in (b) (4) bags x 3 days each

Notes: (1) Cohort 2 (Part A) was administered the same dose level as Cohort 1 using the intended (b) (4) 0.9% saline bags.
(2) The planned 400-mg dose for Cohort 5 was lowered to 300 mg prior to dosing of this group.
(3) For the venous tolerability evaluation (Part D), the first drug infused per the subject's randomization was administered in the right arm and infusions were administered in the left arm for the second drug of the randomization.
(4) There was no PK analysis for Cohort 1.
(5) The safety data for Cohorts 1 and 2 were pooled, giving a denominator of 18 for the 100-mg dose level.
mg = milligrams; mL = milliliters.

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Table 2. Drug Products

Drug Name	TR-701 FA for Injection 200 mg/vial	TR-701 FA Tablet 200 mg	Placebo
Formulation	lyophilized powder	tablet	USP Sterile Saline
Strength	200 mg	200 mg	0.9%
Lot Number	B090135	B090282	C775551, J9K505, J9K709, J9K658, J8N439
Storage Condition	15°C to 30°C	15°C to 30°C	15°C to 30°C
Amount Supplied	399 vials (200 mg/vial)	21 tablets	450 bags[a]
Amount Used	251 vials	8 tablets	303 bags
Amount Remaining	148 vials	13 tablets	147 bags
Disposition	Incinerated (b) (4) (b) (4)	Retained by Clinical Research Unit for 5 years	Transferred to bulk supply at Clinical Research Unit
Supplier	(b) (4)		
Manufacturer			

Inclusion Criteria: Males and females of non-childbearing potential; 18-50 years of age (inclusive); 20-29.9 kg/m^2 (inclusive) in body mass index; and in good health as confirmed by medical history, physical exam, and laboratory evaluations were enrolled.

Subjects were required to abstain from any medications (including over-the-counter remedies) from 7 days prior to Check-in until after completion of the study. Subjects were also required to abstain from alcohol-, grapefruit-, or caffeine-containing foods or beverages or foods or beverages with high levels of tyramine from 72 hours prior to Check-in until after completion of the study. Use of selective serotonin re-uptake inhibitors (SSRIs), monoamine oxidase (MAO) inhibitors, and other serotonergic medications were not allowed within 14 prior to Check-in until after completion of the study.

PK Sample Collection: Plasma and urine samples were collected (**Table 3**) and analyzed for pharmacokinetic purposes.

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Table 3. Pharmacokinetic sampling scheme for Part A (single-dose), Part B (multiple-dose), Part C (bioavailability).

PART A	
DAY 1	**Plasma** • 0 Hour (prior to the start of infusion) • 0.25 and 0.5 hours after the start of infusion; • every 0.5 hours during infusion (as needed); • immediately prior to the end of infusion; • and 0.08, 0.17, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours post-infusion (relative to the end of infusion).
PART B	
DAY 1	**Plasma** • Pre-dose • 0.25 and 0.5 hours after the start of infusion; • every 0.5 hours during infusion (as needed); • immediately prior to the end of infusion; • and 0.08, 0.17, 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 12 hours post-infusion (relative to the end of infusion). **Urine** • -2 to 0 (predose; Day 1 only), 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours after the start of infusion.
DAYS 2-6	**Plasma (Trough)** • 0 Hour (prior to the start of infusion)
DAY 7	**Plasma** • 0 Hour (prior to the start of infusion) • 0.25 and 0.5 hours after the start of infusion; • every 0.5 hours during infusion (as needed); • immediately prior to the end of infusion; • and 0.08, 0.17, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours post-infusion (relative to the end of infusion). **Urine** • 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours after the start of infusion.
PART C	
DAY 1	**Plasma** ***IV*** • Pre-dose • 0.25 and 0.5 hours after the start of infusion; • every 0.5 hours during infusion (as needed); • immediately prior to the end of infusion; and 0.08, 0.17, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours post-infusion (relative to the end of infusion). ***Oral*** • 0 Hour (predose) • 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours post-dose.

Reference ID: 3476543

Analytical Methods: Pharmacokinetic samples were analyzed for TR-701 and TR-700 in plasma/urine by validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) (**Table 4**). For the calculation of summary statistics, all concentrations below the limit of quantification (BLQ) were to be set to zero.

Table 4. Bioanalytical results of TR-701 and TR-700 in plasma /urine

Criterion	TR-701	TR-700	Comments
PLASMA			
Calibration Range	5-1000 ng/mL	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	Satisfactory
Linearity, R^2	≥0.9957	≥0.9983	Satisfactory
Accuracy	Within ±2%	Within ±2%	Satisfactory
Precision, CV	≤5.7 %	≤3.3 %	Satisfactory
Quality Control*	15, 150, 750, 5000(10x) ng/mL	15, 150, 750, 5000(10x) ng/mL	Satisfactory
Accuracy	Within ±2%	Within ±0.7%	Satisfactory
Precision, CV	≤6.3 %	≤6.6%	Satisfactory
Stability	• Maximum duration from sample collection to end of analysis: 86 days • Stability: 156 days at -60 to -80 ºC		Satisfactory
URINE			
Calibration Range	5-1000 ng/mL	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	Satisfactory
Linearity, R^2	≥0.9982	≥0.9998	Satisfactory
Accuracy	Within ±7%	Within ±2%	Satisfactory
Precision	≤5.2 %	≤2.4%	Satisfactory
Quality Control*	15, 150, 750, 5000(10x) ng/mL	15, 150, 750, 5000(10x) ng/mL	Satisfactory
Accuracy	Within ±2.7%	Within ±4.7%	Satisfactory
Precision, CV	≤7.6 %	≤8.3 %	Satisfactory
Stability	• Maximum duration from sample collection to end of analysis: 69 days • Stability: 378 days at -60 to -80 ºC		Satisfactory

**Numbers in the parentheses indicate the dilution factor for QC samples.*

Pharmacokinetic Assessment: For each subject (with the exception of subjects in the Pilot Cohort and the Venous Tolerability Cohort, Part D), the following PK parameters were calculated, whenever possible, based on the plasma concentrations of TR-701 and its active metabolite, TR-700, according to the model independent approach: C_{max}, T_{max}, AUC_{0-24}, AUC_{0-t}, $AUC_{0-\infty}$, F, CL, CL/F, V_{ss} (IV administration only), Vz/F(oral administration only), λ_Z, and $t_{1/2}$. In addition, for Part B the following PK parameters were calculated, whenever possible, on both Day 1 and 7 for each subject based on the urine TR-701 and its active metabolite, TR-700, concentrations: $Ae_{(0-24)}$, CL_R, and $fe_{(0-24)}$. Dose-normalized C_{max} and AUC parameters were also determined for TR-701 and TR-700, wherever possible, by calculating the ratio of C_{max} and AUC ($AUC_{0-\infty}$ for Part A and Part B, Day 1 and AUC_{0-24} for Part B, Day 7) to the dose of TR-701 FA and TR-700 administered (TR-700 dose for a given dose of TR-701 FA = TR-701 FA Dose x [TR-700 MW of 370.34/TR-701 FA MW of 450.32]).

Reference ID: 3476543

Statistical Methods:
Descriptive statistics were calculated for PK parameters of TR-701 and its active metabolite, TR-700. Minimum, maximum, mean, and median values were determined and standard deviations were calculated. Absolute bioavailability was examined between the oral TR-701 FA tablet (Test) and the 200-mg TR-701 FA IV formulation (Reference) in Part C. An analysis of variance (ANOVA) was performed. Data for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ of TR-701 and its active metabolite, TR-700, were natural log-transformed prior to analysis. The 90% confidence intervals (CIs) of the test group PK parameter means relative to the reference group PK parameter means were obtained by taking the antilog of the corresponding 90% CIs for the differences between the means on the natural log scale.

Safety Assessment: Safety procedures included adverse event (AE) assessments, 12-lead electrocardiograms (ECGs), vital signs, physical examinations, and laboratory assessments.

RESULTS
Study Population: In total, 90 subjects were enrolled in the study, 78 subjects completed the study.

Cohort 1 (Subjects 005 through 016) was inadvertently infused using (b) (4) bags. Since compatibility testing had not been performed with the TR-701 FA solution using the (b) (4) bags, Cohort 2 was given the same dose as Cohort 1 (100-mg dose) using the intended (b) (4) saline bag. Pharmacokinetic data from Cohort 1 were not analyzed and reported since the same 100-mg dose was repeated in Cohort 2 and PK data from Cohort 2 were reported. Safety data from Cohorts 1 and 2 were pooled together resulting in a denominator of 18 for the 100-mg dose level.

Based on multiple IV-related AEs in Cohort 5 (300-mg dose/7 days), study drug administration was changed from a 60-minute infusion of 250 mL on Days 1 and 2 to a 120-minute infusion of 500 mL on Days 3 through 6.

In Part B, Subject 045 (200 mg TR-701 FA) withdrew early and did not have all scheduled PK samples drawn. Due to limited data, Subject 045 was excluded from the Day 7 PK analysis for TR-700 and TR-701.

In Part B, Subject 045 (200 mg TR-701 FA), Subjects 051, 052, 054, 055, 056, 058, 059, and 060 (300 mg TR-701 FA) and Subjects 053 and 057 (placebo) did not have plasma or urine collections for Day 7. As a result, plasma and urine PK parameters were calculated for Day 1 only for Subjects 045, 051, 052, 054, 055, 056, 058, 059, and 060 in Part B.

There were no other protocol deviations in the conduct of this study.

Reviewer Comment: These deviations documented do not appear to adversely affect the results of the study.

Reference ID: 3476543

Demographics:
A summary of demographic and baseline characteristics for the study population is presented in **Table 5**. There were no substantial differences in demographic characteristics between the study groups.

Table 5. Demographic Characteristics

Demographic Variable	Pilot N=4	Part A: Single Ascending Dose N=47	Part B: Multiple Ascending Dose N=21	Part C: Absolute Bioavailability N=8	Part D: Venous Tolerability N=10
Mean age (years)	34 (21 to 45)	29 (18 to 48)	29 (18 to 47)	30 (23 to 37)	33 (20 to 43)
Mean weight (kg)	80.7 (70.7 to 90.3)	78.2 (58.5 to 103.8)	74.7 (48.9 to 110.7)	73.2 (60.0 to 85.6)	77.6 (57.2 to 91.1)
Mean height (cm)	178.8 (174 to 188.2)	174.5 (156 to 191.5)	172.3 (152.7 to 193.1)	171.9 (153.4 to 184.0)	176.5 (155.3 to 188.9)
Mean BMI (kg/m^2)	25.4 (22.3 to 29.8)	25.6 (19.8 to 31.5)	25.0 (20.6 to 29.8)	24.7 (22.4 to 26.3)	24.8 (20.9 to 27.5)
Gender (n[%])					
Male	4 (100%)	34 (72.3%)	16 (76.2%)	6 (75%)	8 (80%)
Female	0 (0%)	13 (27.7%)	5 (23.8%)	2 (25%)	2 (20%)
Ethnicity (n[%])					
Hispanic or Latino	0 (0%)	2 (4.3%)	5 (23.8%)	1 (12.5%)	0 (0%)
Not Hispanic or Latino	4 (100%)	45 (95.7%)	16 (76.2%)	7 (87.5%)	10 (100%)
Race (n[%])					
White	4 (100%)	41 (87.2%)	16 (76.2%)	7 (87.5%)	9 (90%)
Black or African American	0 (0%)	6 (12.8%)	3 (14.3%)	1 (12.5%)	1 (10%)
Other	0 (0%)	0 (0%)	2 (9.5%)	0 (0%)	0 (0%)

Pharmacokinetics:
Single Ascending Dose (Part A)
TR-701
Following single IV administration of TR-701 FA at dose levels of 100, 200, and 400 mg in Cohorts 2, 3, and 7 (Part A), respectively, Plasma TR-701 concentrations increased with dose, but were only detectable during infusion and briefly thereafter (generally less than 1 hour at 100 mg or 200 mg, and less than 3 hours at the top dose of 400 mg). Pharmacokinetic parameters are shown in **Table 6** and indicate rapid clearance and small volume of distribution. The mean half-life of the 200 mg dose group was less than 10 minutes.

Reference ID: 3476543

Table 6. Summary of the Mean (Standard Deviation) Plasma Pharmacokinetic Parameter Data for TR-701 (Part A: Single Ascending Dose)

PK Parameter	N	Cohort 2 100 mg TR-701 FA	N	Cohort 3 200 mg TR-701 FA	N	Cohort 7 400 mg TR-701 FA
C_{max} (ng/mL)	9	579 (193)	9	1014 (312)	9	2456 (701)
DN-C_{max} (ng/mL/mg)	9	5.79 (1.93)	9	5.07 (1.56)	9	6.14 (1.75)
T_{max} (hr)[a]	9	0.917 (0.283, 1.50)	9	0.500 (0.250, 1.92)	9	0.500 (0.250, 1.00)
AUC_{0-t} (ng*hr/mL)	9	754 (153)	9	1288 (271)	9	3268 (624)
DN-AUC_{0-t} (ng*hr/mL/mg)	9	7.54 (1.53)	9	6.44 (1.36)	9	8.17 (1.56)
$AUC_{0-\infty}$ (ng*hr/mL)	8	778 (145)	9	1290 (272)	7	3082 (524)
DN-$AUC_{0-\infty}$ (ng*hr/mL/mg)	8	7.78 (1.45)	9	6.45 (1.36)	7	7.71 (1.31)
CL (L/hr)	8	132 (23.7)	9	161 (30.0)	7	133 (22.4)
V_{ss} (L)	8	11.5 (3.29)	9	9.79 (4.41)	7	10.6 (5.57)
λ_z (1/hr)	8	7.94 (3.46)	9	6.74 (3.08)	7	1.86 (1.02)
$t_{½}$ (hr)	8	0.102 (0.0405)	9	0.121 (0.0501)	7	0.682 (0.699)

a Median (minimum, maximum) presented for T_{max}.
Notes: Subjects 5 through 16 (Cohort 1, 100-mg dose) were dosed with (b) (4) *saline bags rather than the intended* (b) (4) *saline bags. As a result, the PK parameter data for Subjects 5 through 16 (Cohort 1) were not reported since the 100-mg PK data were reported from Cohort 2.*

TR-700

As shown in Table 7, following single IV administration of TR-701 FA at dose levels of 100, 200, and 400 mg in Cohorts 2, 3, and 7, respectively, plasma TR-700 concentrations were detectable at the first time point (15 min post start of infusion) indicating rapid conversion of the prodrug into the active form and increased during the infusion (**Figure 1**). Median TR-700 T_{max} values were generally achieved at the end or shortly after infusion and ranged from 1.92 to 2.17 hours across the treatments at the100- to 400-mg dose range and infusion times of 60 to 120 minutes. C_{max} values ranged from 1.2 to 5.1 µg/mL across all doses of TR-701 FA, reflecting a dose-dependent increase. After reaching C_{max}, TR-700 concentrations declined with mean $t_{1/2}$ values ranging from 11.0 to 13.4 hours across the 100- to 400-mg dose range. Mean Vss values ranged from 67 to 74 L across groups. Clearance values were generally dose-independent and ranged from 4.8 to 5.8 L/hr.

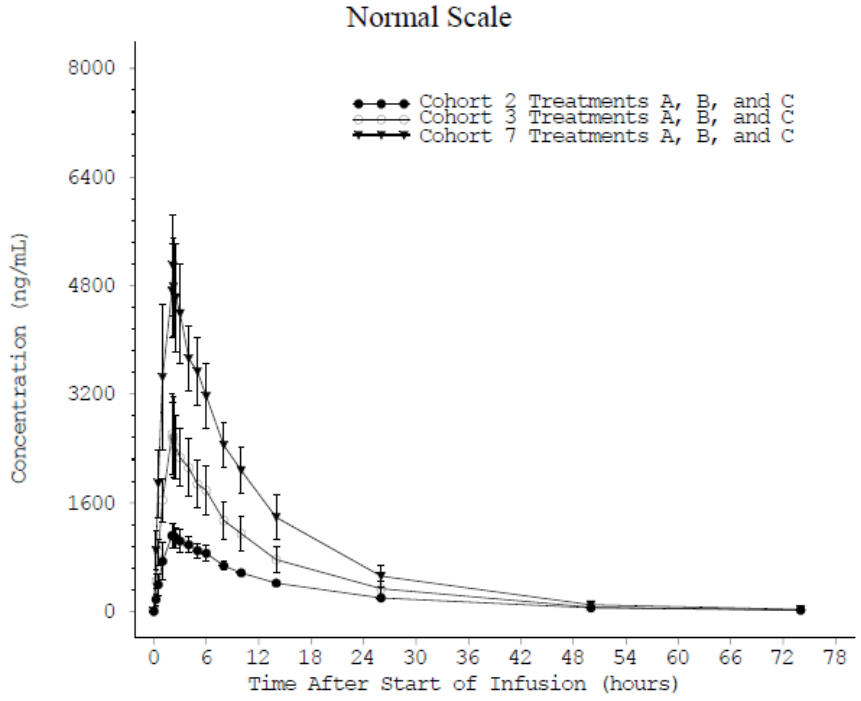
Table 7. Summary of the Mean (Standard Deviation) Plasma Pharmacokinetic Parameter Data for TR-700 (Part A: Single Ascending Dose)

Reference ID: 3476543

PK Parameter	N	Cohort 2 100 mg TR-701 FA	N	Cohort 3 200 mg TR-701 FA	N	Cohort 7 400 mg TR-701 FA
C_{max} (ng/mL)	9	1163 (186)	9	2618 (577)	9	5131 (788)
DN-C_{max} (ng/mL/mg)	9	14.2 (2.27)	9	16.0 (3.52)	9	15.6 (2.40)
T_{max} (hr)[a]	9	1.92 (1.08, 2.25)	9	2.17 (0.917, 2.33)	9	2.10 (0.917, 2.50)
AUC_{0-t} (ng*hr/mL)	9	17017 (1661)	9	29991 (10339)	9	58194 (11382)
DN-AUC_{0-t} (ng*hr/mL/mg)	9	208 (20.3)	9	183 (63.0)	9	177 (34.6)
$AUC_{0-\infty}$ (ng*hr/mL)	9	17359 (1769)	8	32583 (8302)	9	58698 (11590)
DN-$AUC_{0-\infty}$ (ng*hr/mL/mg)	9	212 (21.6)	8	199 (50.6)	9	178 (35.2)
CL (L/hr)	9	4.77 (0.514)	8	5.41 (1.75)	9	5.79 (1.06)
V_{ss} (L)	9	74.5 (9.42)	8	67.1 (15.3)	9	67.5 (12.2)
λ_z (1/hr)	9	0.0522 (0.00442)	8	0.0630 (0.00409)	9	0.0621 (0.00815)
$t_{½}$ (hr)	9	13.4 (1.14)	8	11.0 (0.763)	9	11.3 (1.23)

a Median (minimum, maximum) presented for T_{max}.
Notes: Subjects 5 through 16 (Cohort 1, 100-mg dose) were dosed with (b) (4) *saline bags rather than the intended* (b) (4) *saline bags. As a result, the PK parameter data for Subjects 5 through 16 (Cohort 1) were not reported since the 100-mg PK data were reported from Cohort 2.*

Figure 1. Mean (Standard Deviation) Overall Plasma Concentration-Time Profiles for TR-700, Part A (Single Ascending Dose; 100, 200, and 400 mg)



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Multiple Ascending Dose (Part B)

TR-701

There was no accumulation of TR-701 after 7 days of repeat IV administration of 200 mg TR-701 FA, although plasma concentrations were detectable for a little longer post infusion (up to 3.75 hours longer in one subject on Day 7 compared to duration on Day 1). TR-701 PK values for Day 1 and Day 7 were similar (**Table 8**) except for mean half-life which approximately doubled, but remained small (~23 min). The mean recovery (f_{e0-24}) of TR-701 in urine was ≤0.001% on Days 1 and 7, indicating that urinary excretion of unchanged TR-701 is a minor pathway for its elimination.

Table 8. Summary of Mean (Standard Deviation) Plasma and Urinary Pharmacokinetic Parameter Data for TR-701 (Part B: Multiple Ascending Dose)

	Cohort 4, 200 mg TR-701 FA				Cohort 5, 300 mg TR-701 FA			
PK Parameter	**N**	**Day 1**	**N**	**Day 7**	**N**	**Day 1**	**N**	**Day 7**
C_{max} (ng/mL)	9	1703 (517)	8	1770 (397)	8	2934 (654)	0	ND
DN-C_{max} (ng/mL/mg)	9	8.52 (2.59)	8	8.85 (1.99)	8	9.78 (2.18)	0	ND
T_{max} (hr)[a]	9	0.250 (0.250, 0.917)	8	0.500 (0.250, 0.917)	8	0.917 (0.250, 0.917)	0	ND
AUC_{0-24} (ng*hr/mL)	9	1367 (459)	7	1525 (280)	8	2423 (567)	0	ND
DN-AUC_{0-24} (ng*hr/mL/mg)	9	6.84 (2.29)	7	7.63 (1.40)	8	8.08 (1.89)	0	ND
AUC_{0-t} (ng*hr/mL)	9	1365 (458)	8	1471 (292)	8	2420 (567)	0	ND
DN-AUC_{0-t} (ng*hr/mL/mg)	9	6.83 (2.29)	8	7.36 (1.46)	8	8.07 (1.89)	0	ND
$AUC_{0-\infty}$ (ng*hr/mL)	9	1367 (459)	0	NA	8	2423 (567)	0	ND
DN-$AUC_{0-\infty}$ (ng*hr/mL/mg)	9	6.84 (2.29)	0	NA	8	8.08 (1.89)	0	ND
CL (L/hr)	9	160 (49.9)	7	135 (26.9)	8	131 (38.2)	0	ND
V_{ss} (L)	7	12.1 (3.77)	7	16.6 (9.70)	7	13.7 (3.30)	0	ND
λ_z (1/hr)	9	5.63 (3.48)	7	2.74 (1.68)	8	2.85 (0.937)	0	ND
$t_{½}$ (hr)	9	0.162 (0.0835)	7	0.386 (0.337)	8	0.275 (0.121)	0	ND
A_{e0-24} (mg)	9	0.000870 (0.00261)	8	0.00232 (0.00362)	8	0.00210 (0.00271)	0	ND
CL_R (L/hr)	9	0.000718 (0.00215)	7	0.00208 (0.00308)	8	0.000795 (0.00101)	0	ND
f_{e0-24} (%)	9	0.000435 (0.00131)	8	0.00116 (0.00181)	8	0.000700 (0.000905)	0	ND

a Median (minimum, maximum) presented for T_{max}.

Notes: Day 7 represents Day 7 or the last day dosed. Subjects in Part B, Cohort 5 discontinued dosing prior to Day 7 and did not have Day 7 plasma or urine samples collected.

Reference ID: 3476543

TR-700

The mean TR-700 concentration-time profiles in **Figure 2** illustrate that following 60-min IV infusion of TR-701 FA 200 mg QD for 7 days, TR-700 readily appeared in plasma and was steadily eliminated with a half-life of 9-12 hours. A slightly higher mean $t_{1/2}$ value of 12.4 hours was observed at the 200-mg dose on Day 7, likely due to the longer sample collection interval compared to Day 1 (72 hours post dose on Day 7 versus 24 hours on Day 1). Median T_{max} values were 1.08 and 1.17 hours on Day 1 at 200 and 300 mg dose levels, respectively, and a similar median T_{max} value of 1.17 hours was observed on Day 7 at the 200 mg dose. TR-700 concentrations were slightly greater on Day 7 compared to Day 1 with an accumulation ratio of TR-700 on Day 7 of 1.28 (**Table 9)**. The mean recovery (f_{e0-24}) of TR-700 over 24 hours ranged from 1.05% to 1.23% at the 200- and 300-mg dose levels on Days 1 and 7 (**Table 9)** indicating that urinary excretion of unchanged TR-700 is a minor pathway for its elimination.

Table 9. Summary of Mean (Standard Deviation) Plasma and Urinary Pharmacokinetic Parameter Data for TR-700 (Part B: Multiple Ascending Dose)

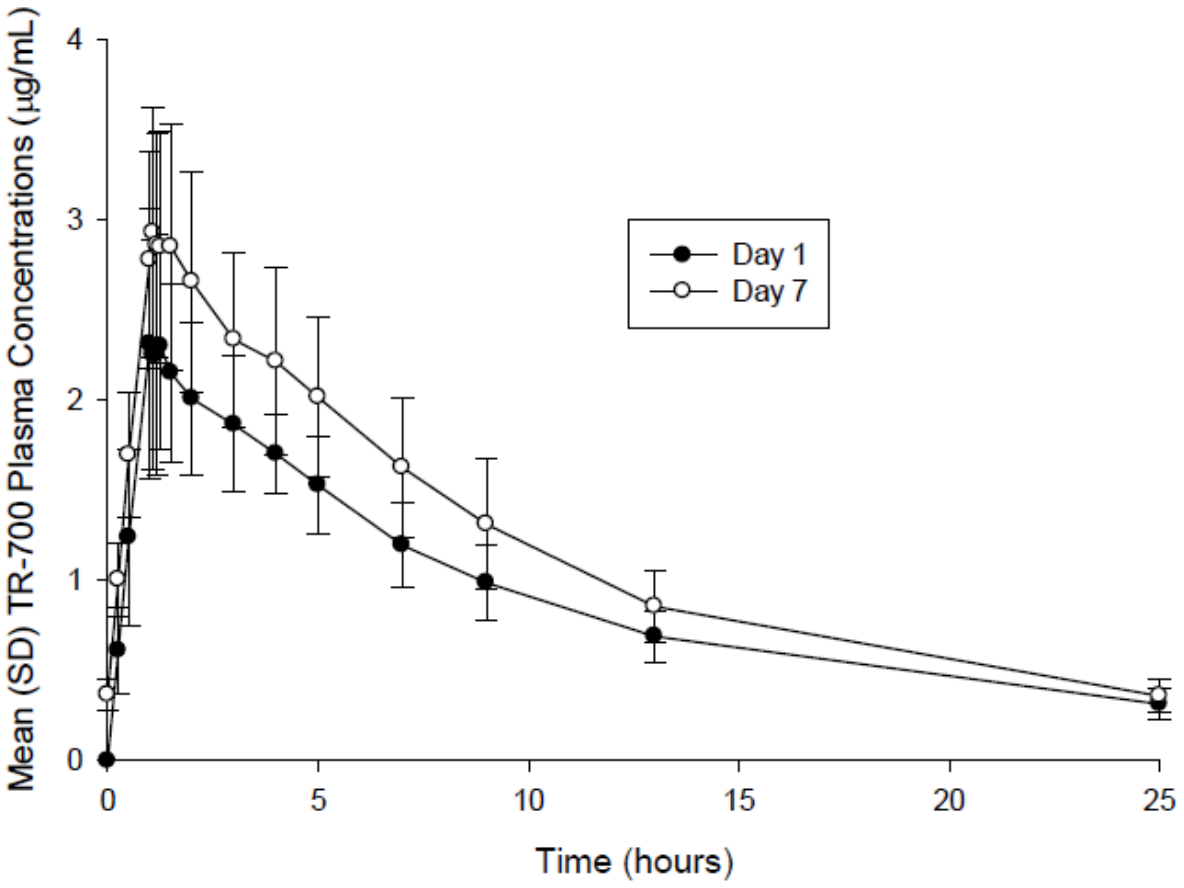
	Cohort 4, 200 mg TR-701 FA				Cohort 5, 300 mg TR-701 FA			
PK Parameter	**N**	**Day 1**	**N**	**Day 7**	**N**	**Day 1**	**N**	**Day 7**
C_{max} (ng/mL)	9	2339 (644)	8	3010 (658)	8	4534 (1066)	0	ND
DN-C_{max} (ng/mL/mg)	9	14.3 (3.93)	8	18.4 (4.01)	8	18.4 (4.32)	0	ND
T_{max} (hr)[a]	9	1.08 (0.917, 1.50)	8	1.17 (0.917, 1.50)	8	1.17 (0.917, 1.50)	0	ND
AUC_{0-24} (ng*hr/mL)	9	22291 (4237)	8	29185 (6220)	8	43493 (9160)	0	ND
DN-AUC_{0-24} (ng*hr/mL/mg)	9	136 (25.8)	8	178 (37.9)	8	176 (37.1)	0	ND
AUC_{0-t} (ng*hr/mL)	9	22242 (4225)	8	36142 (7573)	8	43398 (9140)	0	ND
DN-AUC_{0-t} (ng*hr/mL/mg)	9	136 (25.8)	8	220 (46.2)	8	176 (37.0)	0	ND
$AUC_{0-\infty}$ (ng*hr/mL)	9	26593 (5183)	0	NA	8	51900 (11197)	0	ND
DN-$AUC_{0-\infty}$ (ng*hr/mL/mg)	9	162 (31.6)	0	NA	8	210 (45.4)	0	ND
CL (L/hr)	9	6.37 (1.19)	8	5.87 (1.41)	8	4.92 (0.880)	0	ND
V_{ss} (L)	9	77.6 (15.9)	8	80.1 (21.0)	8	61.2 (15.2)	0	ND
λ_z (1/hr)	9	0.0762 (0.0137)	8	0.0564 (0.00590)	8	0.0763 (0.0110)	0	ND
$t_{½}$ (hr)	9	9.33 (1.50)	8	12.4 (1.25)	8	9.27 (1.44)	0	ND
A_{e0-24} (mg)	9	1.72 (0.547)	8	1.95 (0.785)	8	3.04 (1.38)	0	ND
CL_R (L/hr)	9	0.0779 (0.0234)	8	0.0662 (0.0220)	8	0.0695 (0.0276)	0	ND
f_{e0-24} (%)	9	1.05 (0.332)	8	1.18 (0.477)	8	1.23 (0.558)	0	ND

a Median (minimum, maximum) presented for T_{max}.

Notes: Day 7 represents Day 7 or the last day dosed. Subjects in Part B, Cohort 5 discontinued dosing prior to Day 7 and did not have Day 7 plasma or urine samples collected.

Reference ID: 3476543

Figure 2. Mean (SD) Tedizolid Plasma Concentrations Following Single and Multiple Once-Daily 200 mg Intravenous Administration



Absolute Bioavailability (Part C)

TR-701

Following single oral administration of TR-701 FA at a dose level of 200 mg in Cohort 6, no TR-701 concentrations were detected. As a result, no TR-701 PK parameters were calculated for Cohort 6 following oral administration. Following a single 1 hour infusion of TR-701 FA at a dose level of 200 mg in Cohort 6, a median T_{max} value of 0.375 hours (22 min) was observed. After the end of the infusion, TR-701 concentrations rapidly declined with a mean $t_{1/2}$ value of 0.197 hours (12 min) observed.

TR-700

The mean plasma concentration-time plots of TR-700 following IV and oral administration of TR-701 FA are illustrated in **Figure 3**. A summary of the mean (SD) plasma PK parameter data for TR-700 is presented in **Table 10**. The statistical analysis of TR-700 PK data (oral dose relative to IV) is presented in **Table 11**.

Reference ID: 3476543

Figure 3. Mean (Standard Deviation) Plasma Concentration-Time Profiles for TR-700, Part C (IV and Oral Administration)

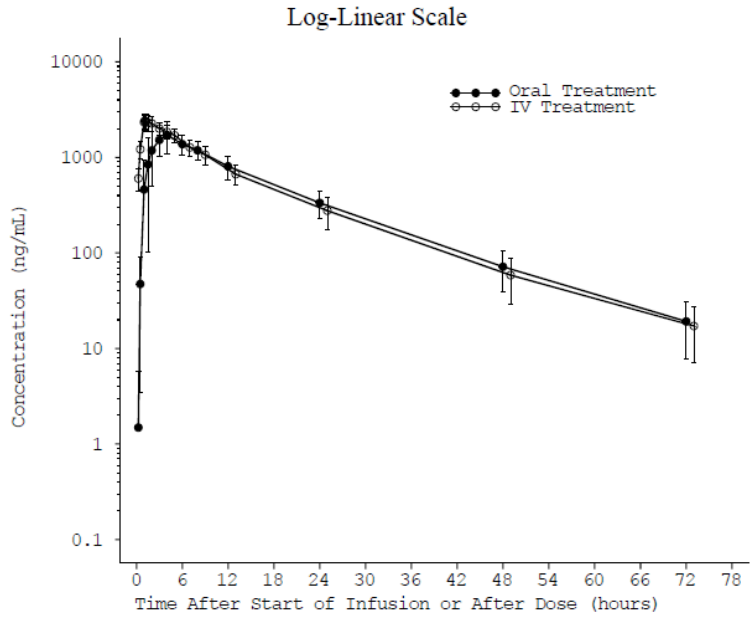


Table 10. Summary of Mean (Standard Deviation) Plasma Pharmacokinetic Parameter Data for TR-700 (Part C: Absolute Bioavailability)

	Cohort 6, 200 mg TR-701 FA			
PK Parameter	**N**	**IV Infusion**	**N**	**Oral Administration**
C_{max} (ng/mL)	8	2481 (414)	8	1911 (442)
DN-C_{max} (ng/mL/mg)	8	15.1 (2.52)	8	11.7 (2.70)
T_{max} (hr)[a]	8	1.17 (0.933, 3.00)	8	3.50 (1.50, 8.02)
AUC_{0-24} (ng*hr/mL)	8	23499 (4227)	8	20386 (4001)
DN-AUC_{0-24} (ng*hr/mL/mg)	8	143 (25.8)	8	124 (24.4)
AUC_{0-t} (ng*hr/mL)	8	28708 (6011)	8	26325 (5877)
DN-AUC_{0-t} (ng*hr/mL/mg)	8	175 (36.7)	8	161 (35.8)
$AUC_{0-\infty}$ (ng*hr/mL)	8	29024 (6135)	8	26673 (6032)
DN-$AUC_{0-\infty}$ (ng*hr/mL/mg)	8	177 (37.4)	8	163 (36.8)
CL (L/hr)	8	5.92 (1.50)	0	NA
V_{ss} (L)	8	71.5 (12.7)	0	NA
λ_z (1/hr)	8	0.0633 (0.0159)	8	0.0654 (0.0163)
$t_{½}$ (hr)	8	11.4 (2.00)	8	11.1 (2.12)
CL/F (L/hr)	0	NA	8	6.52 (1.89)
V_z/F (L)	0	NA	8	100 (17.7)

a Median (minimum, maximum) presented for T_{max}.
NOTE: Subjects were given the following treatments: Cohort 6, Oral 200-mg TR-701 FA tablet and IV 200 mg TR-701 FA in 250 mL USP sterile saline as a 60-minute IV infusion.

Reference ID: 3476543

Table 11. Statistical Analysis of TR-700 Pharmacokinetic Data (Oral Tablet Relative to Intravenous - Part C Absolute Bioavailability)

Parameter (Units)	N[a]	Test Mean[b] (Oral Tablet)	N[a]	Reference Mean[b] (Intravenous)	Test/Reference[c]	90% Confidence Interval[d]
AUC_{0-t} (ng*hr/mL)[e]	8	25649	8	28094	91.30	(86.71 , 96.12)
$AUC_{0-\infty}$ (ng*hr/mL)	8	25972	8	28393	91.47	(86.81 , 96.38)

a N was the number of subjects with evaluable PK data for that treatment.
b Least squares mean from ANOVA. Natural log parameter means calculated by transforming the natural log means back to the linear scale (ie, geometric means).
c Ratio of parameter means for natural-log transformed parameter (expressed as a percent). Natural log transformed ratios transformed back to the linear scale.
d 90% confidence interval for ratio of parameter means of natural-log transformed parameter (expressed as a percent). Natural-log transformed confidence limits transformed back to the linear scale.
e IV duration is 1 hour longer than oral.
Note: AUC = area under the concentration-time curve (over specified time interval); N = number; ng = nanogram; hr = hour; mL = milliliter; t = time of last quantifiable sample up to 72 hours post dose/end of infusion.

The mean TR-700 concentration-time profiles in **Figure 3** illustrate that after IV and oral administration, TR-700 concentrations were generally similar. Following a single 1-hour infusion or oral administration (crossover design) of TR-701 FA at 200 mg in Cohort 6, median T_{max} values for TR-700 of 1.17 and 3.50 hours were observed under fasting conditions following the IV and oral administrations, respectively (**Table 10**). After reaching C_{max}, TR-700 concentrations declined with mean $t_{1/2}$ values of 11.4 and 11.1 hours following the IV and oral administrations, respectively. Mean V_{ss} and CL values of 71.5 L and 5.92 L/hr were observed following IV administration. Mean V_z/F and CL/F values of 100 L and 6.52 L/hr were observed following oral administration (**Table 10**).

Statistical analysis results show least squares (LS) mean AUC_{0-t} values following oral administration (25649 ng*hr/mL) were 8.7% lower than those following IV administration (28094 ng*hr/mL) as shown in **Table 11**. The 90% CI for LS mean AUC_{0-t} (86.71, 96.12) was entirely contained within the FDA defined (FDA Guidance 2003) 80% to 125% equivalence range. Similar results were observed for LS mean $AUC_{0-\infty}$ values. These results indicate that, compared to IV administration, there is no statistically significant difference in the extent of absorption (AUC) for TR-700 following oral administration of 200 mg TR-701 FA. The percent bioavailability of TR-700 following oral administration was 91.47% (based on $AUC_{0-\infty}$).

Reviewer Comment: The study design and data analysis are in agreement with the recommendations by the FDA BA BE guidance (2003). Of note, AUC/MIC was shown to best correlate with efficacy in animal infection models. Given the absolute bioavailability of 91% (based on $AUC_{0-\infty}$), dose adjustment is considered to be unnecessary for intravenous to oral switch.

Safety:
Overall, 56 of the 90 subjects reported at least 1 treatment-emergent (TEAE), the majority of which were mild with 7 moderate AEs and 1 severe AE (vomiting). There were no deaths during the study.

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The AEs most frequently reported were infusion site-related AEs including infusion site pain, infusion site swelling, and infusion site erythema. The incidence of infusion site-related AEs, along with the number and severity of infusion site examination findings, increased with multiple dosing, possibly due to either the presence of the IV catheter, the numerous venous punctures, or the study drug. Visual Infusion Phlebitis scores did not differ between 200 mg TR-701 FA and placebo, indicating that infusions of active drug are as well tolerated as placebo infusions. The number of subjects reporting noninfusion site-related AEs did not appear to increase with increasing dose or exposure to TR-701 FA.

No clinically significant changes or findings were noted from clinical laboratory evaluations, vital sign measurements, physical examinations, or 12-lead ECGs for this study. With the exception of the high number of TEAEs and early discontinuations by subjects receiving multiple doses of 300 mg TR-701 FA IV because of a higher incidence of venous AEs.

SPONSOR'S CONCLUSIONS

- Single doses of 50 to 400 mg TR-701 FA were generally well tolerated in this group of healthy male and female subjects
- Multiple doses of 200 mg TR-701 FA over 7 days were generally well tolerated; however, there was a higher incidence of treatment-related venous AEs and study drug discontinuations due to AEs following multiple doses of 300 mg TR-701 FA
- The AEs most frequently reported following IV TR-701 FA administration were infusion site pain, infusion site swelling, and infusion site erythema
- Generally dose proportional increases in TR-701 and TR-700 C_{max} and AUC were observed
- No accumulation of TR-701 plasma concentrations was observed and accumulation of TR-700 was modest and well predicted by single dose kinetics
- The CL and V_{ss} values for TR-701 and TR-700 appeared independent of dose and the duration of exposure
- Approximately 1% or less of the TR-701 FA dose was excreted in urine as either TR-701 or TR-700
- The absolute bioavailability of TR-700 following administration of a 200-mg TR-701 FA tablet was 91.47%

REVIEWER ASSESSMENT: The Sponsor's conclusions are appropriate based on study results. For labeling, only steady-state pharmacokinetic data of TR-700 for the proposed therapeutic 200 mg QD regimen will be used; pharmacokinetic data of prodrug TR-701 will not be included.

Reference ID: 3476543

STUDY NO.: TR701-108

An Open-Label, Randomized, Single-Dose, 2-Treatment, 2-Sequence, Crossover, Exploratory Pharmacokinetic and Bioavailability Study of 2 Capsule Formulations of TR 701 (Torezolid Phosphate) in Normal Healthy Adults

Date(s): 26 February 2009 to 25 March 2009
Sponsor: Trius Therapeutics, Inc., San Diego, CA
Clinical Site: Covance Clinical Research Unit Inc., Austin, Texas
Analytical Site: (b) (4)

OBJECTIVE(S):

1. To determine the pharmacokinetics (PK) of 2 different TR-701 oral formulations (free acid [FA] powder in capsule and di-sodium salt formulated capsule) after single doses in normal healthy adult subjects;
2. To determine the relative bioavailability (F_{rel}) of TR-701 FA powder in capsule formulation (test) compared to di-sodium salt formulated capsule (reference); and
3. To determine the safety and tolerability of the TR-701 FA powder in capsule formulation and to further evaluate the safety profile of the TR-701 di-sodium salt capsule.

METHODS

Study Design: This was an open-label, randomized, 2-treatment, 2-sequence crossover, PK study conducted in 12 healthy male and female subjects. Subjects were confined to the clinical research unit (CRU) from approximately 24 hours prior to first dose until approximately 72 hours after final dose (approximately 10 days).

Subjects received each of the following treatments in a crossover design:

• Treatment A (Reference): One 200 mg capsule of TR-701 di-sodium salt (equivalent to 150 mg TR-700);
• Treatment B (Test): One 182 mg capsule of TR-701 FA (equivalent to 150 mg TR-700)

There was at least a 5-day washout between dose administrations.

All doses were preceded by an overnight fast (ie, at least 8 hours) from food (not including water) and were followed by a fast from food (not including water) for at least 4 hours postdose. Except as part of oral dose administration, subjects restricted their consumption of water for 1 hour prior to dose and for 2 hours postdose; at all other times during the study, subjects could consume water on an ad libitum basis.

Drug Product: Tedizolid phosphate disodium salt, also known as TR-701 (200 mg oral capsules) and tedizolid phosphate free acid, also known as TR701 FA, powder in capsule (182 mg).

Inclusion Criteria: Males and females of non-childbearing potential; 18-60 years of age (inclusive); 18.5-32 kg/m^2 (inclusive) in body mass index; nonsmokers, and in good health as

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confirmed by medical history, physical exam, 12-lead electrocardiogram (ECG), and laboratory evaluations were enrolled.

Subjects were required to abstain from any medications (including over-the-counter remedies) from 7 days prior to Check-in and during the entire study. Subjects were also required to abstain from alcohol-, grapefruit-, or caffeine-containing foods or beverages or foods or beverages with high levels of tyramine from 72 hours prior to Check-in and during the entire study. Use of selective serotonin re-uptake inhibitors (SSRIs), monoamine oxidase (MAO) inhibitors, and other serotonergic medications were not allowed within 14 days prior to Check-in and during the entire study.

PK Sample Collection: Blood samples were collected at the following timepoints for each study period: 0 Hour (pre-dose), 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 18, 24, 48, and 72 hours post-dose.

Analytical Methods: PK samples were analyzed for TR-700 by validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) (**Table 1**). For the calculation of summary statistics, all concentrations below the limit of quantification (BLQ) were to be set to zero.

Table 1. Bioanalytical results of TR-700 in plasma

Criterion	TR-700	Comments
	PLASMA	
Calibration Range	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	Satisfactory
Linearity, R^2	≥0.9989	Satisfactory
Accuracy	Within ±3%	Satisfactory
Precision, CV	≤2.2 %	Satisfactory
Quality Control*	15, 150, 750, 5000(10x) ng/mL	Satisfactory
Accuracy	Within ±4.8%	Satisfactory
Precision, CV	≤3.8%	Satisfactory
Stability	• Maximum duration from sample collection to end of analysis: 31 days • Stability: 156 days at -60 to -80 ºC	Satisfactory

**Numbers in the parentheses indicate the dilution factor for QC samples.*

Pharmacokinetic Assessment: Analysis included measurements of TR-700 C_{max}, T_{max}, AUC_{0-12}, AUC_{0-24}, AUC_{0-t}, AUC_{0-inf}, λ_Z, and $t_{½}$ for plasma. Relative bioavailability, F_{rel}, was calculated as follows:

$$\frac{\text{AUC (Test)}}{\text{AUC (Reference)}} \times \frac{\text{Dose (Reference)}}{\text{Dose (Test)}}$$

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where Reference is Treatment A (one 200 mg capsule of TR-701 di-sodium salt [equivalent to 150 mg TR-700] and Test is Treatment B (one 182 mg capsule of TR-701 FA [equivalent to 150 mg TR-700].

In addition, to examine a possible weight effect with regards to TR-700 exposure, C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were weight normalized (WN), whenever possible, by first correcting the TR-700 dose for each subject to their respective body weight.

Statistical Methods: Relative bioavailability was examined between Treatment A (Reference) and Treatment B (Test). An analysis of variance (ANOVA) was performed. Data for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were ln-transformed prior to analysis. The 90% confidence intervals (CIs) of the test group means relative to the reference group means were obtained by taking the antilog of the corresponding 90% CIs for the differences between the means on the log scale.

Safety Assessment: Safety and tolerance were assessed at specified timepoints by physical examinations, medical history, clinical laboratory evaluations (Chem-20, complete blood count [CBC], and urinalysis [UA]), 12-lead ECGs, adverse events (AEs), and vital signs.

RESULTS

Study Population: In total, 12 subjects were enrolled in the study and completed the study. A summary of demographic and baseline characteristics for the study population is presented in **Table 2**.

Table 2. Demographic Characteristics

Demographic Variable	
Mean age in years	36 (range 26 to 59 years)
Mean weight in kg	75.1 (range 57.0 to 95.5 kg)
Mean height in cm	168.5 (range 154.3 to 184.2 cm)
BMI in kg/m^2	26.4 (range 20.4 to 30.6 kg/m^2)
Gender (n[%])	
Male	6 (50.0%)
Female	6 (50.0%)
Ethnicity (n[%])	
Hispanic or Latino	6 (50.0%)
Not Hispanic or Latino	6 (50.0%)
Race (n[%])	
White	12 (100%)

The only deviations noted were that respiratory rate was not measured 48 hours postdose for Subject No. 1005 and the time for the 6 hours postdose vital signs measurement was not recorded for Subject No. 1008. Some blood collections were performed late due to

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uncontrollable circumstances, the actual sampling collection times were recorded for PK analysis instead.

Reviewer Comment: These deviations do not appear to affect the validity of PK data.

Pharmacokinetics:
The mean TR-700 concentration time profiles in **Figure 1** illustrate that after oral administration of TR-701 as either Treatment A (200 mg di-sodium salt formulated capsule) or Treatment B (182 mg free acid powder in capsule), TR-700 concentrations declined in a monoexponential manner and were nearly identical between the two treatments.

Following oral administration of 200 mg and 182 mg TR-701 as two different oral formulations (di-sodium salt formulated capsule and FA powder capsule) in Treatments A and B, respectively, overall TR-700 median T_{max} values of 2.50 hours for Treatment A and 3.00 hours for Treatment B were observed (**Table 3**). After reaching C_{max}, TR-700 concentrations readily declined with an overall mean $t_{½}$ of 11.4 hours observed for both Treatments A and B (**Table 3**). The TR-700 mean CL/F and Vz/F values were comparable between the two TR-701 formulations (**Table 3**). As shown in **Table 4**, the 90% CI for LS mean C_{max} (89.70, 101.31) was within the 80% to 125% equivalence range. The 90% CI for LS mean AUC_{0-t} (92.99, 98.77) was within the 80% to 125% equivalence range. Similar results were observed for $AUC_{0-\infty}$. An overall mean relative bioavailability (F_{rel}) of 96%, was observed for the test treatment (Treatment B) compared to the reference treatment (Treatment A). These analyses of the results indicate that the TR-701 FA powder capsule and the TR-701 di-sodium salt formulated capsule are deemed bioequivalent.

As shown in **Table 3,** overall mean WN-C_{max}, WN-AUC_{0-t}, and WN-$AUC_{0-\infty}$ values were comparable between the two formulations. A gender difference was observed in TR-700 PK after TR-701 administration for both Treatments A and B. With males as the reference, females had 27% and 23% greater mean C_{max} values, that partially corrected by normalizing for weight, in Treatments A and B, respectively. In addition, females had 13.6% and 9.5% lower mean V_z/F and 14.6% and 13.1% lower mean $t_{½}$ values compared to males for Treatments A and B, respectively.

Reviewer Comment: Due to the limited sample size (n=12), the gender difference in TR-700 PK will be further evaluated upon review of the Population PK Analysis study report (cln-13-0701-072).

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Figure 1. Mean (SD) Plasma Concentration-Time Profiles for TR-700 Following a Single Oral Dose of 200 mg TR-701 Capsule (Treatment A) and 182 mg TR-701 FA Capsule (Treatment B).

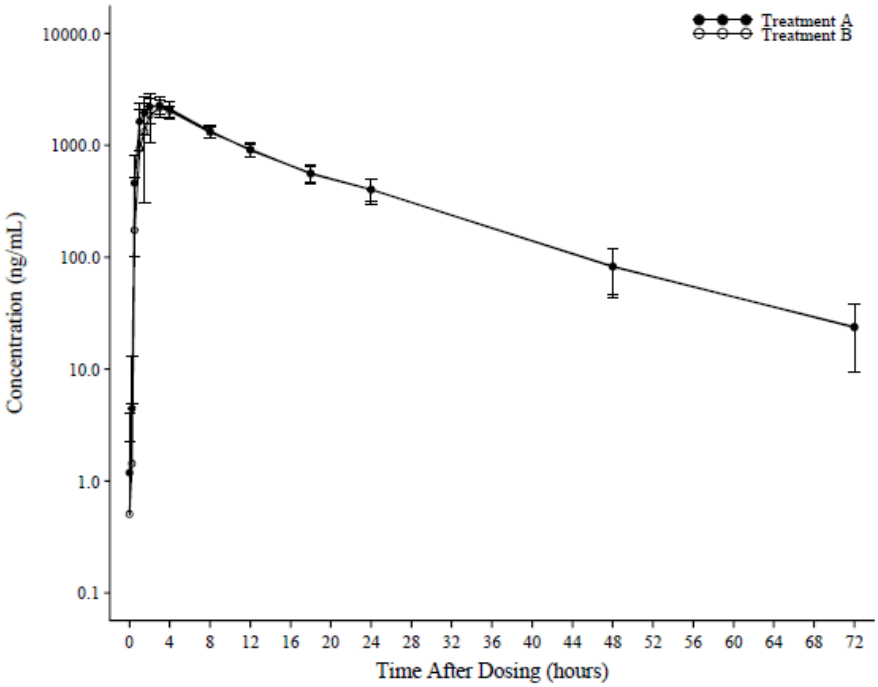

Table 3. Summary of Mean (SD) TR-700 Pharmacokinetic Parameter Data

		TR-701 Oral Formulations					
		Treatment A (Reference) 200 mg di-sodium salt (150 mg TR-700)			Treatment B (Test) 182 mg free acid (150 mg TR-700)		
PK Parameter	Units	Males	Females	Overall	Males	Females	Overall
C_{max}	(ng/mL)	2173 (235)	2762 (447)	2468 (459)	2107 (158)	2595 (484)	2351 (428)
T_{max}[a]	(hr)	2.50 (1.50, 3.07)	2.50 (1.50, 4.00)	2.50 (1.50, 4.00)	3.00 (2.00, 4.00)	2.50 (1.00, 3.00)	3.00 (1.00, 4.00)
AUC_{0-t}	(ng*hr/mL)	32760 (5882)	32653 (4635)	32707 (5049)	31689 (4955)	30829 (4030)	31259 (4329)
$AUC_{0-\infty}$	(ng*hr/mL)	33365 (6210)	32886 (4679)	33125 (5248)	32274 (5283)	31067 (4068)	31670 (4540)
F_{rel}		NA	NA	NA	0.972 (0.0460)	0.948 (0.0616)	0.960 (0.0533)
CL/F	(L/hr)	4.64 (0.932)	4.65 (0.756)	4.64 (0.809)	4.76 (0.795)	4.90 (0.654)	4.83 (0.698)
V_z/F	(L)	80.9 (7.75)	69.9 (10.2)	75.4 (10.4)	82.2 (5.90)	74.4 (8.55)	78.3 (8.11)
λ_z	(1/hr)	0.0570 (0.00712)	0.0664 (0.00331)	0.0617 (0.00724)	0.0577 (0.00773)	0.0658 (0.00299)	0.0618 (0.00701)
$t_{1/2}$	(hr)	12.3 (1.46)	10.5 (0.542)	11.4 (1.43)	12.2 (1.54)	10.6 (0.488)	11.4 (1.38)
WN-C_{max}	(ng/mL)/(mg/kg)	1132 (225)	1317 (232)	1224 (238)	1087 (118)	1235 (244)	1161 (199)
WN-AUC_{0-t}	(ng*hr/mL)/(mg/kg)	17140 (4337)	15554 (2369)	16347 (3433)	16500 (3558)	14777 (2651)	15639 (3124)
WN-$AUC_{0-\infty}$	(ng*hr/mL)/(mg/kg)	17463 (4516)	15665 (2397)	16564 (3572)	16813 (3739)	14892 (2679)	15852 (3259)

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Table 4. Statistical Analysis of TR-700 Pharmacokinetic Data (TR-701 182 mg free acid Relative to 200 mg di-sodium salt)

	Least Squares Means					
	182 mg free acid (Treatment B)		200 mg di-sodium salt (Treatment A)			90% Confidence
Parameter (Units)	N[a]	Test Mean[b]	N[a]	Reference Mean[b]	Test/Reference[c]	Interval[d]
C_{max} (ng/mL)	12	2316	12	2430	95.33	(89.70 , 101.31)
AUC_{0-t} (ng*hr/mL)	12	30983	12	32328	95.84	(92.99 , 98.77)
$AUC_{0-\infty}$ (ng*hr/mL)	12	31371	12	32721	95.87	(93.05 , 98.79)

Safety: There were no deaths or SAEs during the study and no subjects discontinued from the study due to AEs. Four (33.3%) of the 12 subjects enrolled in this study reported at least 1 AE after study drug administration; however, none of the AEs were judged treatment-related. The majority of AEs were mild and no severe AEs were reported. No specific AE was reported by more than 1 subject. No clinically significant changes or findings were noted from clinical laboratory evaluations, vital sign measurements, physical examinations, or 12-lead ECGs for this study.

SPONSOR'S CONCLUSIONS

- The relative bioavailability of TR-700 was 96% between the tested 182 mg TR-701 FA powder capsule (Treatment B) and the reference 200 mg TR-701 di-sodium salt formulated capsule (Treatment A).
- The TR-700 C_{max} and AUC values observed for Treatment B were found to be bioequivalent to those in Treatment A.
- Based on AEs, clinical laboratory evaluations, vital signs, physical examinations, and 12-lead ECGs, single doses of 200 mg TR-701 di-sodium salt and 182 mg TR-701 FA were well tolerated in this group of healthy male and female subjects.

REVIEWER ASSESSMENT: The Sponsor's conclusions are appropriate based on study results.

Reference ID: 3476543

STUDY NO.: TR701-109

A Phase 1 Open-Label Study with Oral TR-701 Free Acid to Assess Pharmacokinetics, Safety, and Tolerability in Elderly Subjects

Date(s): 17 January 2012 – 28 February 2012
Investigator(s): David Subich, M.D.
Clinical Site(s): Covance Clinical Research Unit Inc.; Daytona Beach, FL, US
Analytical Site(s): (b) (4)

OBJECTIVE(S):
To compare the PK profile of TR-700 in elderly subjects versus younger control subjects, and to compare the safety and tolerability of TR-701 FA in elderly subjects and younger control subjects.

METHODS
Study Design: This was an open-label Phase 1 study of a single oral tablet of TR-701 FA 200 mg to compare the TR-700 PK profile in elderly subjects (age 65 years and older, with at least 5 subjects at least 75 years old) and younger control subjects (age 18 to 45 years). To provide 12 evaluable subjects in each age group, up to 28 subjects were to be enrolled. Efforts were made to match the younger control subjects to the elderly subjects by sex, race, and body mass index (BMI). One site participated in this study.

Inclusion/Exclusion Criteria: Subjects ≥65 years of age (at least 5 subjects were required to be ≥75) or matched-control subject ≥18 and ≤45 years of age, with BMI ≥18.0 and ≤35.0 kg/m2, and medically stable were to be enrolled.

Treatment: A single 200 mg oral dose of TR-701 FA was to be administered to each subject in the morning of Day 1. The study drug was to be administered with 120 mL of water after an overnight fast of at least 8 hours. Subjects were to continue fasting until at least 4 hours after study drug administration. Ingestion of water was permitted during the fasting period. The formulation used was as follows:

- TR-701 FA tablets, 200 mg (lot number B090282)

Sample Collection: TR-700 and TR-701 plasma concentrations were determined from blood drawn 30 minutes predose, and at 0.5, 1, 1.5, 1, 3, 4, 6, 8, 12, 24, 36, 48, 60, and 72 hours postdose.

Analytical Methods: Pharmacokinetic samples were analyzed for TR-700 and TR-701 by validated HPLC-MS/MS analytical method (**Table 1**).

Reference ID: 3476543

Table 1. Bioanalytical validation summary for TR-700 and TR-701 in human plasma samples

Criterion	TR-700	TR-701	Comments
Range	5-1000 ng/mL	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	Satisfactory
Linearity	≥0.996	≥0.989	Satisfactory
Accuracy	99.3% - 106% [a] 98.8%-102.4% [b]	94.1% - 107.4% [a] 90.5%-106% [b]	Satisfactory
Precision (RSD)	1.4%-3.8% [a] 1.0% - 5.4% [b]	5.7%-6.9% [a] 2.1% - 7.8% [b]	Satisfactory
Incurred sample reproducibility	pass	pass	Satisfactory
Stability	Stored at -60 to -80 ºC	Stored at -60 to -80 ºC	Satisfactory

[a] QC samples (15.0, 150, 750, and 5000 ng/mL); [b] Standards

PK Assessment:
Summary statistics for plasma concentrations and the following PK parameters were determined for each age group for TR-700 only: Cmax, Tmax, AUC0-t, AUC0-∞, and apparent terminal half-life, λz, AUMC0-∞, CL/F, MRT, Vss/F, and Vz/F.

Statistical Methods:
Descriptive statistics including the number of subjects, mean, standard deviation, coefficient of variation, median, geometric mean, minimum, and maximum were provided for all PK parameters. The parameters Cmax, AUC0-t, and AUC0-∞, were compared between the 2 groups, including calculation of geometric mean ratio (elderly group relative to younger group) and corresponding 90% confidence intervals. Individual subject and group mean concentration-time profiles were generated. The relationships between age and PK parameters such as area under the concentration-time curve (AUC) and Cmax were explored using scatter plots and correlation coefficients, and regression analysis. All p-values ≤0.05 were considered statistically significant.

RESULTS

Study Population: In total, 28 subjects were enrolled. All enrolled completed the study (**Table 2**).

Reference ID: 3476543

Table 2. Summary of subject demographic and baseline characteristics (ITT analysis set)

Characteristic	Elderly Group N=14	Younger Control Group N=14
Age (years)		
Mean	71.9	33.7
SD	5.08	7.25
Median	71.5	32.5
Minimum	66	25
Maximum	78	45
BMI (kg/m^2)		
Mean	28.19	27.11
SD	3.619	2.893
Median	28.65	26.95
Minimum	22.0	23.3
Maximum	33.6	32.8
Sex, n (%)		
Male	7 (50.0%)	7 (50.0%)
Female	7 (50.0%)	7 (50.0%)
Race, n (%)		
White	14 (100.0%)	14 (100.0%)
Ethnicity, n (%)		
Hispanic/Latino	2 (14.3%)	3 (21.4%)
Not Hispanic/Latino	12 (85.7%)	11 (78.6%)

Pharmacokinetics:
TR-700 mean plasma concentration-time profiles for elderly and younger control subjects were similar, as presented in **Figure. 1**. For all subjects at each time point, TR-701 concentrations were BLQ (<5.00 ng/mL).

Comparison of TR-700 plasma PK parameters between groups are presented in **Tables 3 &4**. No statistically significant differences in TR-700 PK parameters were found between the Elderly Group and the Younger Control Group.

Figure 1: TR-700 Mean (± SD) Trough Concentration versus Study Day (Safety Analysis Set)

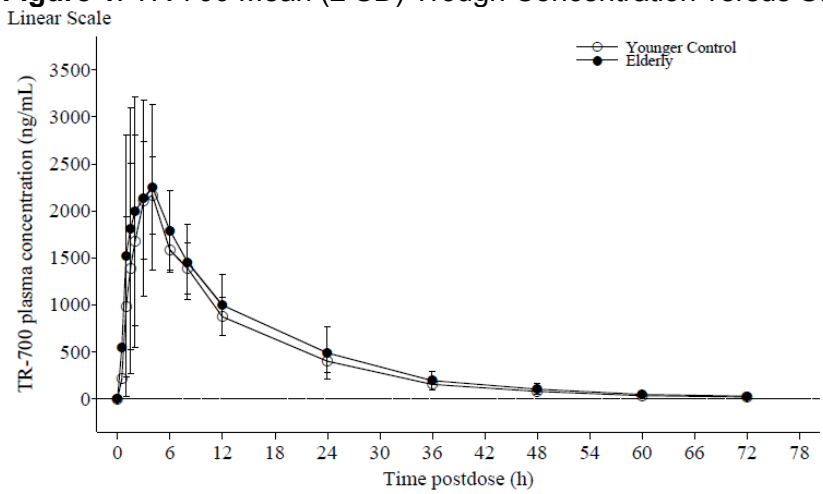

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Table 3. TR-700 Pharmacokinetic Parameters (PK Analysis Set)

Group	Statistic	C_{max} (ng/mL)	T_{max}[1] (hr)	AUC_{0-t} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{½}$ (hr)
Elderly	N	14	14	14	14	14
	Mean	2624	3.00	34156	34681	12.3
	SD	730	1.50-8.00	10346	10586	1.25
	CV	0.278	0.593	0.303	0.305	0.101
Younger Control	N	14	14	14	14	14
	Mean	2357	3.00	29526	29903	11.8
	SD	485	1.5-8.00	5705	5873	1.01
	CV	0.206	0.538	0.193	0.196	0.086

Abbreviations: AUC_{0-t}=area under the plasma concentration-time curve from Hour 0 to the last measurable concentration; $AUC_{0-\infty}$=area under the plasma concentration-time curve from Hour 0 extrapolated to infinity based on the apparent terminal rate constant; C_{max}=maximum observed concentration; CV=coefficient of variation (standard deviation/mean); N=number of subjects; SD=standard deviation; T_{max}=time to occurrence of C_{max}; $t_{½}$=apparent terminal half-life

[1]Median, Range, and CV presented for T_{max}

Table 4. Comparison of TR-700 Plasma PK Parameters between Groups (PK Analysis Set)

Parameter	Elderly		Younger Control		(Elderly/Control)	90% CI
	N	Geometric Mean[a]	N	Geometric Mean[a]	Geometric Mean Ratio[b]	
C_{max} (ng/mL)	14	2517	14	2309	1.091	0.917, 1.297
AUC_{0-t} (ng•hr/mL)	14	32705	14	28972	1.129	0.953, 1.337
$AUC_{0-\infty}$ (ng•hr/mL)	14	33186	14	29328	1.132	0.954, 1.343

Abbreviations: AUC_{0-t}=area under the plasma concentration-time curve from Hour 0 to the last measurable concentration; $AUC_{0-\infty}$=area under the plasma concentration-time curve from Hour 0 extrapolated to infinity based on the apparent terminal rate constant; CI=confidence interval; C_{max}=maximum observed concentration; N=number of subjects

[a]Least squares mean from analysis of variance (ANOVA) model, calculated by transforming the natural log mean back to the linear scale (ie, geometric mean). The ANOVA model included the log of PK parameter as the response and group as a fixed factor with 2 levels.

[b]Ratio of parameter means, transformed back to the linear scale.

Safety: TR-701 FA Tablet, 200 mg was well tolerated, with no TEAEs being reported in elderly or younger control subjects. In addition, no SAEs, including deaths, were reported during the study.

SPONSOR'S CONCLUSIONS: The results of this study demonstrate the overall PK profiles of TR-700 in elderly subjects and younger control subjects are similar following oral administration of a single 200 mg dose of TR-701 FA. Therefore, no dose adjustment is necessary for elderly subjects. Oral TR-701 FA was well tolerated at the 200 mg dose.

Reference ID: 3476543

REVIEWER ASSESSMENT: The Sponsor's conclusions are appropriate based on study results. Following single 200 mg dose of TR-701FA, the PK profile of elderly subjects is similar to that of younger adult subjects.

Reference ID: 3476543

STUDY NO.: TR701-111

A Phase 1, Open-Label, Multicenter, Two-Part, Single-Dose, Parallel-Design, Safety, Tolerance, and Pharmacokinetic Study of Orally and Intravenously Administered TR-701 FA in 12- to 17-Year-Old Adolescent Patients

Date(s): 21 July 2010 – 24 September 2011
Investigators: John Bradley MD, Antonio Arrieta MD, Richard Jacobs, MD, Kevin Spicer, MD
Clinical Site(s): Rady Children's Hospital-San Diego(CA), Children's Hospital of Orange County (CA), Arkansas Children's Hospital (AK), Nationwide Children's Hospital (OH)
Analytical Site(s): (b) (4)

OBJECTIVE(S):

- To describe the single-dose pharmacokinetics (PK) of TR-701 FA and its active metabolite, TR-700, when administered orally (Part A) or intravenously (IV; Part B) in 12- to 17-year-old adolescent patients.
- To evaluate the safety and tolerability of oral (Part A) or IV (Part B) TR-701 FA administration in 12- to 17-year-old adolescent patients.

METHODS:

Study Design: This was an open-label, multicenter, 2-part, parallel-design study conducted to assess the PK, safety, and tolerability of TR-701 FA and its active metabolite, TR-700, following single administration of oral (Part A) or IV (Part B) TR-701 FA in 12- to 17-year-old adolescent patients. It was to be conducted in a parallel configuration at 5 study sites over a period of approximately 14 months.

Inclusion/Exclusion Criteria: Medically stable adolescent patients between 12 and 17 years of age, inclusive, who were receiving prophylaxis for or had a confirmed or suspected Gram-positive bacterial infection and were receiving concurrent antibiotic treatment with Gram-positive antibacterial activity. Patient's weight was to be >5th percentile and <95th percentile by age.

Treatment: All enrolled patients in this open-label study were to receive a single dose of 200 mg TR-701 FA administered either as an oral tablet (Part A) or as a 60-minute IV infusion (Part B), formulations as follows.

- TR-701 FA tablets, 200 mg (lot number B090282)
- TR-701 FA for injection, 200 mg/vial; (lot number B090135)

Sample Collection:

Part A: Blood samples for analysis of TR-700 and TR-701 were collected at the following timepoints: predose, and 1, 2, 4, 6, 8, 12, and 24 hours postdose.
Part B: Blood samples for analysis of TR-700 and TR-701 were collected at the following timepoints: predose, and 0.5, 1, 1.5, 2, 4, 6, 8, 12, and 24 hours postdose.
Urine samples for analysis of TR-700 and TR-701 were collected during the following intervals: predose, and 0 to 4, 4 to 8, 8 to 12, and 12-24 hours postdose.

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Analytical Methods: Pharmacokinetic samples were analyzed for TR-700 and TR-701 by validated HPLC-MS/MS analytical method (**Table 1**).

Table 1. Bioanalytical method validation for TR-700 and TR-701 in human plasma and urine samples

Criterion	TR-700 plasma	TR-701 plasma	TR-700 urine	TR-701 urine	Comments
Range	5-1000 ng/mL	5-1000 ng/mL	5-1000 ng/mL	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	5 ng/mL	5 ng/mL	Satisfactory
Linearity	≥0.997	≥0.988	≥0.998	≥0.994	Satisfactory
Accuracy	98.8 -106.6% [a] 99.4-101.5% [b]	94.1 - 107.4% [a] 94.7-103.5% [b]	99.8 -102.0% [a] 98.5-102.2% [b]	97.3 -103% [a] 97-106% [b]	Satisfactory
Precision (RSD)	5.2%-8.6% [a] 2.3% - 5.0% [b]	5.7%-6.9% [a] 2.2% - 7.0% [b]	2.0%-7.3% [a] 0.5% - 3.8% [b]	5.0%-8.8% [a] 0.4% - 8.0% [b]	Satisfactory
Incurred sample reproducibility	pass	pass	-	-	Satisfactory
Stability	Stored at -60 to -80 ºC	Stored at -60 to -80 ºC	Stored at -60 to -80 ºC	Stored at -60 to -80 ºC	Satisfactory

[a] QC samples (15.0, 150, 750, and 5000 ng/mL); [b] Standards

Statistical Methods:
Individual and mean plasma concentration-time profiles were to be plotted for each patient. Plasma and urine concentration data at each time or interval, respectively, were to be summarized with the number of observations, mean, geometric mean, standard deviation (SD), median, minimum, maximum, and coefficient of variation (CV). PK parameters were to be listed individually and summarized with mean, median, minimum, maximum, SD, and CV. Geometric means were to be calculated for all PK parameters except Tmax. Where data were available, absolute bioavailability was to be examined between the oral TR-701 FA (Test) and the IV TR-01 FA treatments (Reference). An analysis of variance (ANOVA) was to be performed.

RESULTS

Study Population: In total, 20 subjects were enrolled and completed the study (**Table 2**).

Table 2. Summary of subject demographic and baseline characteristics

Demographic Characteristic **Safety Analysis Set**	**Part A** **N=10**	**Part B** **N=10**
Mean age in years	15 (range 12 to 17 years)	14 (range 11[a] to 17 years)
Mean weight in kg	64.9 (range 38.5 to 83.1 kg)	55.3 (range 39.0 to 70.0 kg)
Mean height in cm	167.7 (range 151.0 to 178.0 cm)	162.0 (range 145.0 to 175.0 cm)
BMI in kg/m^2	22.9 (range 16.9 to 27.0 kg/m^2)	21.0 (range 16.0 to 28.4 kg/m^2)
Sex n (%)		
Male	9 (90.0%)	7 (70.0%)
Female	1 (10.0%)	3 (30.0%)
Ethnicity n (%)		
Hispanic or Latino	6 (60.0%)	2 (20.0%)
Not Hispanic or Latino	4 (40.0%)	8 (80.0%)
Race n(%)		
White	8 (80.0%)	9 (90.0%)
Asian	0 (0.0%)	1 (10.0%)
Black or African American	1 (10.0%)	0 (0.0%)
Native Hawaiian or Other Pacific Islander	0 (0.0%)	0 (0.0%)
American Indian/ Alaskan Native	0 (0.0%)	0 (0.0%)
Other	1 (10.0%)	0 (0.0%)

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Pharmacokinetics:
Following a single IV infusion of 200 mg TR-701 FA, TR-701 levels rapidly declined and t1/2 after IV infusion administration could only be reliably estimated for 1 patient due to limited quantifiable TR-701 concentrations. No PK parameters could be derived for TR-701 after a single oral administration of 200 mg TR-701 FA since all TR-701 concentration values were BLQ.

The PK parameters following a single oral or IV infusion administration of 200 mg TR-701 FA are listed in **Table 3**. The absolute bioavailability estimate is 89%. The arithmetic mean TR-700 concentration-time profiles after oral (Part A) and IV infusion (Part B) of 200 mg TR-701 FA are presented in **Figure 1**.

Urine PK parameter data for TR-701, which could only be calculated following IV administration, indicated very low renal elimination of TR-701 with mean fe0-24 values less than 0.01% of the dose. TR-700 urine pharmacokinetic parameters are summarized in **Table 4**. The results indicate that renal elimination is not important for the disposition of TR-700 with mean fe0-24 values less than 1% of the dose when administered by either route.

Table 3. Mean (SD) Plasma PK Parameter Data for TR-700 after Administration of 200 mg TR-701 FA (Oral or IV)

PK Parameters	Units	Oral (Part A)		IV (Part B)	
		Mean (SD)	N	Mean (SD)	N
C_{max}	(ng/mL)	2231 (549)	10	3854 (1506)	10
T_{max}[a]	(hr)	4.00 (1.00, 6.00)	10	1.02 (0.500, 2.02)	10
AUC_{0-24}	(ng·hr/mL)	21017 (6492)	10	25468 (6371)	10
AUC_{0-t}	(ng·hr/mL)	21000 (6503)	10	25482 (6384)	10
$AUC_{0-\infty}$	(ng·hr/mL)	25205 (9153)	10	27820 (7282)	10
$AUC_{ext\%}$	(%)	15.3 (7.14)	10	8.11 (2.12)	10
CL/F[b]	(mL/hr)	7186 (2115)	10	6313 (1808)	10
V_{ss}/F[c]	(mL)	90822 (23103)	10	54222 (10189)	10
V_z/F[d]	(mL)	83498 (28194)	10	59278 (12208)	10
$t_{½}$	(hr)	8.26 (1.99)	10	6.64 (0.689)	10

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Figure 1. Arithmetic mean (+/- SD) TR-700 Concentration-Time Profiles for 200 mg TR-701 FA

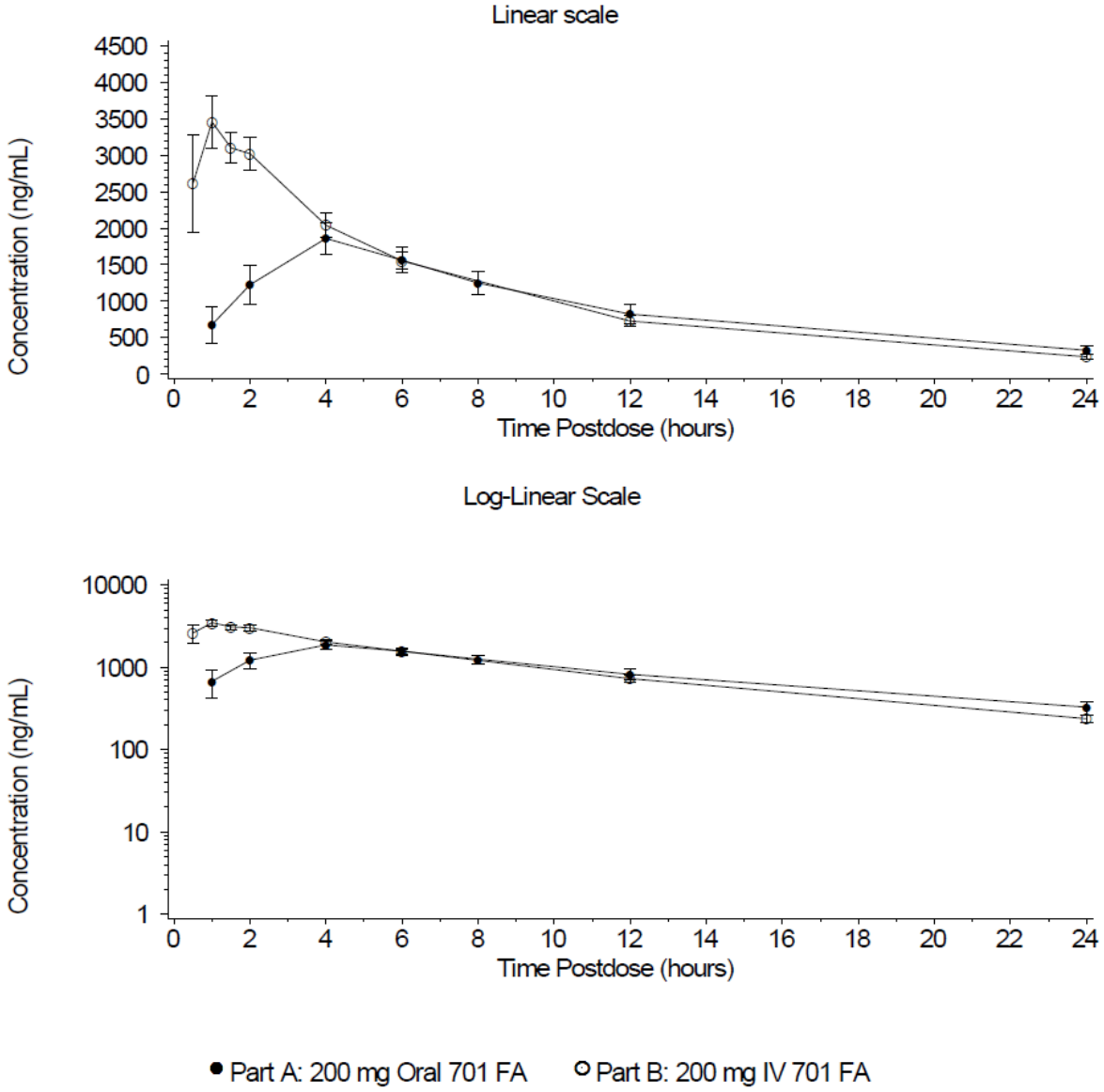

Table 4. Mean (SD) Urine PK Parameter Data for TR-700 after Administration of 200 mg TR-701 FA

PK Parameter	Units	Oral (Part A)	N	IV (Part B)	N
Ae_{0-24}	(mg)	0.765 (0.409)	9	1.28 (0.825)	8
fe_{0-24}	(%)	0.464 (0.247)	9	0.777 (0.503)	8
CL_R	(mL/hr)	37.6 (17.8)	9	51.6 (26.1)	8

Safety: TR-701 FA, administered as an oral tablet or an IV infusion to adolescents, was safe and generally well-tolerated. TEAEs were mild, no deaths and no SAEs were reported, and no patient

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discontinued treatment due to a TEAE. Clinical laboratory evaluations, vital signs measurements, physical examinations, and 12-lead ECGs did not show clinically significant changes.

SPONSOR'S CONCLUSIONS:
1. TR-701 concentrations are only quantifiable for a short period of time following IV administration of TR-701 FA and undetectable following oral administration.
2. TR-700 PK is generally similar for IV infusion or oral administration of 200 mg TR-701 FA, with oral absorption accounting for reduced Cmax and delayed Tmax.
3. Oral bioavailability of TR-700 is high at approximately 89%.
4. Renal excretion is a minor route of elimination for TR-700 and TR-701.
5. TR-701 FA, administered as an oral tablet or an IV infusion to adolescent patients, is safe and generally well-tolerated.

REVIEWER ASSESSMENT: The Sponsor's conclusions are appropriate based on study results.

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STUDY NO.: TR701-114

A Phase 1, crossover study of blood pressure and heart rate response to Pseudoephedrine

Date(s): 23 April 2012 – 15 June 2012
Investigator(s): Bradley Vince, M.D.
Clinical Site(s): Vince and Associates clinical research, Overland Park, KS, US
Analytical Site(s): (b) (4)

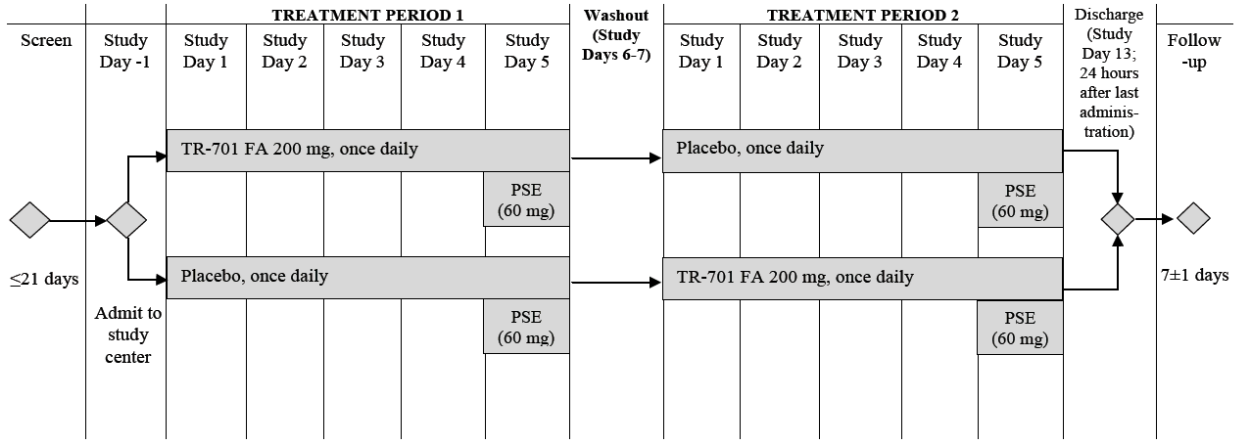
OBJECTIVE(S):

- Primary: To compare systolic blood pressure (SBP) following administration of pseudoephedrine (PSE) concurrent with placebo or steady-state TR-701 free acid (FA) in healthy subjects.
- Secondary: To compare diastolic blood pressure (DBP) and heart rate (HR) following administration of PSE concurrent with placebo or steady-state TR-701 FA in healthy subjects; To compare the pharmacokinetics (PK) of TR-700 at steady state when TR-701 FA is administered alone or concurrent with PSE in healthy subjects; To compare the PK of single-dose PSE in healthy subjects when administered concurrent with placebo or TR-701 FA; To compare the safety following administration of PSE concurrent with placebo or TR-701 FA in healthy subjects; To assess the safety and tolerability of administration of 200 mg oral TR-701 FA when given in combination with tyramine for up to 14 days.

METHODS

Study Design: Subjects were to be randomized to one of the two treatment sequences (N=9 per sequence) on Study Day 1. Subjects were to receive oral 200 mg TR-701 FA or placebo once daily for 5 days during each treatment period, with a 2-day washout between periods (72 hours between doses). On Study Day 5 of each treatment period, subjects were to receive oral 60 mg PSE, a typical therapeutic dose, at the same time as study drug. TR-701 FA, placebo, and PSE were to be administered with water after an overnight fast of at least 8 hours. Subjects were to continue fasting until at least 4 hours after study drug administration. Study schedule is depicted in Table 1.

Table 1. Study Schedule



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Inclusion/Exclusion Criteria: Healthy subjects from 18 to 45 years of age, without clinically significant abnormalities identified by medical history were eligible for enrollment. Subjects were required to have resting systolic blood pressure 90 to 130 mmHg, diastolic blood pressure 60 to 90 mmHg, heart rate 50 to 90 bpm, and QTc interval ≤500 msec. A high tyramine diet and exposure to certain drugs, foods, and beverages or any investigational drug were reasons for exclusion from enrollment.

Treatment: TR-701 FA Tablets, 200 mg (lot number B090282), administered orally once daily for 5 days. PSE, two 30 mg tablets (SudoGest®), administered orally with TR-701 FA on Study Day 5.

Sample Collection: Blood samples were collected at predose, 1, 2, 3, 4, 6, 8, 12, and 24 hour post-dose on Days 4 and 5.

Analytical Methods: Pharmacokinetic samples were analyzed for TR-700, TR-701 and PSE by validated high-performance liquid chromatography (HPLC) with tandem mass spectrometric (MS/MS) detection in plasma (**Table 2**).

Table 2. Bioanalytical results of TR-700 and TR-701 in human plasma

Criterion	TR-700	TR-701	Pseudoephedrine	Comments
Range	5-1000 ng/mL	5-1000 ng/mL	5-500 ng/mL	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	5 ng/mL	Satisfactory
Linearity	≥0.998	≥0.988	≥0.999	Satisfactory
Accuracy	94.7% - 105.8% [a] 97.4%-102% [b]	91.4% - 99.6% [a] 92.6%-107% [b]	100% - 122.3% [a] 99%-102% [b]	Satisfactory
Precision (RSD)	1.3%-6.0% [a] 1.1% - 3.6% [b]	6.7%-9.6% [a] 2.3% - 6.8% [b]	1.2%-13.9% [a] 0.9% - 2.2% [b]	Satisfactory
Selectivity	No interfering peak in the presence of PSE 200 ng/mL or TR-700 1500ng/mL			Satisfactory
Incurred Sample Reproducibility	pass	pass	pass	Satisfactory
Stability	Stored at -60 to -80 ºC	Stored at -60 to -80 ºC	Stored at -60 to -80 ºC	Satisfactory

[a] QC samples; [b] Standards

PK/PD Assessment:

- PK: Plasma concentrations of TR-700 and TR-701 were assessed on Study Days 4 and 5 of the treatment period during which the subject received TR-701 FA, and plasma concentrations of PSE were assessed on Study Day 5 of both treatment periods.
- PD: Blood pressure and HR monitoring (resting at least 10 minutes supine) were to be conducted in triplicate within 15 minutes prior to study drug administration and at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 hours after study drug administration on Study Day 5 of each treatment period.

Statistical Methods:
Standard PK parameters were calculated for TR-700 (Cmax, Cmin, Tmax, AUC) and PSE (Cmax, Tmax, AUC, t1/2) and geometric mean (GM) ratios and 90% CIs were calculated for

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GM ratios for each parameter and compound to determine if there were any drug-drug interactions, ie, (TR-701 FA+PSE)/(TR-701 FA) and (PSE+TR-701 FA)/PSE.

The following PD parameters were summarized by treatment group using descriptive statistics at each time point on Study Day 5 (up to the 24-hour time point):

- Mean SBP, DBP, and HR and mean changes in SBP, DBP, and HR from pre-PSE administration
- Mean maximum SBP, DBP, and HR and mean maximum change in SBP, DBP, and HR from pre-PSE administration.

The average maximum changes in SBP, DBP, and HR after PSE administration on Study Day 5 (up to the 24-hour timepoint) of each treatment period were compared between the placebo and TR-701 FA treatment groups using analysis of variance (ANOVA) models with average maximum change as the dependent variable; average baseline value, treatment (TR-701 FA, placebo), period, and sequence as fixed effects; and subject within sequence as a random effect. A p-value <0.05 was considered statistically significant.

RESULTS

Study Population: Eighteen subjects were randomized, received study drug in both treatment periods, and completed study assessments through the Discharge Visit, and 17 subjects completed the study through the Follow-up Visit (**Table 3**).

Table 3. Demographic and baseline characteristics – ITT analysis set

		Treatment Sequence[a]		
Characteristic		**1 (N=9)**	**2 (N=9)**	**All Subjects (N=18)**
Gender				
Male	n (%)	7(77.8)	8(88.9)	15(83.3)
Female	n (%)	2(22.2)	1(11.1)	3(16.7)
Race				
White	n (%)	4(44.4)	5(55.6)	9(50.0)
Black or African American	n (%)	4(44.4)	4(44.4)	8(44.4)
American Indian or Alaska Native	n (%)	1(11.1)	0(0.0)	1(5.6)
Ethnic Group				
Not Hispanic or Latino	n (%)	9(100.0)	9(100.0)	18(100.0)
Age (years) at Screening				
	Mean (SD)	31.0 (7.57)	37.8 (6.10)	34.4 (7.52)
	Median	32.0	39.0	34.5
	Min, Max	22, 44	29, 45	22, 45
BMI (kg/m^2)				
	Mean (SD)	26.66 (2.591)	26.69 (2.555)	26.67 (2.496)
	Median	27.50	27.30	27.40
	Min, Max	20.8, 29.5	22.9, 29.9	20.8, 29.9

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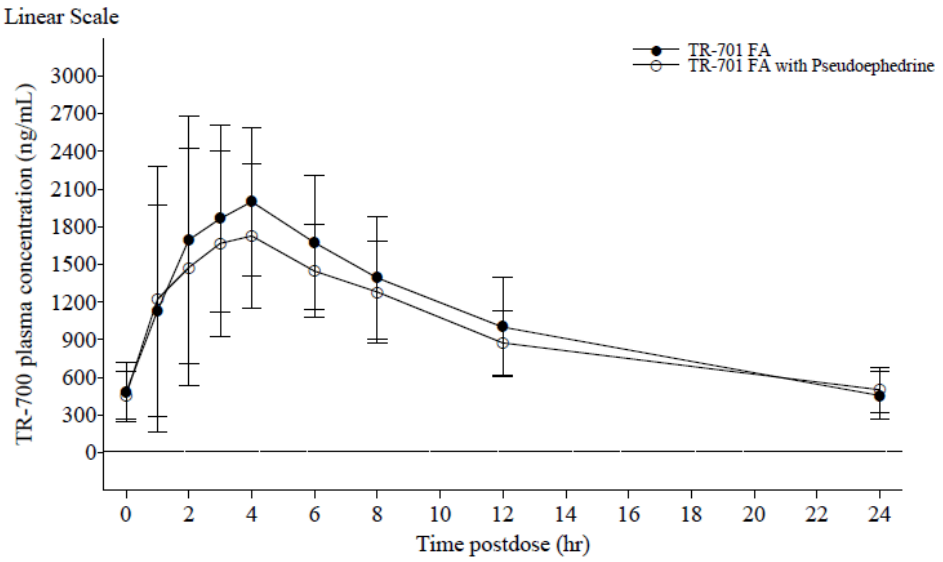
Pharmacokinetics:

TR-700 PK With and Without PSE Co-administration:

Concentrations of TR-701 in plasma were below detection limits in all patients at all timepoints on Days 4 and 5 of the treatment period during which TR-701 FA 200 mg was administered on Days 1 through 5. Mean (SD) plasma concentration of TR-700 over time after administration of TR-701 FA alone on Day 4 and with PSE on Day 5 is shown in (**Figure 1**).

Figure 1: Mean (±SD) TR-700 Concentrations at Steady State – PK Analysis Set



---Lower Limit of Quantification

Note: Postdose refers to time of coadministration of TR-701 FA and placebo on Study Day 4 or TR-701 FA and pseudoephedrine on Study Day 5 of the treatment period during which subjects received daily TR-701 FA

The statistical analysis of PK parameters (**Table 4**) demonstrated similarity of TR-700 Cmax PK with or without PSE. GMRs were within a 0.8 to 1.25 bioequivalence range for the PK parameters Cmin, AUC0-t, and AUC0-24, and the 90% confidence limits were within this range. The coadministration of PSE with TR-701 FA resulted in a minor decrease (approximately 14%) in TR-700 Cmax.

Table 4: Effect of PSE on TR-700 Plasma PK Parameters –PK Analysis Set

	Geometric Mean[a]		Geometric Mean Ratio[b]	
Parameter (units)	**TR-701 FA+PSE (N=18)**	**TR-701 FA (N=18)**	**(TR-701 FA+PSE) / TR-701 FA**	**90% CI[c]**
C_{max} (ng/mL)	1855.8	2157.5	0.860	(0.780, 0.948)
C_{min} (ng/mL)	417.3	401.3	1.040	(1.019, 1.061)
AUC_{0-t} (ng·hr/mL)	22690.9	24578.5	0.923	(0.883, 0.965)
AUC_{0-24} (ng·hr/mL)	22688.4	24598.2	0.922	(0.882, 0.964)

[a] Least squares mean from analysis of variance (ANOVA) model, calculated by transforming the natural log mean back to the linear scale (ie, geometric mean). The ANOVA model included the log of the PK parameter as the response, treatment as a fixed

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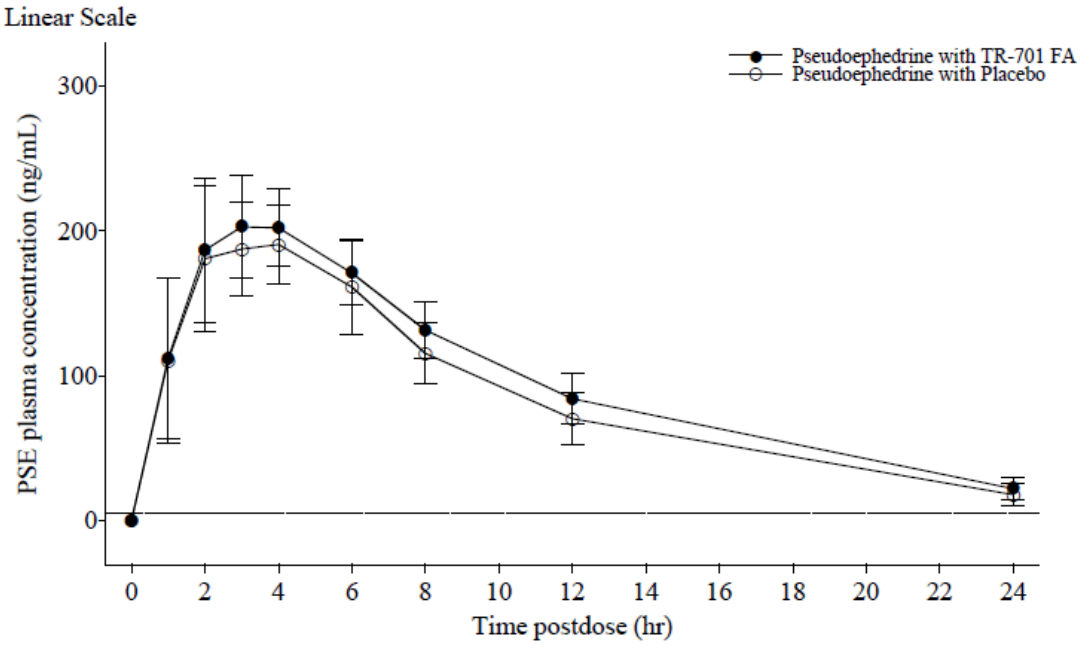
factor, and subject as a random factor. [b] Ratio of parameter means, transformed back to the linear scale. [c] 90% confidence interval for the geometric mean ratio.

PSE PK With and Without TR-700 Co-administration:
Mean (SD) plasma concentration of PSE over time after administration of PSE with placebo or TR-701 FA on Day 5 of the 2 treatment periods is shown in **Figure 2**.

Figure 2: Mean (±SD) PSE Concentrations-time profiles – PK Analysis Set



---Lower Limit of Quantification

Note: Postdose refers to time of coadministration of PSE with placebo or TR-701 FA on Study Day 5 of the corresponding treatment period.

Steady state TR-700 had little effect on exposure to PSE (**Table 5**). There was a small (5%) increase in maximal PSE concentration and 13% increase in exposure over time when PSE was coadministered with TR-701 FA, but lower and upper limits of the GMR 90% CI were within the 0.8 to 1.25 acceptable bioequivalence limits for both Cmax (GMR=1.05; 90% CI=[1.016, 1.084]) and AUC0-∞ (GMR=1.133; 90% CI=[1.071, 1.198]).

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Table 5: Effect of TR-700FA on PSE Plasma PK Parameters –PK Analysis Set

Parameter (Units)	Geometric Mean[a]		Geometric Mean Ratio	
	PSE with TR-701 FA (N=18)	PSE with Placebo (N=18)	PSE + TR-701 FA/ PSE + Placebo[b]	90% CI
C_{max} (ng/mL)	210.2	200.2	1.050	(1.016, 1.084)
AUC_{0-t} (ng·hr/mL)	2227.2	1986.1	1.121	(1.065, 1.180)
$AUC_{0-\infty}$ (ng·hr/mL)	2422.6	2138.9	1.133	(1.071, 1.198)

[a] Least squares mean from analysis of variance (ANOVA) model, calculated by transforming the natural log mean back to the linear scale (ie, geometric mean). The ANOVA model included the log of the PK parameter as the response, sequence, period, and treatment as a fixed factor, and subject as a random factor. [b] Ratio of parameter means, transformed back to the linear scale. [c] 90% confidence interval for the geometric mean ratio

Pharmacodynamics:
Blood pressure responses to PSE were recorded for 24 hours on Day 5 of once-daily administration of TR-701 FA or placebo. Mean maximum increases in blood pressure and heart rate were not significantly different between placebo and tedizolid treatments (**Table 6**). Four (22%) subjects experienced a systolic blood pressure rise ≥15 mmHg when PSE and tedizolid phosphate were administered concomitantly. Similarly, five (28%) subjects experienced an SBP elevation ≥15 mmHg when PSE was administered with placebo.

Table 6: Blood Pressure and Heart Rate Changes Following Pseudoephedrine Administered with Placebo and TR-701 FA

Parameter (unit)	LS Mean Maximum Change from Baseline[a]		Difference (95% CI)	*P*-value
	TR-701 FA (n=18)	Placebo (n=18)		
Systolic BP (mmHg)	11.6	12.1	-0.5 (-3.8, 2.7)	0.7309
Diastolic BP (mmHg)	6.7	6.8	-0.1 (-2.0, 1.7)	0.8966
Heart rate (beats/min)	13.6	15.2	-1.6 (-3.8, 0.7)	0.1689

Note: Baseline is defined as the average Day 5 predose measurement for each period. LS means, differences, and 95% CIs are derived from an analysis of variance model with average maximum change as the dependent variable and average baseline value, treatment (TR-701 FA or placebo), period, and sequence as fixed effects, and subject nested within sequence as a random effect.

SPONSOR'S CONCLUSIONS: TR-701 FA did not potentiate the PSE-induced pressor response in this population of 18 healthy adults. There was a small 5% increase in PSE Cmax and 13% increase in AUC0-∞ with TR-700 at steady state, but both GMRs and 90% confidence intervals were within the 0.8 to 1.25 acceptable bioequivalence range (FDA Guidance March 2003). There was no statistically significant change in SBP, DBP, or HR between TR-701 FA and placebo treatments when coadministered with PSE. PSE effects on TR-700 steady state PK following TR-701 FA administration every 24 hours, were minimal and not expected to affect efficacy or safety.

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REVIEWER ASSESSMENT: The Sponsor's conclusions are appropriate based on study results. The study showed that the coadministration of TR-701FA with vasoconstrictors such as PSE did not potentiate the PSE-induced pressor response in healthy subjects.

Reference ID: 3476543

STUDY NO.: TR701-123

A Phase 1 Open-Label Study with 200 mg Intravenous TR-701 FA to Assess Safety and Pharmacokinetics in Advanced Renal Impairment Subjects

Date(s): 26 October 2011 to 03 March 2012
Sponsor: Trius Therapeutics, Inc., San Diego, CA
Clinical Site: Site 1, Orlando Clinical Research Center (Orlando, FL); Site 2, DaVita Clinical Research (Minneapolis, MN).
Analytical Site: (b) (4)

OBJECTIVE(S): The primary objectives of this study were to assess the pharmacokinetics (PK) and to evaluate the safety of intravenous (IV) infusion of TR-701 FA in subjects with advanced renal impairment compared with matched control subjects with normal renal function.

METHODS

Study Design: This was an open-label, Phase 1 study of IV 200 mg TR-701 FA in subjects with advanced renal impairment (estimated glomerular filtration rate [eGFR] <30.0 mL/min/1.73 m^2), with or without hemodialysis, compared with matched control subjects (eGFR ≥80.0 mL/min/1.73 m^2). Twenty-four subjects were recruited into 1 of 3 groups (n=8 per group) based on their eGFR (using the Modification of Diet in Renal Disease, 4-variable formula [MDRD4]) and hemodialysis status: subjects with normal renal function (eGFR ≥80.0 mL/min/1.73 m^2; Control Group), subjects with advanced renal impairment not receiving hemodialysis (eGFR <30.0 mL/min/1.73 m^2; Nondialyzed Group), and subjects with renal impairment (end-stage renal disease) receiving hemodialysis (eGFR <15.0 mL/min/1.73 m^2; Dialyzed Group). Control Group subjects were matched (by age, sex, and body mass index [BMI]) to Nondialyzed Group subjects. The Screening Visit was scheduled to occur within 21 days of Day 1 (study drug infusion). Eligible subjects were admitted to the study center on Day -1.

Subjects in the Control and Nondialyzed Groups received a single 60-minute infusion of 200 mg TR-701 FA. Subjects were confined to the study center for the duration of the sampling period (Day -1 through 72 hours after the start of the infusion). Blood and urine were collected for PK analysis.

Subjects in the Dialyzed Group received 2 separate 60-minute infusions of 200 mg TR-701 FA in a crossover design, with 1 infusion to start within 1 hour after completion of hemodialysis and another infusion to start 1-1.5 hours prior to hemodialysis. Subjects were assigned to the order in which they were to receive the 2 infusions and an attempt was made to balance the assignments. A minimum 7-day washout period was required. High-flux hemodialysis was performed using a single-use dialyzer (ie, no reuse). Blood was to be analyzed for PK and urine collected (from subjects who produce urine) for PK analysis. In addition, afferent and efferent blood samples were collected prehemodialysis and every 30 minutes during hemodialysis in those subjects receiving TR-701 FA prior to hemodialysis.

For all subjects, a Follow-up Visit occurred 7 days (±1 day) after the last TR-701 FA infusion.

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Reviewer Comment: The study design meets the criteria outlined in the FDA "Guidance for Industry Pharmacokinetics in Patients with Impaired Renal Function: Study Design, Data Analysis, and Impact on Dosing and Labeling".

Drug Product:
Control Group and Nondialyzed Group: A single infusion of TR-701 FA 200 mg in 250 mL sterile saline for injection as a 60-minute IV infusion; lot number B110185.

Dialyzed Group: Two infusions of TR-701 FA 200 mg in 250 mL sterile saline for injection, each as a 60-minute IV infusion, separated by a minimum of 7 days; lot number B110185.

Inclusion Criteria: Males and females of non-childbearing potential; 18-75 years of age (inclusive); 18-40 kg/m^2 (inclusive) in body mass index; medically stable, with stable hemoglobin and hematocrit values for at least 3 months prior to screening and on stable dosage(s) of medication(s) (other than those specifically prohibited) for 30 days were eligible to be enrolled.

Subjects were excluded if they had a functioning transplanted solid organ, malignancy within two years of screening, hypertensive crisis, pheochromocytoma, carcinoid syndrome, or thyrotoxicosis, high tyramine diet, an investigational device present, treatment with investigational medicinal product within 30 days, a history of alcohol or substance abuse or dependence within 12 months, use of medications which are inducers of drug-metabolizing enzymes within 30 days, use of monoamine oxidase inhibitors, tricyclic antidepressants, buspirone, meperidine, selective serotonin reuptake inhibitors, or serotonin 5-hydroxytryptamine receptor agonists (triptans) within 14 days, use of directly- or indirectly-acting sympathomimetic agents, vasopressive agents, dopaminergic agents, or over-the-counter medications containing vasoconstrictive agents within 2 days, a history or presence of a clinically significant abnormal electrocardiogram, or hypersensitivity to oxazolidinones or any component in the study drug formulation.

Additionally, subjects were required to meet Group-specific criteria:

- Control Group: eGFR ≥80.0 mL/min/1.73 m^2
- Nondialyzed Group: eGFR <30.0 mL/min/1.73 m^2 and not on dialysis currently or not anticipated to receive dialysis within 30 days prior to the Screening Visit
- Dialyzed Group: eGFR <15.0 mL/min/1.73 m^2, receiving chronic and stable maintenance hemodialysis for at least 3 months, and Kt/V urea ≥ 1.2

PK Sample Collection: Plasma and urine samples were collected (**Table 1**) and analyzed for pharmacokinetic purposes.

Reference ID: 3476543

Table 1. Pharmacokinetic sampling scheme

Control and Nondialyzed Groups	
	Plasma • Pre-dose (within 30-min preinfusion) • 0.5, 1, 1.5, 2, and 2.5 hours (±3 minutes); 3, 3.5, and 4 hours (±5 minutes); 5, 6, and 8 hours (±10 minutes); and 12, 24, 36, 48, 60, and 72 hours (±15 minutes) from the start of the infusion. **Urine** • -12 to 0, 0-12, 12-24, 24-36, and 36-48 hours after study drug infusion.
Dialyzed Group	
	Plasma • Pre-dose (within 30-min preinfusion) • 0.5, 1, 1.5, 2, and 2.5 hours (±3 minutes); 3, 3.5, and 4 hours (±5 minutes); 5, 6, and 8 hours (±10 minutes); and 12, 24, 36, 48, 60, and 72 hours (±15 minutes) from the start of the infusion. **Urine** • -12 to 0, 0-12, 12-24, 24-36, and 36-48 hours after study drug infusion. Afferent and efferent blood samples were collected every 30 minutes in subjects receiving TR-701 FA prior to hemodialysis, dialysate samples were collected during hemodialysis.

Analytical Methods: PK samples were analyzed for TR-701 and TR-700 by validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) (**Table 2**). For the calculation of summary statistics, all concentrations below the limit of quantification (BLQ) were to be set to zero.

Table 2. Bioanalytical results of TR-701 and TR-700 in plasma and urine

Criterion	**TR-701**	**TR-700**	**Comments**
PLASMA			
Calibration Range	5-1000 ng/mL	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	Satisfactory
Linearity, R^2	≥0.9914	≥0.9965	Satisfactory
Accuracy	Within ±5.0%	Within ±2.0%	Satisfactory
Precision, CV	≤7.0 %	≤4.0%	Satisfactory
Quality Control*	15, 150, 750, 5000(5x), 5000(10x), 5000(100x), 5000(500x) ng/mL	15, 150, 750, 5000(5x), 5000(10x), 5000(100x), 5000(500x) ng/mL	Satisfactory
Accuracy	Within ±9.8%	Within ±8.0%	Satisfactory
Precision, CV	≤8.9 %	≤6.6 %	Satisfactory
Stability	• QC Stability: 934 days at -60 to -80 ºC • Longest possible duration of storage: 266 days		Satisfactory
URINE			
Calibration Range	5-1000 ng/mL	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	Satisfactory
Linearity, R^2	≥0.9959	≥0.9983	Satisfactory

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Accuracy	Within ±6.9%	Within ±4.1%	Satisfactory
Precision	≤7.9 %	≤4.8%	Satisfactory
Quality Control*	15, 150, 750 ng/mL	15, 150, 750, 5000(10x) ng/mL	Satisfactory
Accuracy	Within ±4.0%	Within ±4.0%	Satisfactory
Precision, CV	≤16.1 %	≤3.6 %	Satisfactory
Stability	• QC Stability: 378 days at -60 to -80 ºC • Longest possible duration of storage: 266 days		Satisfactory

**Numbers in the parentheses indicate the dilution factor for QC samples.*

Pharmacokinetic Assessment: The following PK parameters were calculated for TR-701 and TR-700 in each subject using standard noncompartmental methods: C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $AUMC_{0-\infty}$, CL, λ_Z, MRT, $t_{1/2}$, Ae_{t1-t2}, Ae_{0-48}, $fe_{(0-48)}$, V_{ss}, Vz, CLr. The following PK parameters also were calculated for each subject based on unbound (u) plasma concentrations of the affected analyte: $C_{max,u}$, $AUC_{0-\infty,u}$. The following parameters were to be calculated for TR-701 FA and TR-700 for each subject who received hemodialysis: Ae_d, CL_d (d denotes dialysis).

Statistical Methods: Plasma parameters C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, CL (calculated only for TR-701 FA), and apparent terminal half-life; and urinary parameters amount excreted into urine, and renal clearance, were summarized for TR-700 FA and TR-701 by descriptive statistics for each renal function group and the control group.

The parameters $C_{max,u}$ and $AUC_{0-\infty,u}$ were calculated and summarized by descriptive statistics. The parameters C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$, (and $C_{max,u}$ and $AUC_{0-\infty,u}$ if calculated) were compared between each renal function group and the control group, including calculation of geometric mean ratio and corresponding 90% confidence intervals. Individual subject and group median plasma concentration-time profiles (linear and semi-logarithmic scales) were generated. The relationships between renal function measurements (eGFR and CLcr) and PK parameters for TR-700 and TR-701, such as AUC and C_{max}, were explored using scatter plots and correlation coefficients.

Dialysate was to be analyzed for drug concentration and amount of drug recovered in dialysate (Ae_d). Arterio-venous differences and dialysate clearance of TR-700 and TR-701 were calculated for subjects in the Dialyzed Group. The parameters C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, (and $C_{max,u}$ and $AUC_{0-\infty,u}$ if calculated) were compared between the 2 single-dose infusions for subjects in the Dialyzed Group, including calculation of geometric mean ratio and corresponding 90% confidence intervals. Differences in TR-700 and TR-701 drug concentrations between afferent and efferent blood samples were summarized. Protein binding percentages were also summarized by group.

Safety data were tabulated and summarized.

Safety Assessment: Safety and tolerance were assessed at specified timepoints by physical examinations, medical history, clinical laboratory evaluations (Chem-20, complete blood count [CBC], and urinalysis [UA]), 12-lead ECGs, adverse events (AEs), and vital signs.

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RESULTS

Study Population: A total of 24 subjects were enrolled (admitted to the study center) to provide 8 evaluable subjects in the Control Group, 8 evaluable subjects in the Nondialyzed Group, and 8 evaluable subjects in the Dialyzed Group. All subjects were included in the 3 analysis sets (Intent-to-Treat [ITT], Safety, and Pharmacokinetic [PK]).

A summary of demographic and baseline characteristics for the study population is presented in **Table 3**. Subjects were predominantly White (45.8%) or Black or African American (50.0%), male (62.5%), and overweight (>75% with BMI in the overweight to morbidly obese range), with a mean age of 56.4 years (range, 40 to 74 years). More subjects in the Control Group were White (87.5%) than Black or African American (12.5%), whereas all subjects in the Dialyzed Group were Black or African American. In the Nondialyzed Group, 50.0% of subjects were White, 37.5% were Black or African American, and 12.5% were American Indian/Alaska Native. The proportion of male and female subjects was balanced in the Control Group and the Nondialyzed Group; in the Dialyzed Group, there were more male subjects (n=7, 87.5%) than female subjects (n=1, 12.5%).

Reviewer Comment: Population PK analysis shows that tedizolid PK are not significantly altered by race or gender, thus, differences in demographic and baseline characteristics between treatment groups do not appear to affect the results of this study.

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Table 3. Demographic Characteristics

Characteristic	Control Group N=8	Nondialyzed Group N=8	Dialyzed Group N=8	Overall N=24
Age (years)				
Mean	57.8	61.0	50.4	56.4
SD	7.29	9.99	8.12	9.34
Median	59.0	63.0	47.0	59.0
Minimum	44	40	42	40
Maximum	68	74	63	74
BMI (kg/m^2)				
Mean	28.68	29.35	27.49	28.50
SD	3.940	5.376	5.209	4.733
Median	28.50	27.50	26.85	27.70
Minimum	23.9	22.7	21.0	21.0
Maximum	36.2	39.6	37.5	39.6
eGFR (mL/min/1.73 m^2)				
Mean	92.6	18.3	7.9	39.6
SD	9.07	6.45	2.17	39.06
Median	89.5	20.0	7.5	20.0
Minimum	81	7	5	5
Maximum	105	27	12	105
Sex, n (%)				
Male	4 (50.0)	4 (50.0)	7 (87.5)	15 (62.5)
Female	4 (50.0)	4 (50.0)	1 (12.5)	9 (37.5)
Race, n (%)				
White	7 (87.5)	4 (50.0)	0 (0.0)	11 (45.8)
Black or African American	1 (12.5)	3 (37.5)	8 (100.0)	12 (50.0)
American Indian/Alaskan Native	0 (0.0)	1 (12.5)	0 (0.0)	1 (4.2)
Ethnicity, n (%)				
Hispanic/Latino	1 (12.5)	2 (25.0)	0 (0.0)	3 (12.5)
Not Hispanic/Latino	7 (87.5)	6 (75.0)	8 (100.0)	21 (87.5)

One subject in the Dialyzed Group (Subject 002-203) withdrew from the study after completing the Dialyzed Postinfusion PK assessment and did not complete the crossover PK assessment (ie, Dialyzed Preinfusion). As a result, only concentrations in the postinfusion period were included in the PK assessment for this subject.

Pharmacokinetics:

Plasma PK

TR-700 plasma concentration-time profiles and PK parameters were similar between nondialyzed subjects with advanced renal impairment (**Figure 1 and Table 4**). Approximately 8% decrease in AUC and nearly identical C_{max} were observed in nondialyzed subjects with advanced renal impairment relative to matched controls (**Table 5)**.

Following IV administration of TR-701 FA, similar PK results were demonstrated in dialyzed subjects with TR-701 FA administration pre- or post-dialysis (**Figure 1 and Tables 4**). TR-700 exposure (AUC and C_{max}) in Dialyzed Group subjects was lower than in Control Group subjects and Nondialyzed Group subjects, by up to approximately 35%. This difference is not of a sufficient magnitude to warrant alteration of the TR-701 FA dose in dialyzed patients and likely represents normal, between-group variability, rather than an increase in clearance in subjects with end-stage renal disease.

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In Dialyzed Group subjects, the difference in TR-700 concentration in afferent and efferent samples was approximately 10%. That is, changes in TR-700 concentration due to dialyses were approximately 10%. In subjects dialyzed postinfusion, 14.7 mg of TR-700 was measured in dialysate, which is <10% of TR-700 content in a 200 mg dose of TR-701 FA, which is comparable to the approximate 10% change in PK as determined by comparison of afferent and efferent concentrations, and exposures (AUC and C_{max}) between Dialyzed Preinfusion and Dialyzed Postinfusion subjects.

Figure 1. Plasma Concentration-Time Profiles for TR-700

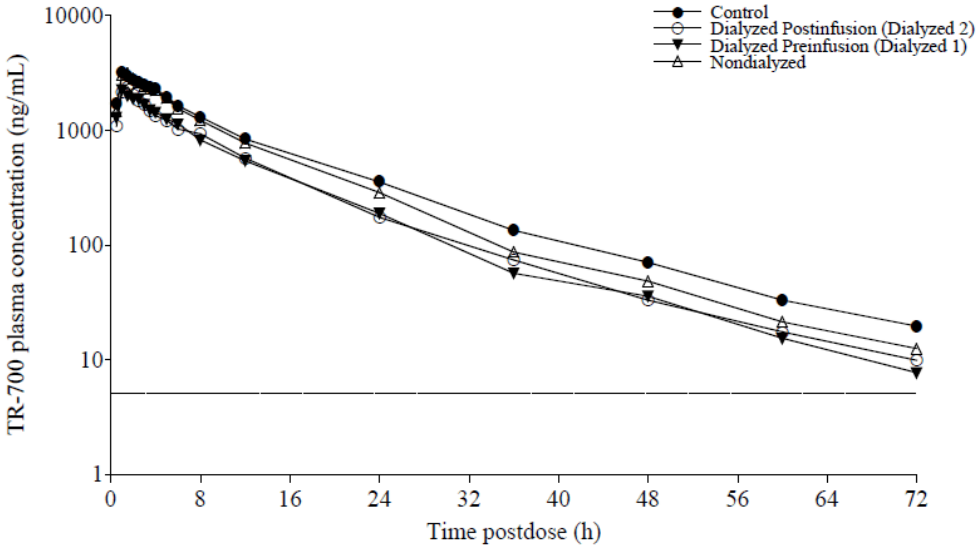

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Table 4. TR-700 Pharmacokinetic Parameters

Group	Statistic	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr•hr/mL)	$t_{½}$ (hr)
Control	n	8	8	8	8	8
	Mean	3105.0	1.438	32022.1	32426.4	12.252
	SD	751.28	0.6232	9322.29	9530.08	2.0389
	Median	3175.0	1.000	32023.6	32397.9	12.007
	Range	2090–4080	1.00–2.50	17941–42983	10852–43330	9.89–15.02
Nondialyzed	n	8	8	8	8	8
	Mean	3122.5	1.315	29693.3	29989.5	12.849
	SD	852.55	0.3701	8932.78	8971.79	2.2804
	Median	3220.0	1.258	28681.8	28926.9	12.656
	Range	1700–4200	1.00–2.00	17886–40815	18102–41402	9.72–17.58
Dialyzed Preinfusion	n	7	7	7	7	7
	Mean	2532.9	1.0000	22973.1	23152.9	11.407
	SD	954.49	0.28868	8024.57	8096.92	1.7810
	Median	2170.0	1.0000	19806.0	19911.0	11.438
	Range	1810–4590	0.500–1.500	15156–38828	15265–39092	8.69–13.72
Dialyzed Postinfusion	n	8	8	8	8	8
	Mean	2857.5	1.313	20814.3	21013.0	11.725
	SD	1009.78	0.2588	4654.86	4708.20	2.3290
	Median	2435.0	1.500	21611.3	21742.0	11.272
	Range	2150–5220	1.00 –1.50	14036–27586	14196–27720	9.29–17.04

Table 5. Comparison of TR-700 Plasma PK Parameters between Groups

Comparison	Parameter	n	Test Geometric Mean[a]	n	Reference Geometric Mean[a]	Geometric Mean Ratio[b]	90% CI[c]
Nondialyzed[d] vs. Control[e]	C_{max} (ng/mL)	8	3005.8	8	3023.0	0.994	0.777, 1.273
	AUC_{0-t} (ng•hr/mL)	8	28445.8	8	30708.8	0.926	0.699, 1.227
	$AUC_{0-\infty}$ (ng•hr/mL)	8	28742.4	8	31071.7	0.925	0.698, 1.227
	$C_{max,u}$ (ng/mL)	8	730.4	8	783.7	0.932	0.745, 1.166
	$AUC_{0-\infty,u}$ (ng•hr/mL)	8	6984.1	8	8055.2	0.867	0.669, 1.124
Dialyzed Postinfusion[d] vs. Dialyzed Preinfusion[e]	C_{max} (ng/mL)	8	2740.5	7	2386.4	1.148	1.053, 1.252
	AUC_{0-t} (ng•hr/mL)	8	20335.6	7	22280.7	0.913	0.828, 1.006
	$AUC_{0-\infty}$ (ng•hr/mL)	8	20527.4	7	22489.8	0.913	0.827, 1.007
	$C_{max,u}$ (ng/mL)	8	645.7	7	534.0	1.209	1.004, 1.456
	$AUC_{0-\infty,u}$ (ng•hr/mL)	8	4836.7	7	4973.1	0.973	0.829, 1.141

[a] *Least squares mean from analysis of variance (ANOVA) model, calculated by transforming the natural log mean back to the linear scale (ie, geometric mean). For each comparison between a renal function group and Control, the ANOVA model included the log of the PK parameter as the response and group as a fixed factor with two levels. For*

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comparison between the Dialyzed groups, the ANOVA model included the log of the PK parameter as the response, group and period as fixed factors, and subject as a random effect.
[b] *Ratio of parameter means (Test/Reference), transformed back to the linear scale.*
[c] *90% confidence interval for the geometric mean ratio.*
[d] *Test*
[e] *Reference*

Reviewer Comment: The Sponsor's rationale of the 35% decrease in AUC observed in dialyzed subjects (pre- or post-infusion), compared to control and nondialyzed subjects, appears reasonable. In addition, the average AUC values in dialyzed patients (21-23 µg•hr/ml) are comparable to those observed in overall populations studied in the tedizolid clinical program (26.6±5.2 µg•hr/ml). Therefore, the magnitude of decrease in AUC observed in dialyzed subjects (pre- or post-infusion) appears to be unlikely of clinical significance.

Following IV administration, TR-701 remains quantifiable for approximately 1.5 hours across all groups. TR-701 concentration changes with dialysis were greater than for TR-700, with approximately 30% lower TR-701 exposure in Dialyzed Postinfusion subjects than in Dialyzed Preinfusion subjects. In the Dialyzed Postinfusion subjects, TR-701 concentrations varied from about 50% lower to about the same concentrations as in Dialyzed Preinfusion subjects.

Reviewer Comment: Given that TR-701 is microbiologically inactive, the difference in TR-701 exposure between dialyzed pre-and post-infusion subjects is unlikely of clinical significance.

Protein Binding
The geometric mean $C_{max,u}$ of TR-700 in plasma was approximately 7%, 32%, and 18% lower in the Nondialyzed, Dialyzed Preinfusion, and Dialyzed Postinfusion Groups versus the Control Group, respectively. The geometric mean $AUC_{0-\infty,u}$ was approximately 13%, 38%, and 40% lower in the Nondialyzed, Dialyzed Preinfusion, and Dialyzed Postinfusion Groups versus the Control Group, respectively (**Table 5**). The mean (SD) TR-700 fraction bound to plasma proteins was 73.2% (7.9%), 75.6% (1.7%), and 76.8% (2.2%) in the Control, Nondialyzed, and Dialyzed (Preinfusion + Postinfusion) Groups, respectively. It is unlikely that these small differences among the groups of subjects would be of any clinical significance.

Urine PK
In all treatment groups, mean values of less than 1 mg of TR-700 was excreted in urine. Measurable levels of TR-701 in urine were found in one 1 subject (Subject 001-102) in the Nondialyzed Group and in no subjects in any other group.

Correlation Analyses of Renal Function and PK Exposure
Across the Control, Nondialyzed, and Dialyzed Preinfusion subjects, there did not appear to be a relationship between eGFR values and TR-700 exposure. Statistically significant slope values for the regression analysis suggest a linear relationship between eGFR and AUC (AUC0-t, AUC0-∞) (p=0.0187 and p=0.0179, respectively, for the slope); however, the small slopes and high degree of variability in AUC across the entire range of eGFR render it unlikely that the relationship is clinically meaningful.

Reviewer Comment: The relationship of renal function and TR-700 exposure were further explored in the Pop PK study. The population pharmacokinetic analysis shows that the evaluated

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covariates (age, gender, race, ethnicity, renal or liver function) did not result in any clinically relevant changes (>20%) in tedizolid exposure.

Across the Control, Nondialyzed, and Dialyzed Pre- and Post-infusion groups, there did not appear to be a linear relationship between CLcr values and TR-700 exposure.
Safety: Three types of adverse events (AEs) were related to TR-701 FA in subjects in the Nondialyzed Group (nausea, vomiting, and headache), and 3 types of AEs were related to TR-701 FA in the Dialyzed Group (diarrhea, vomiting, and headache). There were no serious adverse events or discontinuations due to an AE in this study. No clinically significant differences were observed across groups with regard to laboratory assessments, vital signs, or ECGs, and in subjects with advanced renal impairment, there were no unexpected or clinically meaningful changes in laboratory assessments, vital signs, or ECGs.

SPONSOR'S CONCLUSIONS
The results of this study suggest that TR-701 FA dose adjustment is not necessary in patients with advanced renal impairment. Pharmacokinetics of TR-701 FA were essentially unchanged in nondialyzed subjects with advanced renal impairment relative to a matched control group. Similar PK results were demonstrated in dialyzed subjects with TR-701 FA administration pre- or post-dialysis, showing that TR-700 is not markedly extracted by hemodialysis. The disposition of TR-701 FA does not appear to be altered in patients with advanced renal impairment. IV TR-701 FA was well tolerated at the single 200 mg dose.

REVIEWER ASSESSMENT: The Sponsor's conclusions are appropriate based on study results. No dose adjustment is necessary in subjects with severe renal impairment and subjects on hemodialysis.

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STUDY NO.: TR701-124

A Phase 1 Open-Label Study with Oral TR-701 FA to Assess Pharmacokinetics and Safety in Subjects with Moderate or Severe Hepatic Impairment

Date(s): 18 March 2008 to 15 May 2008
Sponsor: Trius Therapeutics, Inc., San Diego, CA
Clinical Site: Site 1, Orlando Clinical Research Center (Orlando, FL); Site 2, DaVita Clinical Research (Minneapolis, MN).
Analytical Site: (b) (4)

OBJECTIVE(S): The objectives of this study were to assess the single-dose pharmacokinetics (PK) and safety of TR-701 free acid (FA) in subjects with moderate or severe hepatic impairment compared with matched control subjects with normal hepatic function.

METHODS

Study Design: This was an open-label Phase 1 study of a single oral dose of TR-701 FA 200mg in subjects with moderate or severe hepatic impairment. Subjects were enrolled to provide 8 evaluable subjects with moderate hepatic impairment (Child-Pugh classification B; score of 7-9), eight evaluable subjects with severe hepatic impairment (Child-Pugh classification C; score of 10–15), and 16 age-, sex-, and body mass index (BMI)-matched control evaluable subjects with normal hepatic function. Subjects with moderate hepatic impairment were enrolled prior to enrollment of subjects with severe hepatic impairment. Matched control subjects were enrolled after subjects with hepatic impairment. The Screening Visit occurred within 28 days of Day 1 (study drug administration). Eligible subjects were admitted to the study center on Day -1. On Day 1, subjects received a single 200 mg oral dose of TR-701 FA under fasting conditions and remained confined to the study center for blood PK sampling and urine collection for possible PK analysis through Day 5. The Follow-up Visit occurred on Day 7 (±1 day).

Reviewer Comment: The study design meets the criteria outlined in the FDA "Guidance for Industry Pharmacokinetics in Patients with Impaired Hepatic Function: Study Design, Data Analysis, and Impact on Dosing and Labeling".

Drug Product: Tedizolid phosphate free acid 200 mg tablet

Inclusion Criteria: Males and females of non-childbearing potential; 18-70 years of age (inclusive); 18-40 kg/m^2 (inclusive) in body mass index; with moderate hepatic impairment (Child-Pugh score of 7 to 9, inclusive, and with medical history, physical examination, laboratory results, and other tests consistent with their hepatic impairment), severe hepatic impairment (Child-Pugh score of 10 to 15, inclusive, and with medical history, physical examination, laboratory results, and other tests consistent with their hepatic impairment), or as a control subject (matched control subject, free from clinically meaningful illnesses or disease, and with medical history, physical examination, laboratory results, and other tests consistent with overall good health and normal liver function) were enrolled.

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Subjects were excluded if they had autoimmune hepatic disease, Wilson's disease, or cholestatic liver disease; alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level ≥5 times upper limit of normal (ULN) (moderate hepatic impairment subjects), ≥8 times ULN (severe hepatic impairment subjects), or above normal limits (control subjects); total bilirubin concentration >5 mg/dL (moderate), no limit (severe), or above normal limits (control); hemoglobin concentration <10 g/dL (moderate), <9 g/dL (severe), or <0.8 times lower limit of normal (control); evidence of acute deterioration of hepatic function within 8 weeks prior to the Screening Visit; recipient of a transplanted solid organ, or an active candidate for liver transplantation; evidence of hepatic carcinoma, hepatorenal syndrome, or hepatopulmonary syndrome; surgically implanted portacaval or portosystemic shunts; creatinine clearance <50 mL/min, as calculated by the Cockcroft-Gault formula; tense ascites, significant pleural effusion, significant bleeding diathesis, esophageal bleeding (unless banded) within the past 8 weeks; significant degree of central nervous system impairment (e.g., grade 3 or 4 encephalopathy) or other signs of hepatic function deterioration; malignancy within 2 years except for basal cell or squamous cell skin carcinoma with clean surgical margins; severe or life-threatening condition or disease (eg, cardiac, respiratory insufficiency, advanced malnutrition); positive serum hepatitis B surface antigen (HBsAg) test or a positive human immunodeficiency virus (HIV) test; ECG finding of QTc interval >500 msec, or other clinically significant ECG abnormality at the Screening Visit; hypertensive crisis, pheochromocytoma, carcinoid syndrome, or thyrotoxicosis.

Subjects were also required to abstain from directly and indirectly acting sympathomimetic agents (eg, pseudoephedrine, phenylpropanolamine), vasopressive agents (eg, epinephrine, norepinephrine), dopaminergic agents (eg, dopamine, dobutamine), or over-the-counter medications containing vasoconstrictive agents, or high tyramine diet from 2 days prior to Day 1 and during the entire study. Use of selective serotonin re-uptake inhibitors (SSRIs), monoamine oxidase (MAO) inhibitors, and other serotonergic medications were not allowed within 14 days prior to Day 1 and during the entire study.

PK Sample Collection: Blood samples were collected at the following timepoints for each study period: within 30 min prior to study drug administration (pre-dose), 0.5, 1, 1.5, and 2 hours (±3 minutes); 3 and 4 hours (±5 minutes); 6 and 8 hours (±10 minutes); and 12, 24, 36, 48, 60, 72, 84, and 96 hours (±15 minutes) postdose. Urine samples were collected within 12 hours prior to study drug administration on Day 1, and 0-12, 12-24, 24-36, and 36-48 hours after study drug administration.

Analytical Methods: PK samples were analyzed for TR-701 and TR-700 by validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) (**Table 1**). For the calculation of summary statistics, all concentrations below the limit of quantification (BLQ) were to be set to zero.

Table 1. Bioanalytical results of TR-701 and TR-700 in plasma and urine

Criterion	TR-701	TR-700	Comments
PLASMA			
Calibration Range	5-1000 ng/mL	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	Satisfactory
Linearity, R^2	≥0.9916	≥0.9983	Satisfactory

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Accuracy	Within ±5.4%	Within ±1.4%	Satisfactory
Precision, CV	≤6.7 %	≤4.4%	Satisfactory
Quality Control*	15, 150, 750, 5000 (10x) ng/mL	15, 150, 750, 5000 (10x) ng/mL	Satisfactory
Accuracy	Within ±9.5%	Within ±5.8%	Satisfactory
Precision, CV	≤7.8 %	≤5.9 %	Satisfactory
Stability	• QC Stability: 934 days at -60 to -80 ºC • This duration provides sufficient stability for the storage of the study samples.		Satisfactory
URINE			
Calibration Range	5-1000 ng/mL	5-1000 ng/mL	Satisfactory
LLOQ	5 ng/mL	5 ng/mL	Satisfactory
Linearity, R^2	≥0.9841	≥0.9979	Satisfactory
Accuracy	Within ±9.1%	Within ±1.1%	Satisfactory
Precision	≤6.4 %	≤5.8%	Satisfactory
Quality Control*	15, 150, 750 ng/mL	15, 150, 750 ng/mL	Satisfactory
Accuracy	Within ±1.3%	Within ±2.0%	Satisfactory
Precision, CV	≤16.8 %	≤6.1 %	Satisfactory
Stability	• QC Stability: 378 days at -60 to -80 ºC • This duration provides sufficient stability for the storage of the study samples.		Satisfactory

**Numbers in the parentheses indicate the dilution factor for QC samples.*

Pharmacokinetic Assessment: The following PK parameters were calculated for TR-701 FA (if possible) and TR-700 in each subject using standard noncompartmental methods: C_{max}), T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, λz, $t_{1/2}$. The following PK parameters also were calculated for each subject based on the unbound plasma concentration of TR-700 (and also for TR-701 if measurable in plasma): $C_{max,u}$, $AUC_{0-\infty,u}$. In addition, the following PK parameters calculated for TR-701 FA (if possible) and TR-700: amount excreted into urine over a specified time interval, calculated as the product of concentration and volume ($A_{e(t1-t2)}$), cumulative amount excreted into urine over the 0 to 48 hour sampling period ($A_{e(0-48)}$), renal clearance (Cl_R), calculated as $A_{e(0-48)}AUC_{0-\infty}$, percent excreted into urine over the 0 to 48 hour sampling interval ($f_{e(0-48)}$).

Statistical Methods: Plasma parameters C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, and $t_{1/2}$ were summarized for TR-700 by descriptive statistics for each enrolled hepatic function group and the matched control group. The same plasma parameters were calculated and summarized for TR-701 if it was measureable in plasma. Descriptive statistics also were to be provided for $C_{max,u}$ and $AUC_{0-\infty,u}$ if these unbound parameters were calculated (ie, if plasma protein binding was affected by hepatic impairment). The parameters C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$, (and $C_{max,u}$ and $AUC_{0-\infty,u}$ if calculated) were to be compared between each hepatic function group and the matched control group, including calculation of geometric mean ratio and corresponding 90% confidence intervals. Individual subject and group mean plasma concentration-time profiles (linear and semi-logarithmic scales) were to be generated. The relationships between hepatic function measurements and PK parameters such as AUC and C_{max} were explored using scatter plots and correlation coefficients, and regression analysis. Safety data were summarized.

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Safety Assessment: Safety and tolerance were assessed at specified timepoints by physical examinations, medical history, clinical laboratory evaluations (Chem-20, complete blood count [CBC], and urinalysis [UA]), 12-lead ECGs, adverse events (AEs), and vital signs.

RESULTS

Study Population: In total, thirty-two (n=8 per group) subjects were enrolled in the study and completed the study. A summary of demographic and baseline characteristics for the study population is presented in **Table 2**. There were no substantial differences in demographic characteristics between the study groups. Of note, subjects in the Moderate Hepatic Impairment Group and the matched group had similar mean BMIs (29.8 and 27.3 kg/m^2, respectively), and on average were overweight. Subjects in the Severe Hepatic Impairment Group and the match group had higher mean BMIs (34.0 and 32.9 kg/m^2, respectively), and thus on average were obese.

Table 2. Demographic Characteristics

Characteristic	Moderate Hepatic Impairment (N=8)	Control 1: Moderate Match (N=8)	Severe Hepatic Impairment (N=8)	Control 2: Severe Match (N=8)	Overall (N=32)
Age (years)					
Mean	53.8	55.3	55.8	53.0	54.4
SD	3.92	7.01	8.41	8.65	6.98
Median	52.5	55.0	59.0	52.0	53.5
Range	48–60	43–67	39–64	39–66	39–67
BMI (kg/m^2)					
Mean	29.81	27.34	34.04	32.86	31.01
SD	5.133	4.200	3.541	4.266	4.896
Median	30.35	28.15	32.95	34.70	31.45
Range	22.9–36.3	21.0–31.7	29.4–38.9	24.1–37.1	21.0–38.9
Sex, n (%)					
Male	7 (87.5)	7 (87.5)	5 (62.5)	5 (62.5)	24 (75.0)
Female	1 (12.5)	1 (12.5)	3 (37.5)	3 (37.5)	8 (25.0)
Race, n (%)					
White	8 (100.0)	5 (62.5)	7 (87.5)	6 (75.0)	26 (81.3)
Black/African American	0	3 (37.5)	0	2 (25.0)	5 (15.6)
American Indian/ Alaskan Native	0	0	1 (12.5)	0	1 (3.1)
Ethnicity, n (%)					
Hispanic/Latino	0	0	2 (25.0)	1 (12.5)	3 (9.4)
Not Hispanic/Latino	8 (100.0)	8 (100.0)	6 (75.0)	7 (87.5)	29 (90.6)
CLcr (mL/min)					
Mean	130.13	111.91	140.54	132.11	128.67
SD	30.142	23.932	39.058	31.136	31.785
Median	138.10	110.90	133.55	143.55	125.70
Range	84.4–169.0	88.0–164.0	100.0–228.0	86.0–167.4	84.4–228.0
Child-Pugh Grade, n (%)					
B (7–9 points)	8 (100.0)	NC	0	NC	8 (25.0)
C (10–15 points)	0	NC	8 (100)	NC	8 (25.0)

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No PK-related protocol deviations were identified.

Pharmacokinetics:

Plasma PK

TR-700 mean plasma concentration-time profiles (linear scale and semi- logarithmic scale) are presented in **Figure 1**. As shown in **Table 3** and **4**, the largest PK differences between subjects with hepatic impairment and matched controls were seen in AUC, which was approximately 34% higher in subjects with severe hepatic impairment compared to matched controls, and 22% higher in subjects with moderate hepatic impairment compared to matched controls. Mean C_{max} was 1% lower in subjects with severe hepatic impairment compared with matched controls and 9% higher in subjects with moderate hepatic impairment compared with matched controls.

Figure 1. Plasma Concentration-Time Profiles for TR-700 in Patients with Moderate and Severe Hepatic Impairment and Respective Matched Control Subjects.

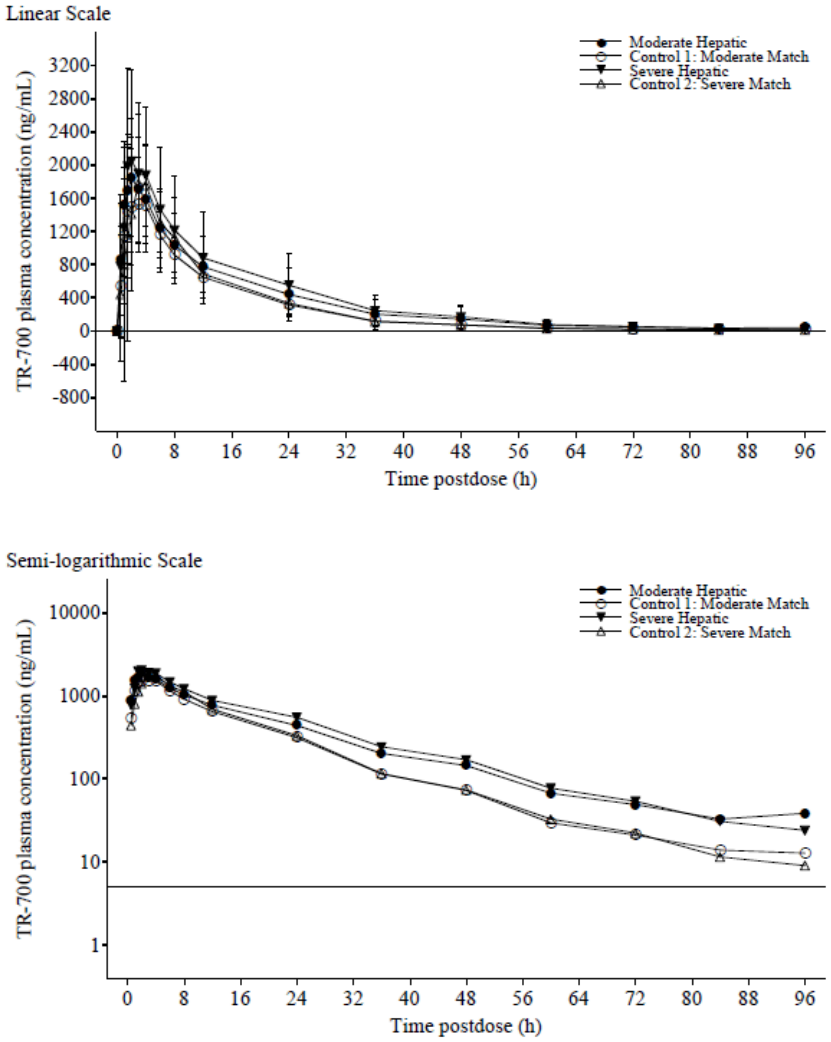

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Table 3. TR-700 Pharmacokinetic Parameters

Group	Statistic	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{½}$ (hr)
Moderate Hepatic Impairment	n	8	8	8	8	8
	Mean	2076.3	1.6250	29886.1	30471.5	14.94
	Geometric Mean	1974.5	NC	26779.6	27166.2	14.58
	SD	742.16	0.79057	16757.29	17496.66	3.486
	Median	1900.0	1.7500	22501.8	22646.0	14.81
	Range	1360–3370	0.500 –3.000	16493–65929	16618–68100	10.3 –19.3
Moderate Match	n	8	8	8	8	8
	Mean	1853.8	2.063	22796.7	22998.0	13.421
	Geometric Mean	1805.8	NC	22144.5	22335.0	12.917
	SD	491.88	1.0155	5634.37	5704.82	3.9316
	Median	1780.0	2.000	23204.2	23384.3	13.434
	Range	1380–2960	1.00–4.00	14264–28659	14349–28847	8.61–20.02
Severe Hepatic Impairment	n	8	8	8	8	8
	Mean	2201.3	1.7542	34800.1	35231.2	14.191
	Geometric Mean	2004.5	NC	31013.5	31356.7	13.901
	SD	1074.79	0.75759	20724.22	21126.72	2.9240
	Median	1955.0	2.0000	32601.4	33036.2	15.165
	Range	1090–4440	0.500–3.000	18399–82950	18498–84376	9.19–17.79
Severe Match	n	8	8	8	8	8
	Mean	2122.5	3.250	24371.4	24563.2	13.682
	Geometric Mean	2021.5	NC	23192.3	23384.5	13.199
	SD	804.41	2.1876	8029.63	8045.54	3.7146
	Median	1955.0	3.000	23971.8	24174.8	13.356
	Range	1320–4010	1.00–8.00	13055–38885	13159–39014	7.04–20.59

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Table 4. Comparison of TR-700 Plasma PK Parameters between Groups

Comparison	Parameter	Test n	Test Geometric Mean[a]	Reference n	Reference Geometric Mean[a]	Geometric Mean Ratio[b]	90% CI[c]
Moderate Hepatic Impairment[d] vs. Moderate Match[e]	C_{max} (ng/mL)	8	1974.5	8	1805.8	1.093	0.849, 1.408
	AUC_{0-t} (ng•hr/mL)	8	26779.6	8	22144.5	1.209	0.863, 1.695
	$AUC_{0-\infty}$ (ng•hr/mL)	8	27166.2	8	22335.0	1.216	0.862, 1.716
	$C_{max,u}$ (ng/mL)	8	596.5	8	486.9	1.225	0.881, 1.703
	$AUC_{0-\infty,u}$ (ng•hr/mL)	8	8206.5	8	6022.2	1.363	0.905, 2.053
Severe Hepatic Impairment[d] vs. Severe Match[e]	C_{max} (ng/mL)	8	2004.5	8	2021.5	0.992	0.703, 1.400
	AUC_{0-t} (ng•hr/mL)	8	31013.5	8	23192.3	1.337	0.925, 1.932
	$AUC_{0-\infty}$ (ng•hr/mL)	8	31356.7	8	23384.5	1.341	0.927, 1.939
	$C_{max,u}$ (ng/mL)	8	358.1	8	398.2	0.899	0.678, 1.192
	$AUC_{0-\infty,u}$ (ng•hr/mL)	8	5601.0	8	4606.8	1.216	0.920, 1.607

Reviewer Comments:

1) *Despite the 22 and 34% mean increase in AUC in patients with moderate and severe hepatic impairment compared to the respective controls, this AUC is within the range of exposure observed at doses that demonstrated safety in Phase 1 studies (tedizolid AUC values range from 17.36 to 123.10 µg*hr/ml at 100-1200 mg of single prodrug dose) and Phase 3 studies (mean tedizolid $AUC_{ss(0-24)}$ of 22 with a range of 6.57 to 49.86 µg*hr/ml at 200 mg QD prodrug dose).*
2) *Higher degree of variability in AUC was observed in patients with moderate and severe hepatic impairment compared to the respective controls (Table 3). The largest $AUC_{0-\infty}$ (84.4 µg•hr/ml) was observed in one patient (out of a total of 8 patients) in the severe hepatic impairment group. This larger variability in subjects with hepatic impairment could be in part due to more extensive medical histories and use of concomitant medications in this population. Given the modest inter-individual variability (<31%) based on the Population PK analysis, this large variability is not expected to be seen in the ABSSSI population.*

Protein Binding

There were no marked changes in between-group assessments of unbound PK parameters relative to results of bound PK parameters. As shown in **Table 4**, the geometric mean $C_{max,u}$ of TR-700 in plasma was approximately 21% higher and the geometric mean $AUC_{0-\infty,u}$ was approximately 35% higher in the Moderate Hepatic Impairment Group compared with the Moderate Match Group. Differences between the Severe Hepatic Impairment Group and the Severe Match Group in TR-700 geometric mean $C_{max,u}$ and geometric mean $AUC_{0-\infty,u}$ were -10% and 22%, respectively, and these collective differences appear to be due to variability in determination of protein binding. The mean (SD) TR-700 fraction bound to plasma proteins was 69.5% (4.3%), 72.5% (3.7%), 81.9% (3.0%), and 80.3% (1.5%) in the Moderate Hepatic Impairment, Moderate Match, Severe Hepatic Impairment, and Severe Match groups, respectively, and 76.4% (4.8%) for all control subjects combined.

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Urine PK
In all groups, the mean fraction of TR-700 excreted in urine in 48 hours (fe_{0-48}) was less than 1%. While there was numerically greater renal excretion of TR-700 in subjects with hepatic impairment compared with matched controls, renal elimination was negligible (**Table 5**). Measurable levels of TR-701 in urine were found in one 1 subject in the Moderate Hepatic Impairment Group and in no subjects in any other group.

Table 5. TR-700 Urine Pharmacokinetic Parameters

Group	Statistic	Ae_{0-48} (mg)	fe_{0-48} (%)	CL_R (mL/hr)
Moderate Hepatic Impairment	n	8	8	8
	Mean	1.1639	0.7076	46.98
	SD	0.45171	0.27463	19.516
	Median	1.0947	0.6656	44.20
	Range	0.637–1.862	0.387 –1.132	27.5–80.9
Moderate Match	n	8	8	8
	Mean	0.6650	0.4043	32.60
	SD	0.16696	0.10151	13.148
	Median	0.5946	0.3615	29.46
	Range	0.468–0.943	0.284–0.573	20.2–56.3
Severe Hepatic Impairment	n	8	8	8
	Mean	0.9145	0.5560	34.081
	SD	0.43414	0.26395	17.6249
	Median	0.9767	0.5938	37.584
	Range	0.242–1.552	0.147–0.944	4.89–58.39
Severe Match	n	8	8	8
	Mean	0.5738	0.3489	24.32
	SD	0.27193	0.16533	9.111
	Median	0.6118	0.3720	22.77
	Range	0.206–0.921	0.125–0.560	15.4–39.9

Correlation Analyses of Hepatic Function and PK Exposure
The relationships between hepatic parameters (AST, ALT, bilirubin, and albumin) and TR-700 PK parameters AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were explored using regression analyses, as presented in **Figure 2a** (AST), **Figure 2b** (ALT), **Figure 2c** (bilirubin), and **Figure 2d** (albumin). While the linear relationships between total bilirubin or albumin and both AUC (AUC_{0-t} and $AUC_{0-\infty}$) and C_{max} were statistically significant, it is unlikely that the relationships are clinically meaningful.

Reviewer Comment: The relationship of hepatic function and TR-700 exposure were further explored in the Pop PK study. The population pharmacokinetic analysis identified ideal body weight and total bilirubin as significant covariates for tedizolid exposure. However, the impact of these two covariates (ideal body weight and total bilirubin) as well as all other evaluated covariates (age, gender, race, ethnicity, renal or liver function) did not result in any clinically relevant changes (>20%) in tedizolid exposure.

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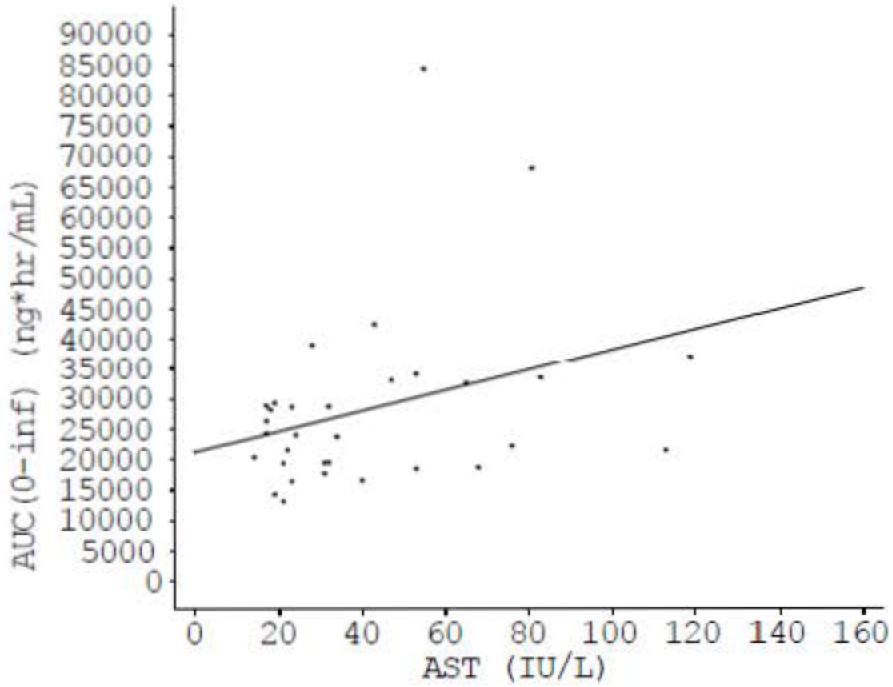
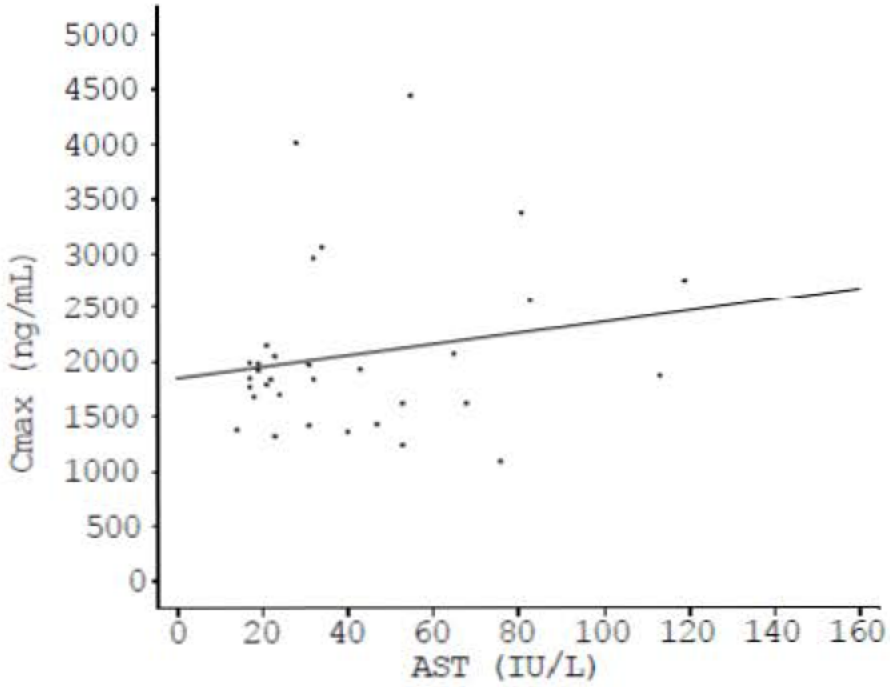
Figure 2. Relationship of TR-700 Exposure (AUC and C_{max}) and Hepatic Function Parameters, AST (a), ALT (b), Bilirubin (c), and Albumin (d).

a)





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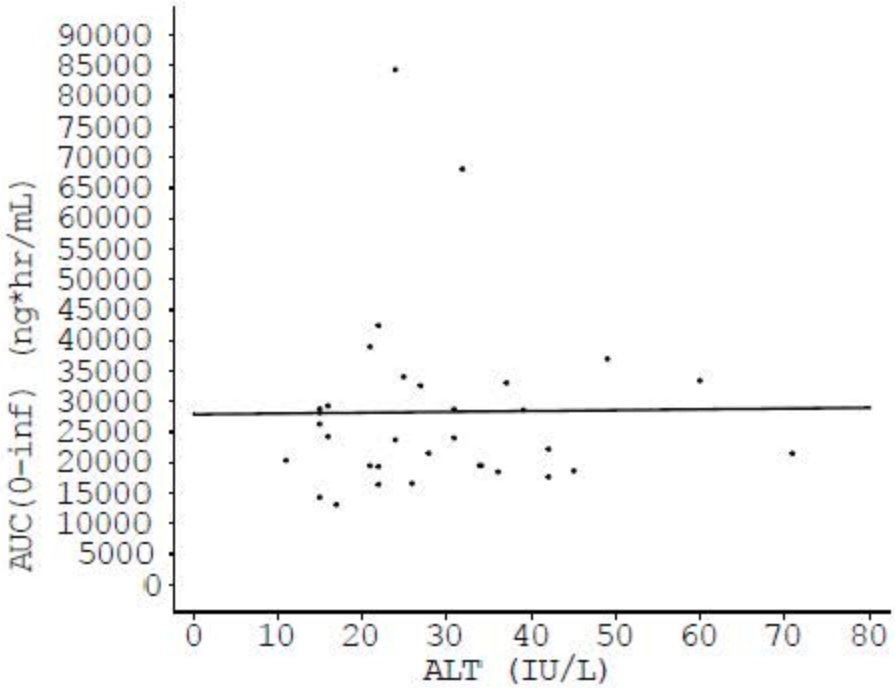
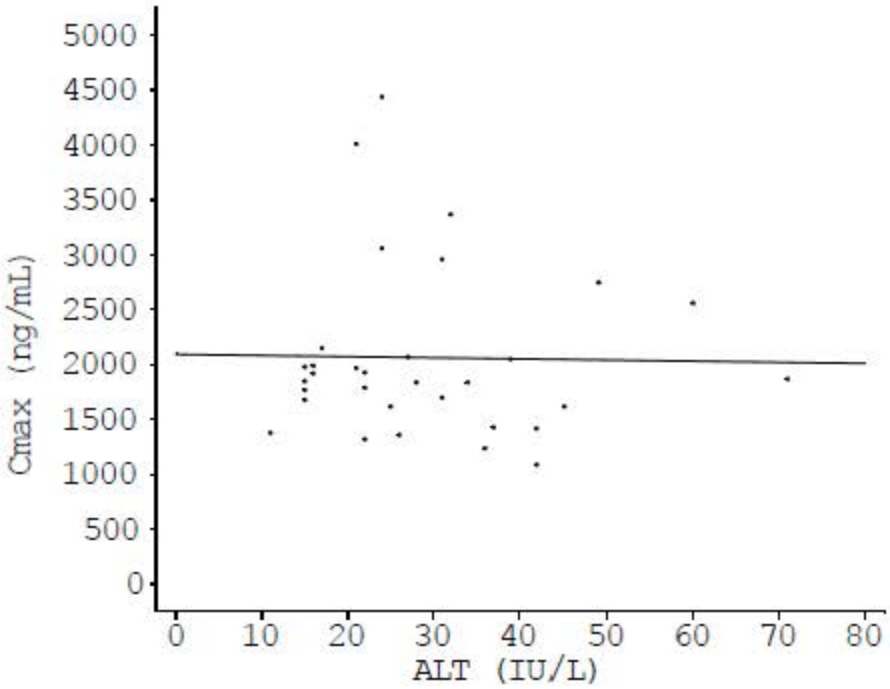
b)





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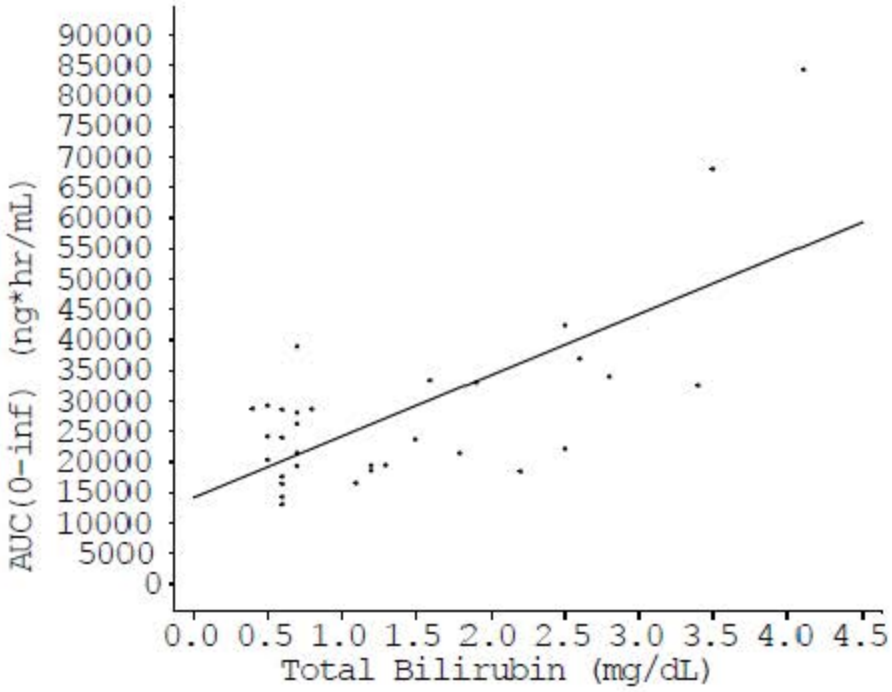
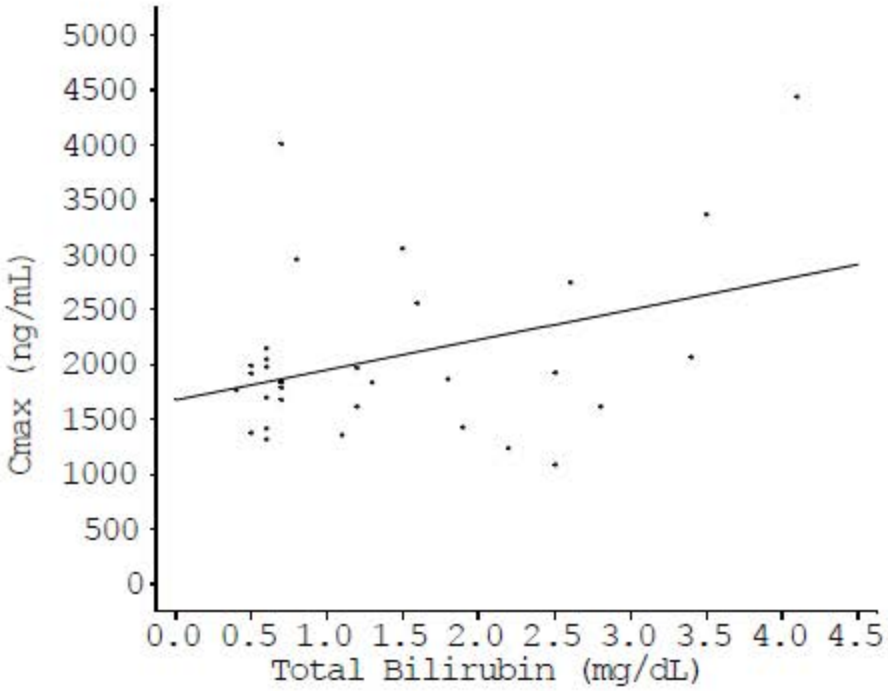
c)





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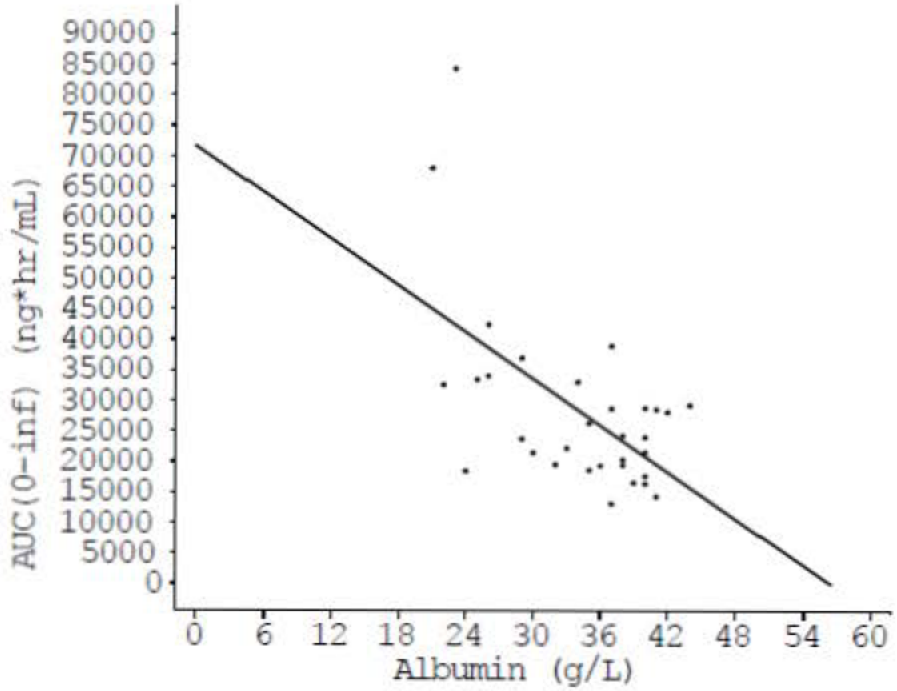
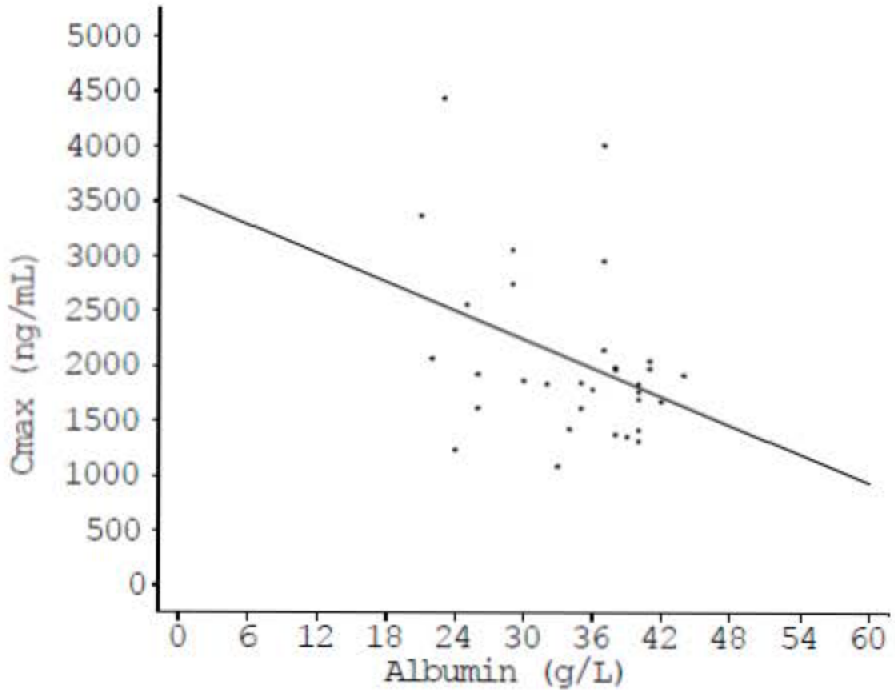
d)





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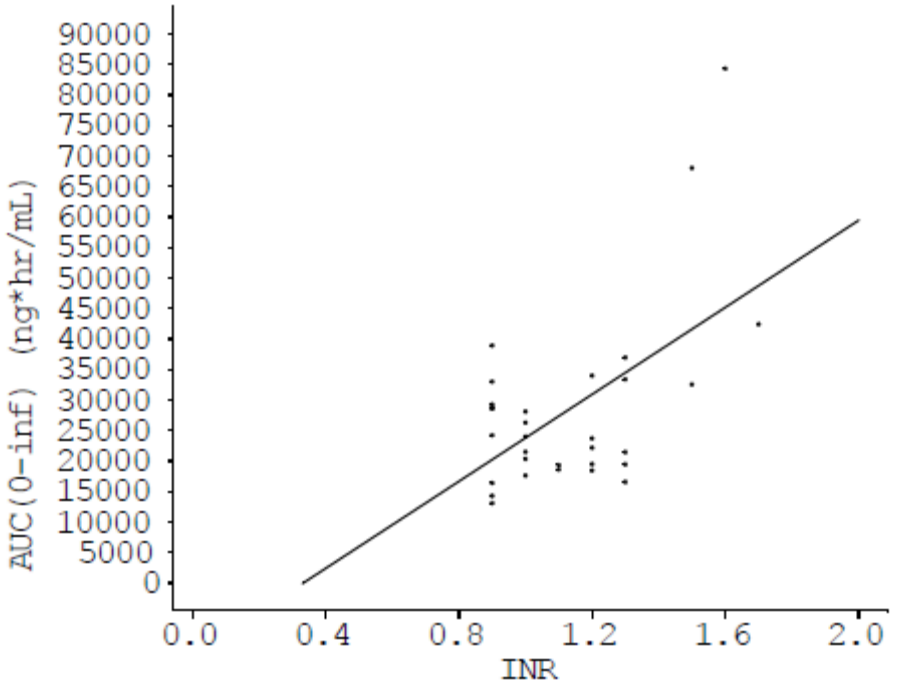
Correlation Analyses of Coagulation Function and PK Exposure
The relationship between coagulation function (INR) and TR-700 PK parameters AUC0-t, $AUC_{0-\infty}$, and C_{max} was explored using regression analyses, as presented in **Figure 3**.
The linear relationships between INR and AUC were statistically significant. However, given the high degree of variability in AUC, particularly at the upper range of INR, it is unlikely that the relationship is clinically meaningful.

Reviewer Comment: Given the small size and high degree of variability in AUC in the study, the correlation of coagulation function and TR-700 exposure may not be conclusive.

Figure 3. Relationship of Coagulation Function and TR-700 Exposure



Safety: TR-701 FA Tablet, 200 mg was well tolerated by subjects with hepatic impairment and matched controls. Most AEs were assessed by the Investigator as mild in severity, and no serious or severe AEs or deaths related to TR-701 FA were reported during the study. One subject experienced a serious adverse event (SAE) of epidural abscess that had an onset 14 days after receiving a single oral 200 mg dose of TR-701 FA and was considered unrelated to study drug. No clinically significant differences were observed across groups with regard to laboratory assessments, vital signs, physical examinations, or ECGs.

SPONSOR'S CONCLUSIONS
Oral TR-701 FA was well tolerated at the single 200 mg dose. The results of this study demonstrate the overall PK of TR-700 in subjects with moderate or severe hepatic impairment is similar following oral administration of a single 200 mg dose of TR-701 FA. In subjects with hepatic impairment, C_{max} and AUC were within the pre-specified 50–200% no effect boundaries. Therefore, no dose adjustment is warranted in subjects with moderate or severe hepatic impairment.

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REVIEWER ASSESSMENT:

1) The Sponsor's pre-specified 50–200% no effect boundary for C_{max} and AUC appears appropriate from a safety perspective based on the range of C_{max} and AUC values at doses that demonstrate safety in clinical trials.
2) Despite the 22 and 34% mean increase in AUC in patients with moderate and severe hepatic impairment compared to the respective controls, this AUC is within the range of exposure observed at doses that demonstrated safety in Phase 1 studies (tedizolid AUC values range from 17.36 to 123.10 µg*hr/ml at 100-1200 mg of single prodrug dose) and Phase 3 studies (mean tedizolid $AUC_{ss(0-24)}$ of 22 with a range of 6.57 to 49.86 µg*hr/ml at 200 mg QD prodrug dose).
3) Higher degree of variability in AUC was observed in patients with moderate and severe hepatic impairment compared to the respective controls (Table 3). The largest $AUC_{0-\infty}$ (84.4 µg•hr/ml) was observed in one patient (out of a total of 8 patients) in the severe hepatic impairment group. This larger variability in subjects with hepatic impairment could be in part due to more extensive medical histories and use of concomitant medications in this population. Given the modest inter-individual variability (<31%) based on the Population PK analysis, this large variability is not expected to be seen in the ABSSSI population.
4) Despite of the increase in, and large variability of, TR-700 exposure observed in patients with moderate/severe hepatic impairment, TR-701 FA Tablet, 200 mg, appeared to be well tolerated by subjects with hepatic impairment.
5) Results from the Phase 1 trial (TR701-101) showed that single oral doses of 200, 400, 600, 800, and 1200 mg TR-701 were generally well-tolerated in this group of healthy male and female subjects.
6) Results from the Phase 2 trial (TR701-104) showed that once daily oral doses of 200, 300, or 400 mg of TR-701 was safe and well tolerated, with no apparent dose-related toxicity.
7) Taken together, the Sponsor's conclusions are appropriate based on study results. No dose adjustment is necessary in subjects with moderate or severe hepatic impairment.

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4.2 Pharmacometric Review

Pharmacometric Review will be provided later as an addendum.

Reference ID: 3476543

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/s/

ZHIXIA YAN
03/24/2014

KIMBERLY L BERGMAN
03/24/2014

JEFFRY FLORIAN
03/24/2014

OFFICE OF CLINICAL PHARMACOLOGY: PHARMACOMETRIC REVIEW

NDA:	205,435 (oral) & 205, 436(intravenous [IV])
Drug	Tedizolid Phosphate
Trade Name	SIVEXTRO
PM Reviewer	Fang Li, Ph.D.
PM Team Leader	Jeffry A. Florian, Ph.D.
Clinical Pharmacology Review	Zhixia (Grace) Yan, Ph.D.
Clinical Pharmacology Team Leader	Kimberly Bergman, Pharm.D.
Sponsor	Trius Therapeutics, Inc., San Diego, CA
Submission Type; Code	Original New Drug Application (New Molecular Entity), Priority
Indication	For the treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by susceptible isolates of Gram-positive microorganisms.
Dosage and Administration	200 mg administered once daily for six (6) days either orally (with or without food) or as a 1-hour IV infusion in patients (b) (4)

1 SUMMARY OF FINDINGS

1.1 Key Review Questions

The purpose of this review is to address the following key questions.

1.1.1 Are there any covariates that significantly influence tedizolid PK parameters and exposure?

Yes, as shown in Figure 1, ideal body weight and total bilirubin are two factors identified to have significant effects on tedizolid clearance based on the population PK analysis. However, the impact of ideal body weight and total bilirubin on clearance were not predicted to be clinically significant. As such, no dose adjustments are recommended for tedizolid based on these two covariates. In addition, other demographic and clinical covariates, such as sex, race, ethnicity, age, weight, BMI, renal and hepatic function, were not found to influence tedizolid clearance significantly.

The sponsor conducted population PK analyses using data from four Phase 1 studies, one Phase 2 study, and two Phase 3 studies. A two-compartment model with sigmoidal absorption, and linear elimination was found to adequately describe the data. Individual

Reference ID: 3476316

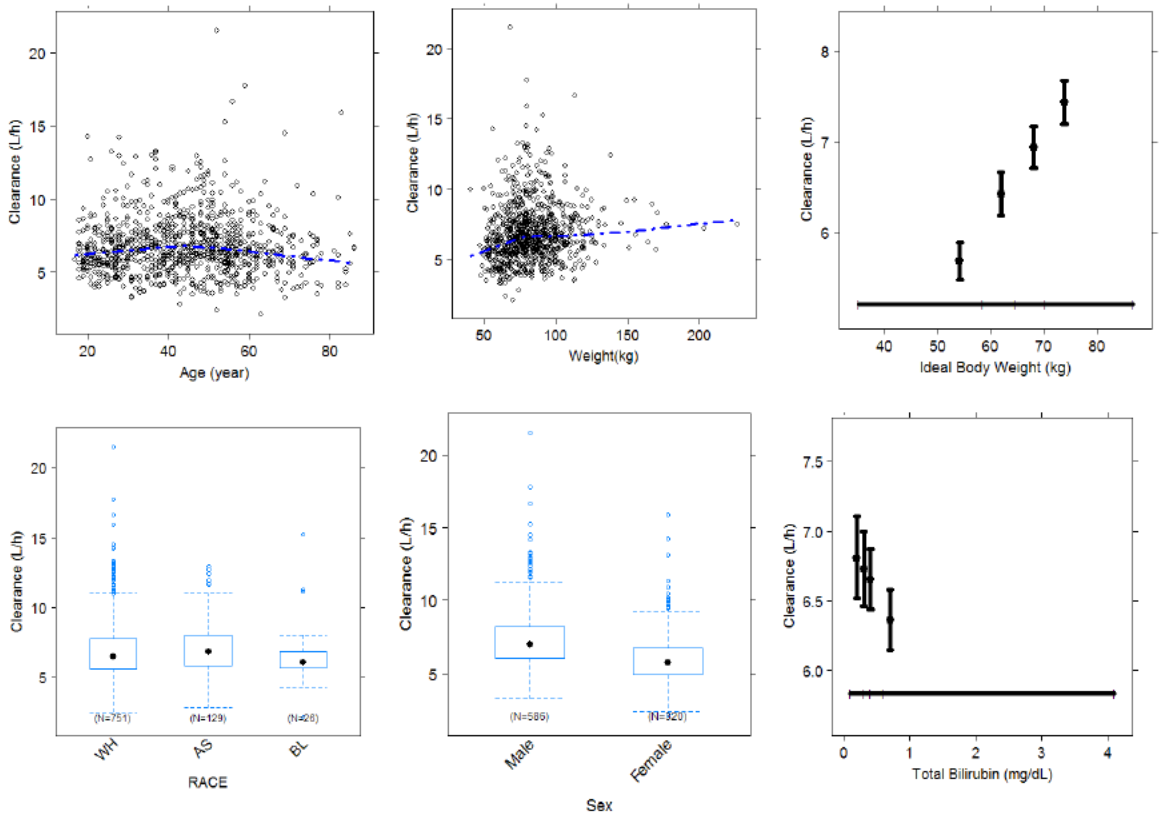
PK parameters were estimated with the sponsor's final population PK model. The relationship between tedizolid clearance, central volume of distribution and covariates of interest, such as age, body weight, BMI, ideal body weight, race, ALT, AST, total bilirubin, and creatinine clearance (CLcr) were explored. The ideal body weight was calculated using the following equations:

$$Males\ IBW(kg) = 51.65 + 1.85 \times \left(\frac{height(cm)}{2.54} - 60 \right) \quad (1)$$

$$Females\ IBW(kg) = 48.67 + 1.65 \times \left(\frac{height(cm)}{2.54} - 60 \right) \quad (2)$$

As shown in Figure 1, tedizolid clearance increased with increasing ideal body weight, but decreased with increasing total bilirubin.

Figure 1: PK parameter-covariates (age, body weight, ideal body weight, race, sex, and total bilirubin) relationships for tedizolid.



The effect of these covariates on tedizolid exposure was explored by the reviewer (see section 4.4.3). Model-predicted $AUC_{ss(0-24)}$ after 200 mg tedizolid was summarized in Table 6 for age, sex, and race and plotted against covariates of interest in Figure 10. Except for ideal body weight and total bilirubin, the values of $AUC_{ss(0-24)}$ were similar in patients with different age, sex, or race. Because exposure differences due to variations in ideal body weight and total bilirubin did not translate into differences in clinical efficacy, no dose adjustment is necessary based on these covariates.

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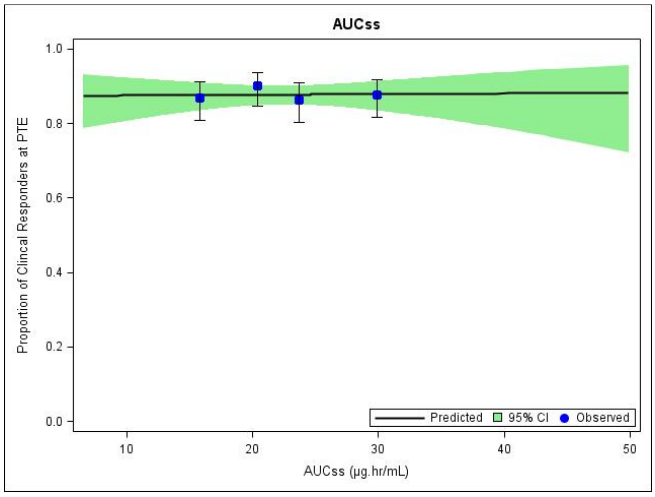
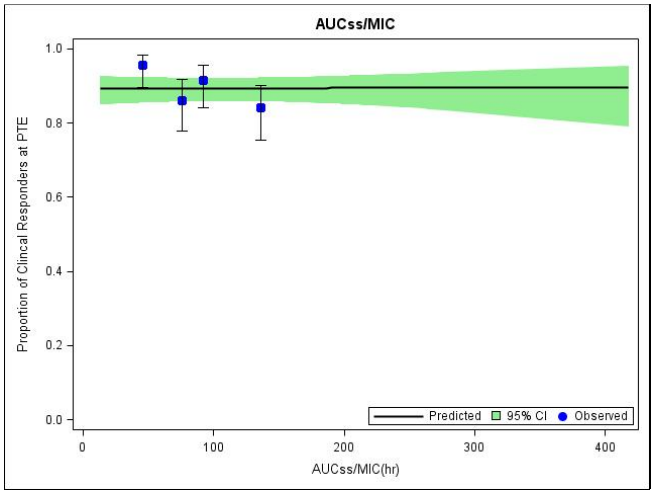
1.1.2 Is there any evidence of exposure-response relationship for efficacy?

Overall, PK/PD analyses of Phase 3 data showed a flat exposure-response relationship for a variety of efficacy endpoints such as clinical response at post treatment (PTE), early clinical response at 48-72 hour(with ≥20% decrease in lesion), and microbiological response at post treatment. This may be due to the limited range of exposures observed from the Phase 3 trials due to evaluation of a single fixed dose (200 mg q.d.). In addition, exposures achieved in Phase 3 trials may be on the plateau of the exposure-response curve as a Phase 2 trial evaluating daily doses of 200, 300, and 400 mg also did not demonstrate a dose-response relationship, though it should be noted this study was in patients with complicated skin and skin structure infections (cSSSI) rather than patients with ABSSSI.

Individual tedizolid exposures for patients in the Phase 3 trials were estimated based on the final population PK model (see Section 3). Estimated tedizolid exposures for each patient included AUC_{0-24}, AUC_{ss}, and C_{max}. Both minimum inhibitory concentrations (MIC), a measure of pathogen susceptibility to treatment, and ratio of exposure/susceptibility measures (AUC_{ss}/MIC, AUC_{0-24}/MIC) were calculated and evaluated as independent measures for predicting treatment outcome. Efficacy endpoints, such as early clinical response at 48-72 hour, clinical response at post treatment (PTE), microbiologic response at end of treatment (EOT), were evaluated as dependent response measures using logistic regression analyses. No evidence of a significant exposure-response relationship was identified for efficacy between these tedizolid exposures or exposure/susceptibility measures and the selected endpoints, as shown in **Figure 2**. The exposure-response relationships between the proportion of microbiologic responders at EOT and tedizolid AUC_{ss} or AUC_{ss}/MIC were similar (data now shown here).

Figure 2: Relationship between proportion of clinical responders at post treatment and tedizolid exposure (AUC_{ss} and AUCss/MIC). The blue points are observed mean proportion of responders in each quartile (95% CI) plotted at the mean value within each quartile. The solid black line and green shaded areas are model-predicted mean and 95% CI based on a univariate logistic regression analysis





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1.1.3 Is the selected dosing regimen appropriate?

The proposed dosing regimen for tedizolid for the treatment of ABSSSI is 200 mg once daily orally or as an intravenous infusion over 1 hour for 6 days. Intravenous to oral switch may be instituted at the discretion of the physician, and no dose adjustment is necessary when switching between the intravenous and oral formulation. The dosing regimen is supported by results from the pivotal Phase 3 trials and the above noted exposure-response analyses:

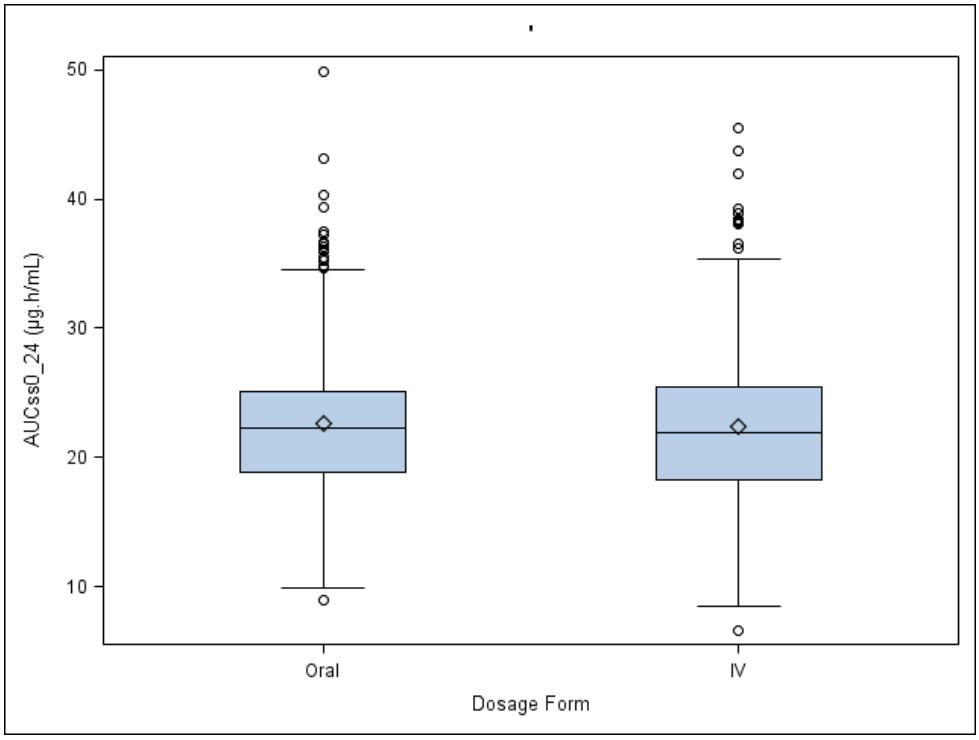
1) The 200 mg tedizolid dose showed comparable efficacy to linezolid 1200 mg in two pivotal Phase 3 studies (early clinical response rate of 79.4% for tedizolid versus 79.5% for linezolid in study TR701-112, and 85.2% for tedizolid versus 82.6 for linezolid in study TR701-113);
2) Higher tedizolid doses of 300 mg and 400 mg did not shown additional efficacy in Phase 2 study (Study 104) compared to 200 mg (89% with clinical cure for 200 mg compared to 89% and 86% for 300 and 400 mg, respectively; note, this was for cSSSI rather than ABSSSI);
3) Exposure-response analysis for efficacy based on the Phase 3 trials showed a flat relationship and higher exposure was not associated with a higher rate of clinical response;
4) Dose-response analyses for safety from Phase 2 showed an increase in gastrointestinal adverse events with increasing dose with higher doses associated with higher probability of adverse events.

Dose adjustment when switching from the IV-to-Oral formulation is not necessary. First, switching was prospectively studied in TR701-113 with 63% of patients received 1-2 days of intravenous treatment before switching to oral dosing and 18% of subjects receiving 6 days of intravenous treatment. No difference in response rates with respect to time prior to switching to oral treatment was observed in TR701-113.

In addition, the absolute bioavailability of tedizolid is 0.86 as estimated by the population PK analysis. Estimated steady-state exposure ($AUCss_{(0-24)}$) to tedizolid in patients in Phase 3 studies did not show significant difference among different dosage forms (Figure 3). A difference in C_{max} was observed between the intravenous and oral formulations (3.0 (0.7) µg/mL compared to 2.2 (0.6) µg/mL). However, this difference in C_{max} is not expected to influence efficacy as AUC_{ss}/MIC was identified as the most significant response parameter from nonclinical evaluation and study TR701-112 demonstrated non-inferiority between oral tedizolid and linezolid.

Reference ID: 3476316

Figure 3: Steady-State Exposure to Tedizolid in Phase 3 Studies by Dosage Form



1.1.4 Is the proposed criterion for interpretation of susceptibility of *S. aureus* derived by the sponsor acceptable?

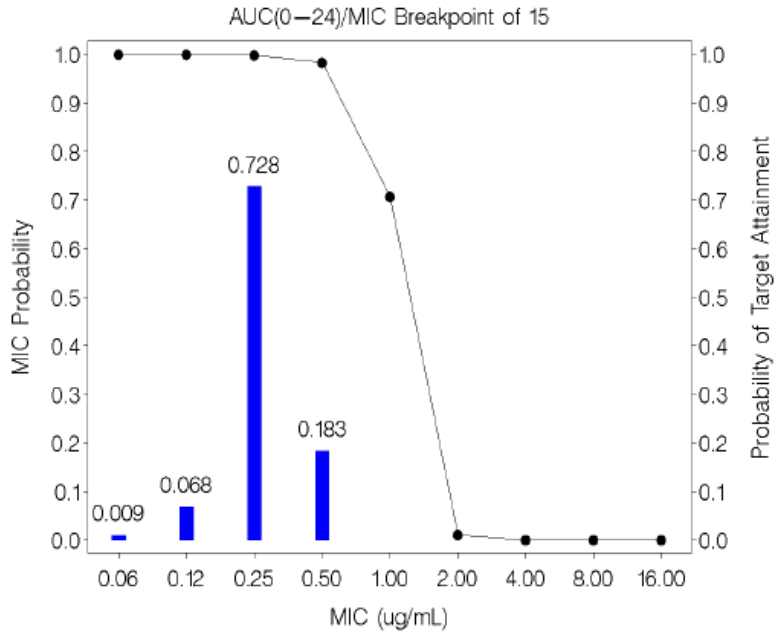
Yes, the proposed susceptibility breakpoint of at least 0.5 µg/mL tedizolid for *S. aureus* is acceptable. The tedizolid $AUC_{0\text{-}24}$/MIC ratio of 15 was determined as the PK/PD target associated with the efficacy of tedizolid against *S. aureus* in the non-neutropenic mouse thigh model of infection. Probability of target attainment analysis was performed to estimate the probability of attaining this PK/PD target measure to support the definition of MIC interpretive breakpoints for *S. aureus.* This probability of target of attainment analysis predicts that 98.3% of subjects achieve an $AUC_{0\text{-}24}$/MIC ratio greater than 15 for an MIC value of 0.5 ng/mL, with the prediction dropping to 70.7% for an MIC value of 1.0 ng/mL.

Based on the final population PK model and the distributions of the demographic covariates determined to be significant predictors of tedizolid PK (total bilirubin and ideal body weight) of acute bacteria skin and skin structure infections (ABSSSI) patients enrolled in Phase 3 studies (TR701-112 and TR701-113), individual estimates of $AUC_{0\text{-}24}$ on Day 1 and Day 6 (at steady state) were simulated by Monte Carlo simulation for a total of 100 trials of 1000 virtual patients each (100,000 patients total). MIC values between 0.06 and 16 µg/mL were considered to encompass a broad range of susceptibility for the target attainment analysis. The distribution of the percentage of patients with AUC/MIC observations above the target value of 15 for each MIC value was examined. The mean percentage of patients with simulated $AUC_{0\text{-}24}$/MIC ratios above the target, across all 100 datasets, was reported as the PK/PD target attainment at each MIC value. The MIC susceptibility breakpoint was determined to be the highest

clinically relevant MIC value with a probability of PK/PD target attainment of at least 90% (i.e., 90% of subjects exceeding an AUC_{0-24}/MIC ratio of 15).

The estimated mean target attainment for the AUC_{0-24}/MIC ratio of 15 was 100%, 100%, 99.8%, 98.3%, and 70.7% for MIC values of 0.06, 0.12, 0.25, 0.5, and 1 µg/mL, respectively (**Figure 4**). The simulated probabilities of target attainment at an MIC value of ≥2 µg/mL approached zero and are not shown. Based on this analysis, an MIC value of 0.5 µg/mL was associated with at least a 90% probability of target attainment. Also, in the ABSSSI clinical studies 82% of patients with an MIC less than 0.5 µg/mL had an early clinical response, defined as a ≥20% decrease in lesion area at the 48-72 hour visit. In contrast, 87% of patients with an MIC greater than or equal to 0.5 µg/mL (almost all patients in this group had an MIC equal to 0.5 µg/mL) had an early clinical response. Based on these considerations, a susceptibility breakpoint of 0.5 µg/mL for *S. aureus* for tedizolid is proposed by the sponsor and appears to be supported by the data. However, it should be noted that the determination of breakpoints involves multiple disciplines providing clinical and microbial interpretations in addition to the above PK-PD observations. The ultimate determination of the tedizolid breakpoint should depend on the totality of information provided by each discipline and continues to be assessed as of the completion of this review.

Figure 4: Probability of PK/PD Target Attainment for Tedizolid against *Staphylococcus aureus* at the AUC_{0-24}/MIC Ratio Breakpoint of 15



Source: Adapted from the Sponsor's report CLN-13-0701-102, Figures 32.

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1.2 Recommendations

The Division of Pharmacometrics (Office of Clinical Pharmacology) has reviewed this application from a clinical pharmacology perspective and recommends approval of 200 mg tedizolid as an intravenous or oral administration over 6 days. In addition, the information provided by the sponsor supports a transition from intravenous to oral administration without a tedizolid dose adjustment. Finally, the reviewer agrees with the sponsor's conclusions from the population PK analyses and exposure-response analysis that no dose adjustment are necessary for tedizolid based on the evaluated covariates (body weight, age, race, ideal body weight, total bilirubin, and race).

1.3 Label Statements

None

2 PERTINENT REGULATORY BACKGROUND

On October 18, Trius Therapeutics (San Diego, CA) submitted two NDA applications to U.S. FDA to seek approval of tedizolid phosphate for the treatment of acute bacterial skin and skin structure infections (ABSSSI). One submission was for tedizolid phosphate oral tablets (NDA205435), and the other was for tedizolid phosphate intravenous solution (NDA205436).

Tedizolid phosphate (TR-701 FA) is a prodrug antibiotic that is rapidly converted *in vivo* by phosphatases to TR-700 (tedizolid), a moiety that has been shown to be microbiologically active against a variety of primarily gram-positive pathogens.

Tedizolid is a member of the oxazolidinone class of antibacterial agents, which are protein synthesis inhibitors that interact with the 50S subunit of the bacterial ribosome, thereby preventing the initiation of translation by inhibiting formation of the initiation complex. The only approved drug in this class of drug is linezolid. The proposed dosing regimen for tedizolid is 200 mg once daily for 6 days.

Prior to initiation of the TR-701 FA Phase 3 program, most *in vivo* nonclinical and initial clinical studies were conducted using an earlier solid form of the prodrug designated as tedizolid phosphate disodium salt (TR-701). TR-701 FA is a free acid prodrug (b) (4)

The two solid forms of the prodrug were found to result in similar TR-700 (tedizolid) pharmacokinetics (PK) in a relative bioavailability study (Study TR701-108) and in a comparison across studies). Therefore, PK results from TR-700 following oral administration of the two prodrug forms are integrated.

Two pivotal phase 3 studies were conducted in support of this submission. The first Phase 3 study (TR701-112) was a non-inferiority, global, multicenter, and randomized, double-blind study to evaluate the efficacy and safety of oral TR-701 FA 200 mg once daily for 6 days versus oral linezolid 600 mg every 12 hours for 10 days for the treatment of ABSSSI in adult patients. The sponsor concluded that TR701-FA was non-inferior to

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linezolid for the primary efficacy analysis of early clinical response (79.5% for TR-701 FA versus 79.4% for linezolid).

The second Phase 3 study (TR701-113) was a non-inferiority, global, multicenter, randomized, double-blind study to compare the efficacy and safety of IV to oral 200 mg TR-701 FA administered once daily for 6 days or IV to oral 600 mg linezolid administered twice daily for 10 days for the treatment of ABSSSI. TR-701 FA was also found to be non-inferior to linezolid for the primary efficacy analysis of early clinical response (85.2% for TR-701 FA and 82.6% for linezolid).

3 RESULTS OF SPONSOR'S ANALYSIS

The pharmacometric analyses in the sponsor's application package include population PK analyses and exposure-response analysis for efficacy and safety. The results of the analysis are summarized below.

3.1 Population PK Analysis

Objectives: The objective of the population PK analysis was to develop a model to characterize the PK of TR-700 and the effect of subject characteristics on the PK variability of TR-700. The final model was used to estimate individual TR-700 exposure in Phase 2/3 studies for further assessment of exposure-response relationships for safety and efficacy of TR-700.

Data: The population PK dataset was pooled from four Phase 1 studies (Studies TR701-107, TR701-109, TR701-123, and TR701-124) in healthy subjects and sparsely sampled data from one Phase 2 study (Study TR701-104) in subjects with cSSSI and two Phase 3 studies (Study TR701-112 and TR701-113) in subjects with ABSSSI. A total of 968 subjects were included in the final dataset, among which, 631 subjects (65.2%) were male and 337 subjects (34.8%) were female. The datasets consisted of 82.9% white, 14.3% black, and 2.9% categorized as "other". The summary of subject characteristics and laboratory values is demonstrated in **Table 1**:

Table 1: Summary of Subject Characteristics of the Final Population Dataset

Subject Characteristic		Study							Overall
		107	109	123	124	104	112	113	
Age (years)	Mean (SD)	28.57 (8.39)	52.79 (20.37)	56.38 (9.34)	54.44 (6.98)	36.21 (12.15)	43.71 (14.99)	46.01 (15.76)	43.09 (15.50)
	Median	26.00	55.50	59.00	53.50	35.00	43.50	46.00	43.00
	Min, Max	18.0, 48.0	25.0, 78.0	40.0, 74.0	39.0, 67.0	18.0, 68.0	18.0, 86.0	17.0, 86.0	17.0, 86.0
	n	61	28	24	32	175	328	320	968
Weight (kg)	Mean (SD)	76.33 (12.41)	79.21 (14.32)	83.75 (19.05)	94.10 (19.91)	81.62 (14.49)	82.34 (18.31)	84.04 (24.41)	82.73 (19.81)
	Median	76.10	79.70	78.80	97.25	80.00	80.30	79.75	80.00
	Min, Max	48.9, 110.7	58.5, 111.8	49.7, 125.3	58.6, 150.9	47.0, 118.0	47.7, 138.3	40.5, 226.4	40.5, 226.4
	n	61	28	24	32	175	328	320	968
Ideal body weight (kg)	Mean (SD)	65.97 (7.52)	61.71 (8.37)	63.72 (9.98)	66.24 (8.49)	65.70 (7.85)	64.09 (7.93)	64.59 (8.27)	64.66 (8.12)
	Median	66.70	61.25	64.80	68.20	66.40	64.50	65.20	65.15
	Min, Max	49.3, 81.3	49.2, 75.1	46.5, 81.2	48.7, 86.3	47.0, 86.8	35.1, 84.9	40.6, 85.6	35.1, 86.8
	n	61	28	24	32	175	328	320	968
Body mass index (kg/m^2)	Mean (SD)	25.22 (2.90)	27.65 (3.26)	28.50 (4.73)	31.01 (4.90)	27.01 (4.37)	27.88 (5.34)	28.46 (7.85)	27.86 (6.05)
	Median	25.50	27.45	27.70	31.45	26.70	27.43	26.88	27.04
	Min, Max	19.8, 31.5	22.0, 33.6	21.0, 39.6	21.0, 38.9	18.5, 36.0	16.0, 40.0	15.6, 69.9	15.6, 69.9
	n	61	28	24	32	175	328	320	968

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Total bilirubin (mg/dL)	Mean (SD)	0.56 (0.25)	0.60 (0.26)	0.63 (0.30)	1.41 (1.03)	0.42 (0.27)	0.45 (0.26)	0.51 (0.33)	0.51 (0.38)
	Median	0.50	0.50	0.60	0.95	0.40	0.40	0.40	0.40
	Min, Max	0.2, 1.3	0.3, 1.3	0.2, 1.5	0.4, 4.1	0.1, 1.7	0.1, 2.0	0.1, 2.0	0.1, 4.1
	n	61	28	24	32	175	328	320	968
Indirect bilirubin (mg/dL)	Mean (SD)	NA (NA)	0.48 (0.21)	0.46 (0.26)	0.94 (0.58)	0.29 (0.22)	0.29 (0.19)	0.31 (0.23)	0.33 (0.26)
	Median	NA	0.40	0.40	0.65	0.30	0.30	0.30	0.30
	Min, Max	NA,NA	0.2, 1.1	0.1, 1.2	0.3, 2.2	0.0, 1.4	0.0, 1.4	0.0, 1.4	0.0, 2.2
	n	0	28	24	32	175	328	320	907

Source: Adapted from the Sponsor's report CLN-13-0701-072, Table 14

Population PK Model

Base Model:

The selected base model was a 2-compartment model with sigmoidal absorption and first-order elimination. An exponential model was used to describe the inter-individual variation (IIV) in ka, CL, and V_c . Two separate log error models were used for the residual error following IV and oral administration, respectively. The population mean parameter estimates for the base PK model are summarized in **Table 2**. The corresponding goodness-of-fit plots are shown in **Figure 5.**

Table 2: Parameter Estimates and Standard Errors from the Base Structural Pharmacokinetic Model for TR-700

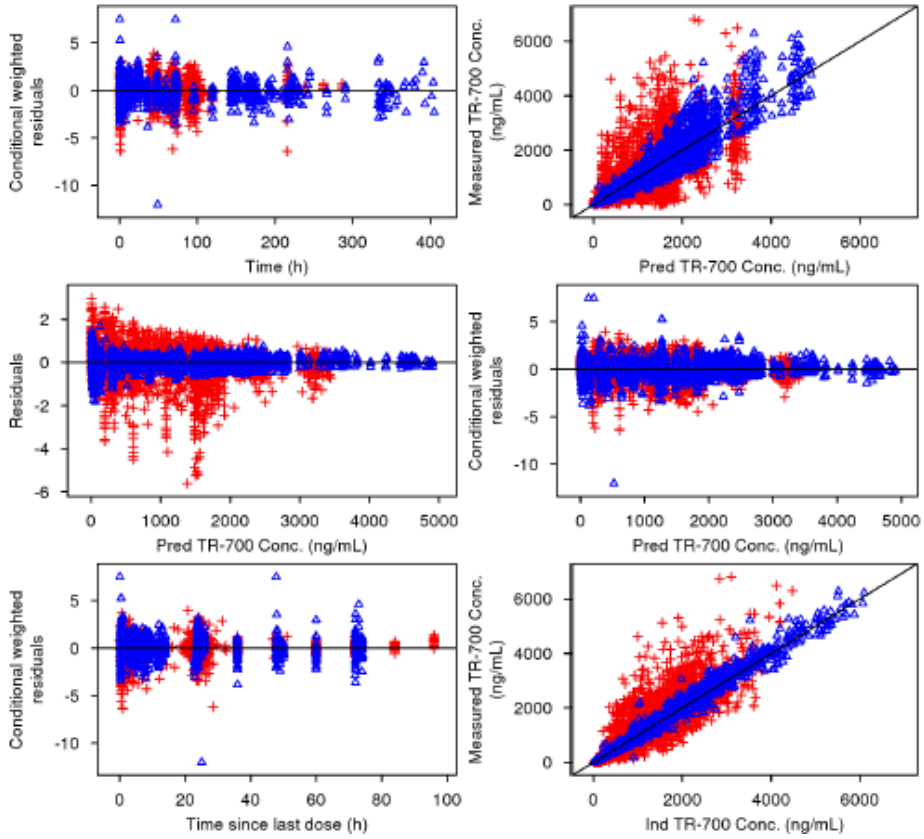
	Final Parameter Estimate		Interindividual Variability / Residual Variability	
Parameter	**Typical Value**	**%SEM**	**Magnitude**	**%SEM**
k_a: First-order absorption rate constant (1/h)	1.86	10.6	169 %CV	12.6
CL: Clearance (L/h)	5.72	2.70	29.1 %CV	9.05
V_c: Central volume (L)	63.1	2.54	21.4 %CV	10.3
Q: Intercompartmental CL (L/h)	0.961	7.52	NE	NE
V_p: Peripheral volume (L)	13.2	5.68	NE	NE
F1: Absolute bioavailability	0.758	3.20	NE	NE
D1: Duration of zero order absorption (h)	1.71	0.184	68.9 %CV	8.08
RV for IV data (log units)	0.0178	15.7	0.134 SD	NA
RV for oral data (log units)	0.223	6.70	0.473 SD	NA
Minimum value of the objective function = -4463.956				

Abbreviations: IV, intravenous; NA, Not Available; NE, Not Estimated; RV, residual variability; %SEM, standard error of the mean expressed as a percentage; SD, standard deviation.

Source: the Sponsor's report CLN-13-0701-072, Table 7

Reference ID: 3476316

Figure 5: Goodness-of-fit Plots for the Base Structural PK Model



Source: Adapted from the Sponsor's report CLN-13-0701-072, Figures 9 (the blue triangles are for intravenous administration while the red pluses are for oral administration)

Final Model

Full population PK model was constructed via forward inclusion of covariates of interest and then reduction step by removing covariates to check MVOF. The resulted model was then refined to the final model. The parameter estimates of the final model were summarized as shown in the **Table 3**. The magnitude of the intersubject variability was large for ka (194% CV), but was modest for CL (31.0% CV) and V_c (13.4% CV). The calculated η-shrinkage values were 35.5% for ka, 16.6% for CL, and 66.9% for Vc. The model was evaluated by goodness-of-fit plots and vpc (**Figure 6 and Figure 7).**

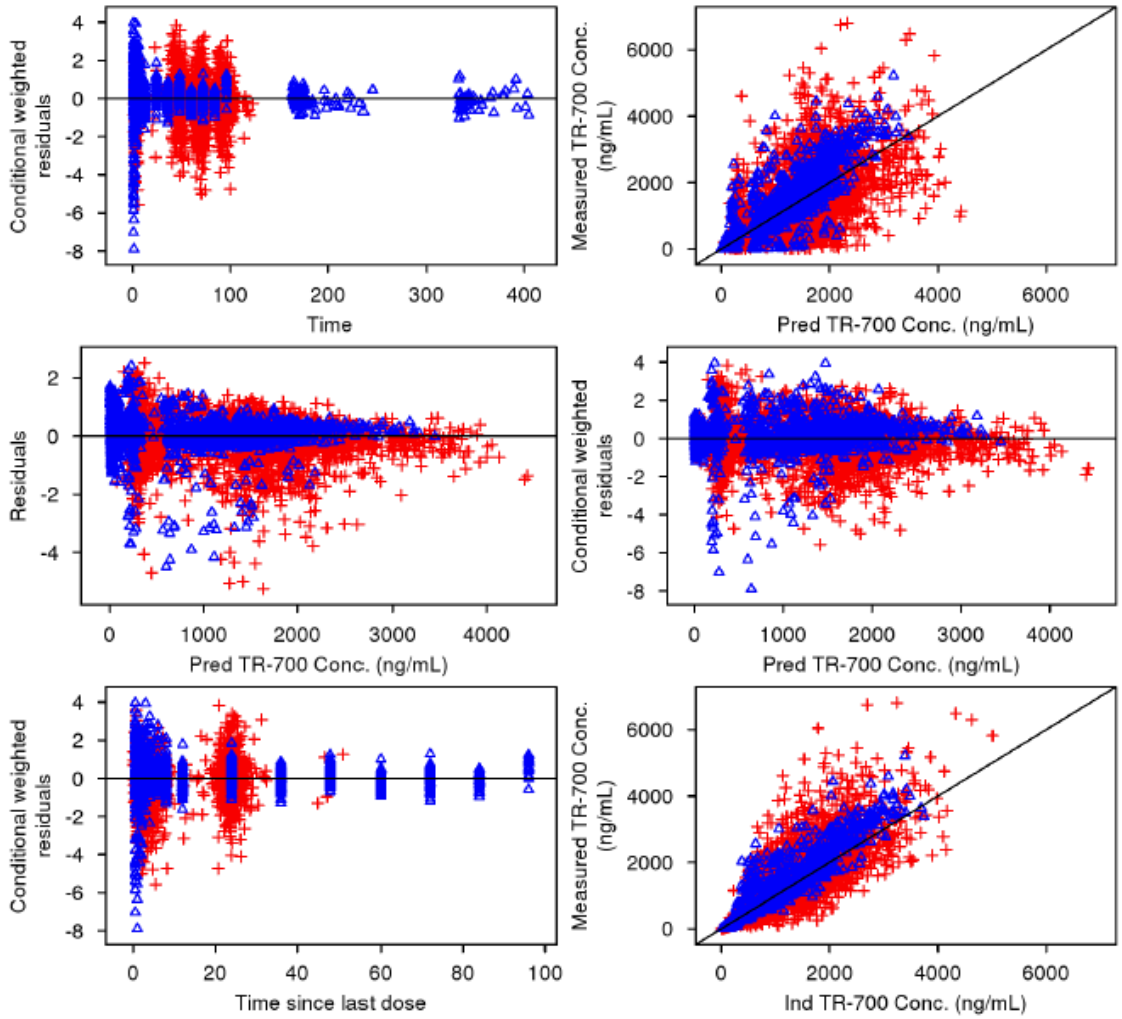
Table 3: Parameter Estimates and Standard Errors from the Final PK Model for TR-700

Parameter	Final Parameter Estimate		Interindividual Variability / Residual Variability	
	Typical Value	%SEM	Magnitude	%SEM
k_a: First-order absorption rate constant (1/h)	1.99	13.0	194 %CV	11.0
CL: Clearance (L/h)	6.69	2.28	31.0 %CV	8.70
Power term for the effect of IBW on CL	0.811	11.9		
Slope for the effect of total bilirubin on CL	-0.851	13.1		
V_c: Central volume (L)	69.0	2.58	13.4 %CV	33.1
Power term for the effect of IBW on Vc	1.32	8.92		
Q: Intercompartmental CL (L/h)	0.959	10.3	NE	NE
V_p: Peripheral volume (L)	13.6	6.30		
F1: Absolute bioavailability	0.859	2.47		
DUR: Duration of zero order absorption (h)	1.62	8.90		
RV for Phase 1 studies (log units)	0.148	17.1	0.384 SD	NA
RV for Phase 2/3 studies (log units)	0.210	5.37	0.458 SD	NA
Minimum value of the objective function = -754.773				

Abbreviations: IBW, ideal body weight; NA: Not Available; NE: Not Estimated; RV, residual variability; %SEM, standard error of the mean expressed as a percentage.

Source: the Sponsor's report CLN-13-0701-072, Table 16

Figure 6: Goodness-of-Fit Plots for the Final PK Model of TR-700



Source: Adapted from the Sponsor's report CLN-13-0701-072, Figures 17 (the blue triangles are for observations in Phase 1 studies while the red plus signs are for observations in Phase 2/3 studies)

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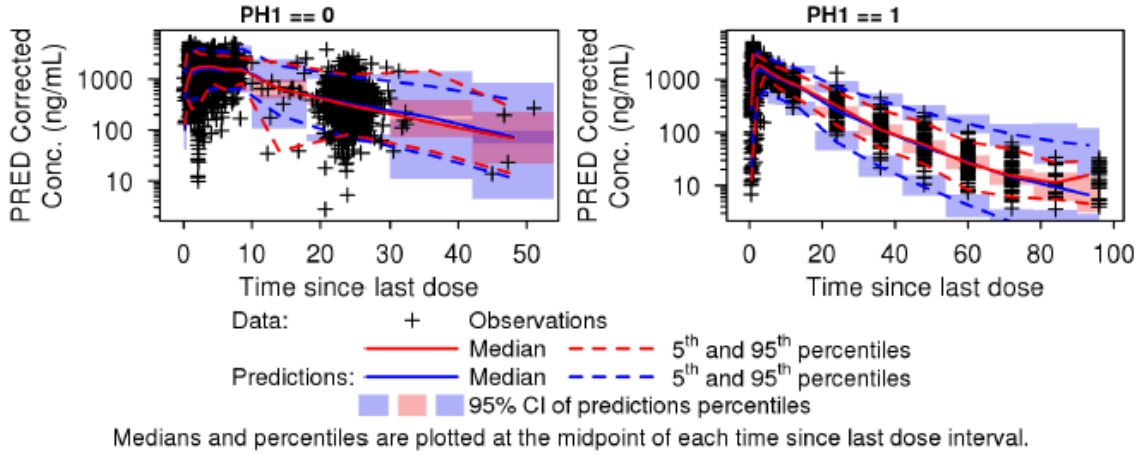
Figure 7: Median and 90% Prediction Intervals of Simulated Data From the Prediction-Corrected Visual Predictive Check of the Final Population PK Model Overlaid on the Median (5th and 95th Percentiles) of Observed TR-700 Data



PH1= = 0: Phase 2/3 studies,; PH1==1: Phase 1 studies.

Source: Adapted from the Sponsor's report CLN-13-0701-072, Figures 18

Reviewer's Comment:

- *The final population PK dataset in Phase 3 did not include subjects less than 17 years old. The PK profile of TR-700 in pediatrics can not be characterized in the population PK analysis*

- *The population PK analysis seems adequate in characterizing the PK profile of tedizolid after IV and Oral administration of TR701 FA. The final model reasonably described the observed data. The estimated PK parameters, such as CL/F and Vc/F, are reasonable and consistent with noncompartmental analyses. The sponsor's analyses were verified by the reviewer, with no discordance identified.*

- *Inter-subject variability, expressed as percent coefficient of variation (%CV), was modest for tedizolid clearance (CL, 31.0% CV) and volume of distribution of the central compartment (V_c, 13.4% CV). The variability of CL and V_c is similar among healthy volunteers and patients (CL: 28.2% CV and 30.0% CV, respectively; V_c: 22.2 CV% vs. 16.4% CV, respectively). Ideal body weight and total bilirubin are two covariates that contributed significantly to the variability of CL; ideal body weight also contributed significantly to the variability of V_c. Tedizolid CL increases with ideal body weight according to a power function, but decreases in proportion to total bilirubin. Tedizolid V_c increases with increasing ideal body weight.*

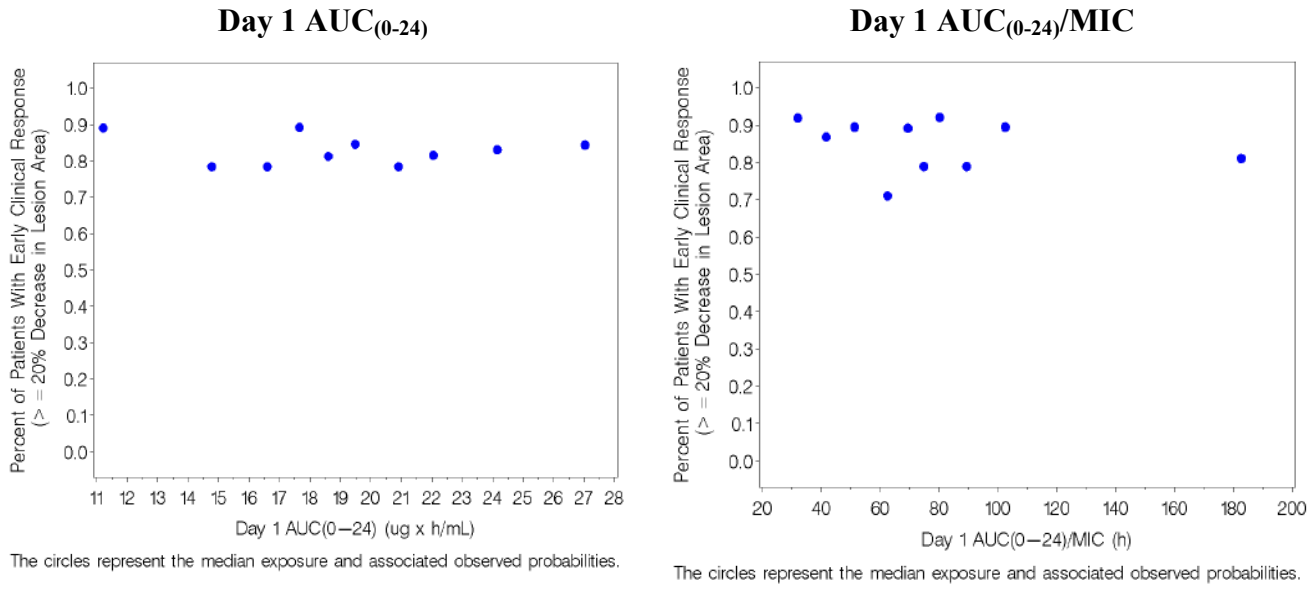
- *While differences in tedizolid clearance resulted in variations in exposure to tedizolid, significant differences in clinical efficacy, evaluated as a variety of*

efficacy endpoints such as early clinical response at 48-72hr, clinical response at post treatment, were not identified. Therefore, dose-adjustment is not recommended based on the identified factors that influence tedizolid clearance (i.e., ideal body weight and total bilirubin).

3.2 Exposure-response analysis for efficacy

Relationships between model-estimated steady-state AUC and proportion of clinical responders using various clinical efficacy end points (Clinical response at post-treatment, early clinical response with ≥20% decrease in lesion) were explored by the sponsor. Higher exposure to tedizolid at the 200 mg dose was not associated with higher efficacy (**Figure 8**). In addition, no relationship was observed between AUC/MIC, a ratio of exposure and susceptibility, and efficacy from the Phase 3 data.

Figure 8: Percent of Patients with Early Clinical Response at 48-72 Hours (≥20% Decrease in Lesion Area) or Clinical Response at Post Treatment Versus Exposure Measurements (AUC_{0-24} and AUC_{0-24}/MIC)



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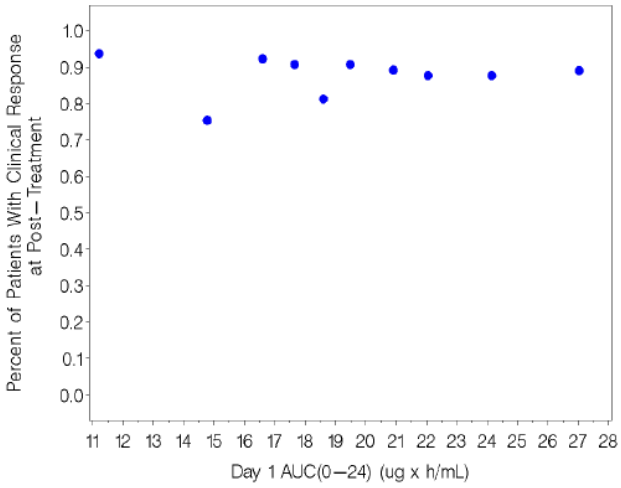
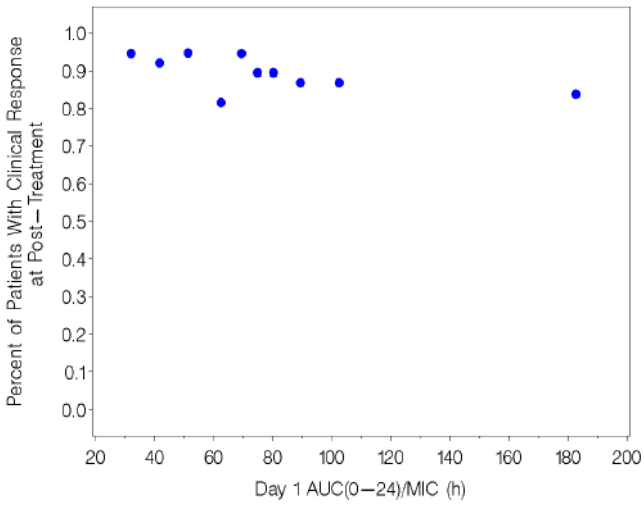


The circles represent the median exposure and associated observed probabilities.



The circles represent the median exposure and associated observed probabilities.

Source: Adapted from the Sponsor's report CLN-13-0701-102, Figures 4 and 8.

- *PK/PD analyses of Phase 3 data showed a similar percentage of patients with a clinical response at PTE across the range of tedizolid therapeutic exposures (as measured by $AUC_{ss(0-24)}$: median of 22.5 with a range of 6.57 to 49.86 µg*hr/ml) In addition, no trend was observed between $AUC_{ss(0-24)}$/MIC and efficacy parameters, either. These observations were further evaluated in the reviewer's analysis in Section 1 and Section 4.*

4 REVIEWER'S ANALYSIS

4.1 Introduction

The Division of Pharmacometrics has reviewed the data and reports submitted in the population PK study (CLN-13-0701-072). The pharmacometrics reviewer further explored the population PK analysis and the exposure-response relationship for efficacy and safety to determine whether the proposed dosing regimen is appropriate.

4.2 Objectives

The reviewer conducted independent analyses with objectives of:

1) evaluating the adequacy of the sponsor's population PK model
2) evaluating the effect of covariates of interest on tedizolid exposure
3) evaluating the exposure-response relationship for efficacy and safety to determine whether the proposed dosing regimen is appropriate.

4.3 Methods

4.3.1 Data Sets

Data sets used are summarized in 4.

Reference ID: 3476316

Table 4: Analysis Data Sets

Study Number	Name	Link to EDR
alpk-flagoutl-2-e1gt6-nmdat.txt	popPK final dataset	\\Cdsesub1\evsprod\NDA205435\0000\m5\datasets\pk\analysis\legacy\programs
adae.xpt adsl.xpt	Integrated summary of safety	\\Cdsesub1\evsprod\NDA205435\0000\m5\datasets\iss\analysis\adam
effcmf.xpt	Exposure and efficacy	\\Cdsesub1\evsprod\NDA205435\0000\m5\datasets\pk-pd-ef\analysis\legacy\datasets

4.3.2 Software

NONMEM (Version 7.2) installed on a 48-core Linux cluster was used for the population PK analysis. An R package "popPK" developed by FDA was used for population PK graphing and reporting; SAS for windows 9.3 was used for all other graphing and statistical analyses.

4.3.3 Models

The sponsor's final population PK datasets and final models were used for testing the adequacy of the submitted final model and evaluation of parameter estimates.

4.4 Results

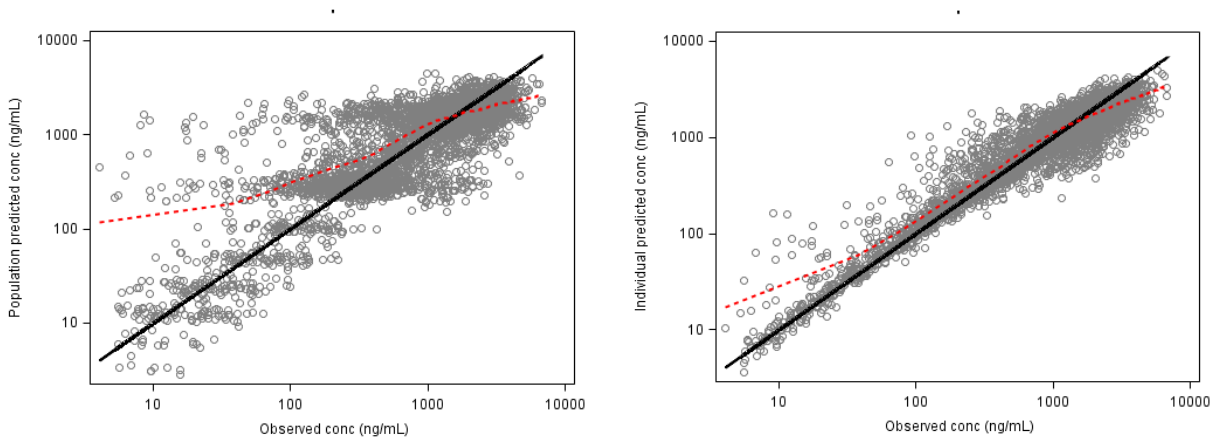
4.4.1 Population PK Analysis

The final model was evaluated by assessing the goodness-of-fit plots as shown in **Figure 9**. The predictions for observations in Phase 1 studies (Study 109, 123, 124) appear better than those in Phase 2/3 studies (Study 104, 112, 113).

4.4.2 Goodness-of-fit plots for the final model

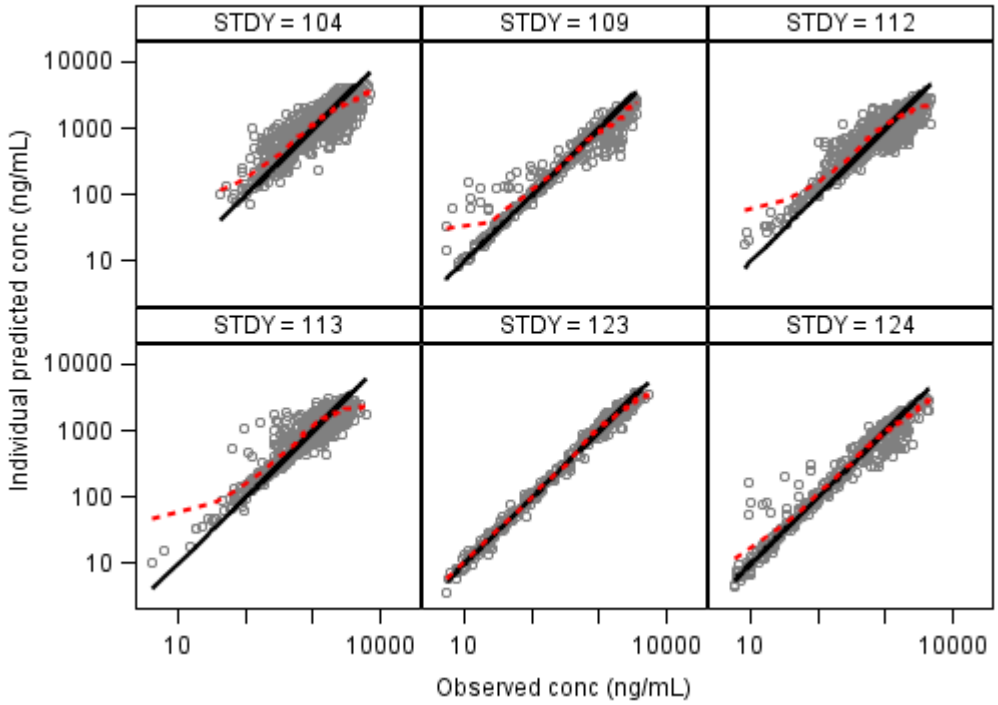
Figure 9: Predicted versus Observed Plasma Tedizolid concentrations (the black lines are line of identity and the dashed red lines are smooth lines)

Population Predicted **Individual Predicted**



Reference ID: 3476316

Individual Predicted Concentrations versus Observed Plasma Concentrations by Study



4.4.3 Parameter Estimates

The reviewer's population PK parameter estimates for the final PK model are shown in **Table 5**, which were similar to those from the sponsor. Summary of post-hoc estimates of tedizolid individual clearance and $AUC_{0\text{-}24}$ after 200 mg IV dose is shown in **Table 6** and **Figure 10**. There was no significant difference in exposure among different race, sex or age groups.

Table 5: Reviewer's final PK model parameter estimates.

Parameter	Estimate	RSE(%)	CI95
Fixed-Effects Parameter Estimates			
Ka: First order absorption rate	1.99	13.6	(1.46-2.52)
CL (Clearance (L/hr))	6.69	2.3	(6.39-6.99)
V2 (Central volume of distribution (L))	68.97	2.6	(65.51-72.43)
Q (Inter-compartmental clearance (L/hr))	0.96	10.0	(0.77-1.15)
F1 (Relative bioavailability)	0.86	2.5	(0.82-0.90)
CL-IBW (Power of IBW on CL)	0.81	11.9	(0.62-1.00)
V2-IBW (Power of IBW on Central Volume	1.32	9.0	(1.09-1.56)

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Slope of Total Bilirubin	-0.85	12.9	(-1.07-0.64)
Inter-Individual Variability Estimates (omega^2)			
Omega(Ka)	3.75	11.2	(2.93-4.56)
Omega(CL)	0.096	8.8	(0.079-0.112)
Omega(V2)	0.018	32.0	(0.0067-0.0290)
Shrinkage			
Omega(Ka)	35.5%		
Omega(CL)	16.6%		
Omega(V2)	67.0%		

Table 6: Summary of individual empirical Bayes' estimates

Parameters		N	Mean	SD	Lower 95%	Upper 95%
CL/F (L/h)	White	751	6.84	2.1	6.69	6.99
	Black	129	7.14	1.86	6.82	7.46
	Other	26	6.69	2.50	5.68	7.70
	Male	586	7.34	2.10	7.17	7.51
	Female	320	6.02	1.73	5.83	6.22
	< 65 years	812	6.93	2.08	6.79	7.07
	≥ 65 years	94	6.41	2.00	6.00	6.82
$AUCs_{s(0-24)}$ (μg.h/mL)	White	751	31.7	8.99	31.1	32.4
	Black	129	29.9	7.79	28.5	31.2
	Other	26	33.7	14.1	28.0	39.4
	Male	586	29.2	7.69	28.6	29.86
	Female	320	35.7	9.81	34.6	36.77
	< 65 years	812	31.3	9.01	30.6	31.9
	≥ 65 years	94	33.7	9.02	31.8	35.5

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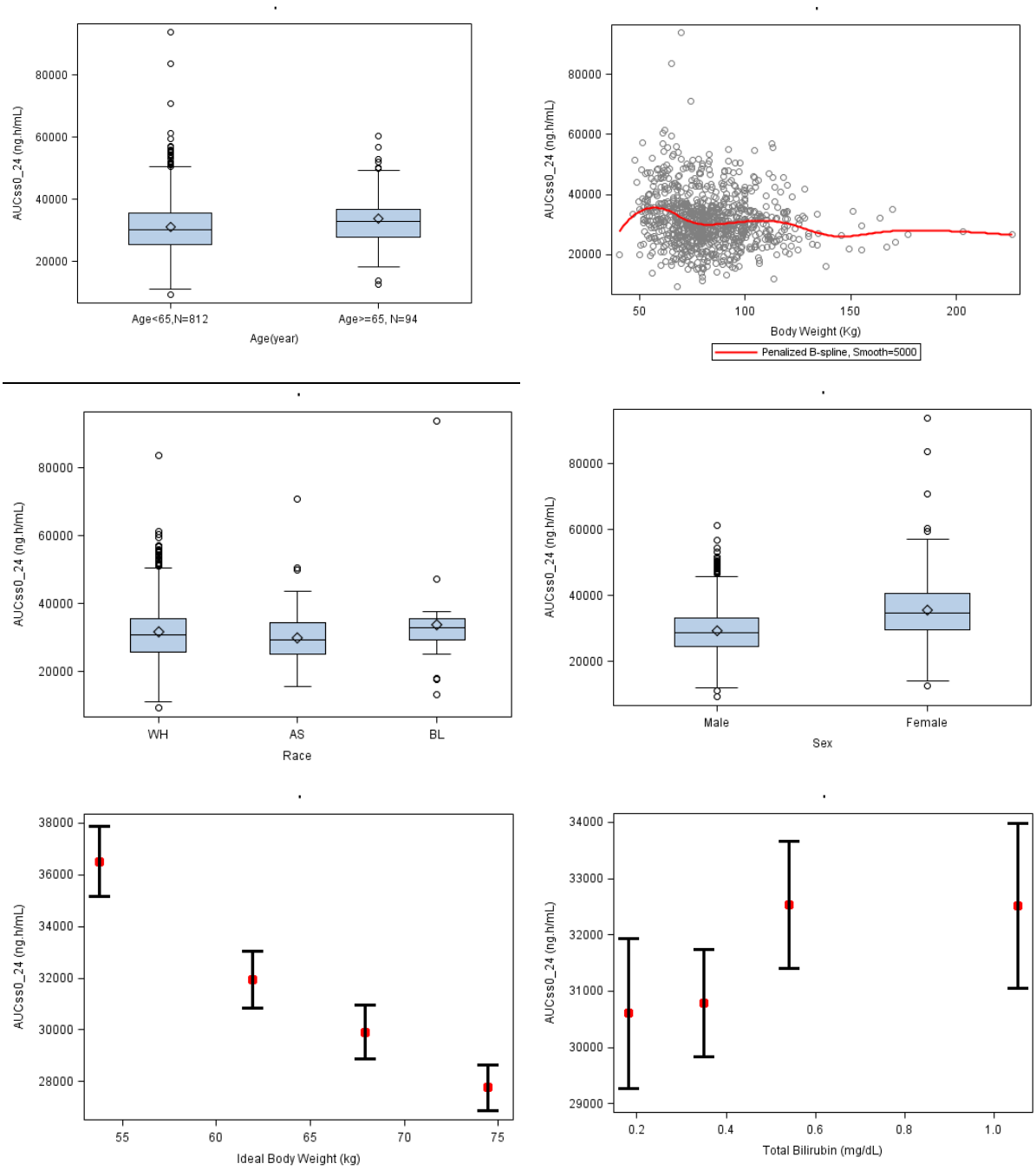
Figure 10: Model-predicted $AUC_{ss(0_24)}$ versus Demographic Parameters



Reviewer's Comments:

The sponsor's model is acceptable. The reviewer's analysis is consistent with that of the sponsor.

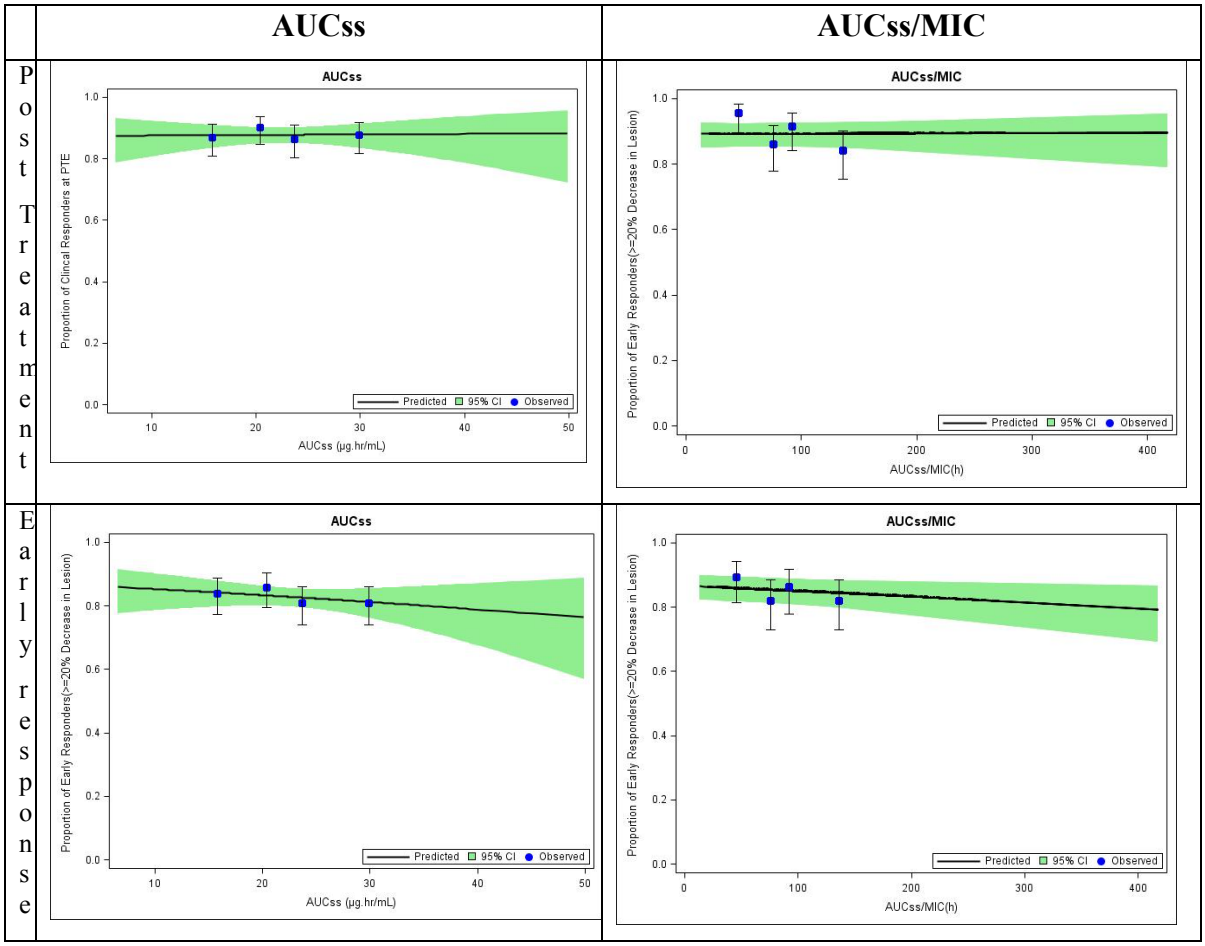
4.4.4 Exposure-response analysis for efficacy

Relationships between model-estimated steady-state AUC and proportion of clinical responders using various clinical efficacy end points (Clinical response at post-treatment,

Reference ID: 3476316

Early clinical response with >=20% decrease in lesion, and microbiological response at post treatment) were explored by the reviewer. Neither AUC_{ss} nor AUC_{ss}/MIC was found to be a significant predictor of clinical efficacy (**Figure 11**).

Figure 11: Proportion of Post-treatment Responders (top) and Early Clinical Responders (bottom) versus Model-predicted AUC_{ss} (left) or AUC_{ss}/MIC of Tedizolid



4.4.5 Exposure-response analysis for safety

The exposure-response analysis for safety that described the relationship between tedizolid exposure and the probability of gastrointestinal AEs, and laboratory measurements was conducted using data from Phase 2 Study TR701-104 and Phase 3 Studies TR701-112 and TR701-113. The analysis dataset consisted of 1644 AE records from 822 patients, of which, 533 patients (65%) were male and 289 (35%) were female. A total of 686 patients (83.5%) in the pooled population were white; 13.6% of patients were black, and 0.9% of patients were Asian. The majority of patients (71.8%) were from North America, 20.0% were from Europe, 5.4% were from South Africa.

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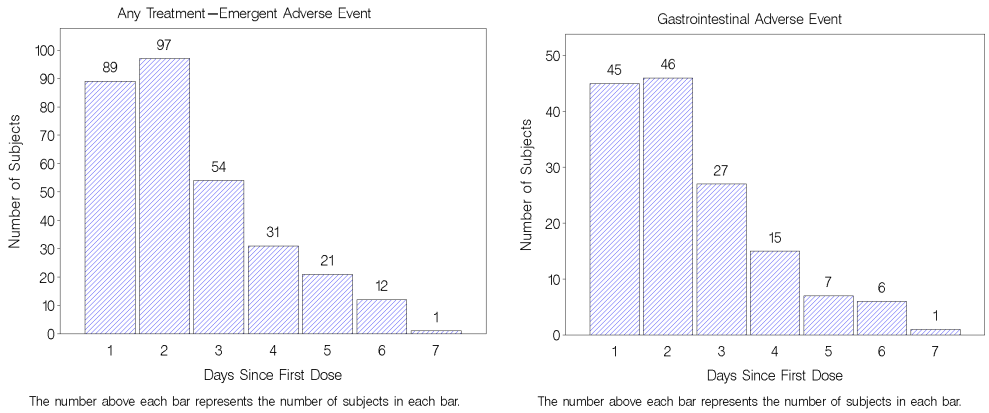
A total of 147 patients (18%) had least 1 occurrence of a GI AE such as vomiting, nausea, and diarrhea during the treatment period. GI AEs accounted for approximately 33% of all TEAEs, and most GI AEs (62%) occurred within the first two days of treatment with tedizolid (Figure 12).

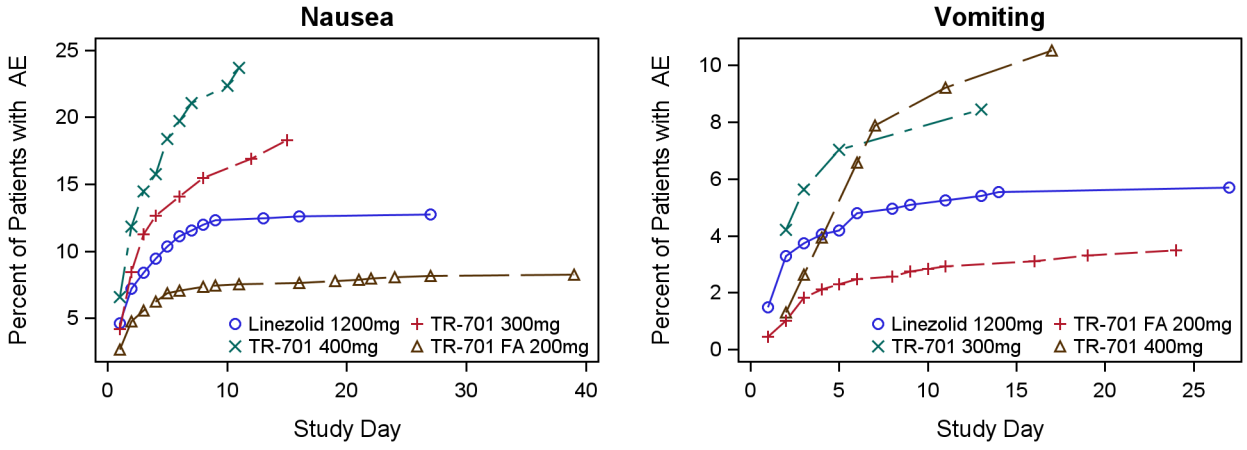
Figure 12: Frequency Distribution of the Time to the First Occurrence of Treatment Emergent and Gastrointestinal Adverse Events



Note: Adapted from the Sponsor's report CLN-13-0701-102, Figures 13 and 20.

The time course of adverse events after treatments with tedizolid 200 mg and linezolid 1200 mg was explored by the reviewer using the integrated safety dataset provided by the sponsor. The treatment with tedizolid 200 mg showed a comparable time course to linezolid 1200 mg. However, when tedizolid dose increased from 200 mg to 400 mg, an increase in the incidence for headache and GI adverse events, including vomiting, nausea, and diarrhea was observed (Figure 13).

Figure 13: Time Course of Selected Adverse Events after Treatment with Tedizolid and Linezolid (note Phase 2 and Phase 3 study results are combined below)



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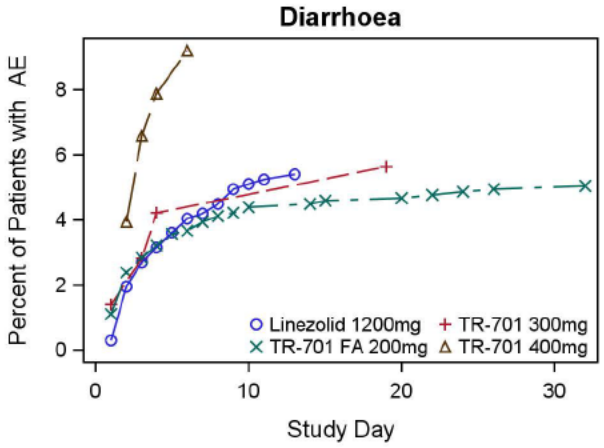

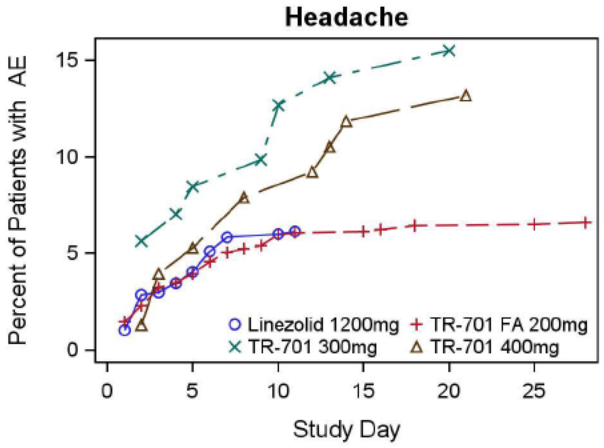

Reference ID: 3476316

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/s/

FANG LI
03/24/2014

JEFFRY FLORIAN
03/24/2014

BIOPHARMACEUTICS REVIEW Office of New Drug Quality Assessment			
Application No.:	NDA 205435	**Reviewer:** Minerva Hughes, Ph.D.	
Submission Date:	18 October 2013		
Division:	Division of Antimicrobial Products	**Team Leader:** Angelica Dorantes, Ph.D.	
		Acting Supervisor: Richard Lostritto, Ph.D.	
Sponsor:	Trius Therapeutics	**Secondary Reviewer:** Team Leader	
Trade Name:	TBD (Sivextro proposed)	**Date Assigned:**	29 Oct 2013
		GRMP Date: **Alt. Goal Date:** **PDUFA Date:**	5 May 2014 23 Mar 2014 20 Jun 2014
Generic Name:	Tedizolid phosphate	**Date of Review:**	11 Mar 2014
Indication:	Acute Bacterial Skin and Skin Structure Infections	**Type of Submission: 505(b)1, NME Priority NDA**	
Formulation/strengths	200 mg Tablet		
Route of Administration	Oral		
Biopharmaceutics Review Focus: Formulation bridging studies, dissolution method and acceptance criterion.			

***SUBMISSION*:**

NDA 205435 was submitted in accordance with Section 505(b) of the FDC Act for the use of tedizolid phosphate tablets in the treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by various gram positive bacteria. This NDA was submitted in concert with NDA 205436, a 200 mg IV formulation of tedizolid phosphate. The IV to tablet switch is intended to occur at the discretion of the physician and no dose adjustments are proposed.

Tedizolid phosphate is a novel oxazolidinone prodrug antibiotic that is converted in vivo by phosphatases to the microbiologically active moiety tedizolid, which is a protein synthesis inhibitor that interacts with the 50S subunit of the bacterial ribosome. Animal studies have shown that oral administration of the active moiety leads to very low exposures. Thus, the prodrug approach is critical for maximal bioavailability.

The proposed commercial product is an immediate release film-coated tablet comprised of the drug substance (200 mg) and the excipients microcrystalline cellulose, mannitol, povidone, crospovidone, magnesium stearate (b) (4)

BIOPHARMACEUTIC REVIEW SUMMARY:

An overview of the Biopharmaceutics review conclusions is as follows.

- Formulation bridging bioavailability/bioequivalence studies
  - During clinical development, the drug substance was changed from the salt-form to the free base. A relative bioavailability study was completed to bridge the formulation change, and the results satisfactorily demonstrated bioequivalence

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between the salt and free base forms.

- In addition to changing the drug substance form, the dosage form was changed from the capsule to tablet. However, the Applicant failed to provide any relative bioavailability or bioequivalence data to bridge the different dosage forms, which is generally required. Because the proposed commercial formulation was used in the pivotal efficacy and safety studies, and bioavailability data are available on the final formulation, the impact of this deficiency appear limited to the adequacy of the food-effect study and it does not impact the reliability of the clinical efficacy and safety data to support approval. The acceptability of the submitted food-effect study will be addressed by the Dr. Zhixia Yan, Clinical Pharmacology Reviewer.

- Dissolution method and acceptance criterion
  - The overall data support the approval of the following dissolution method and acceptance criterion.

Parameter	Criterion
Appartus	USP 2
Medium	pH 6.8 Phosphate Buffer, 0.05M
Agitation	60 rpm
Sampling Time/ Acceptance Criterion	Q = (b) (4) at 20 minutes
Analytical	HPLC/UV

- Drug product shelf-life (dissolution stability during storage only).
  - Dissolution performance is adequate on storage and is not limiting for setting an expiration dating period. A final recommendation on the expiry period will be addressed by Dr. Rajiv Agarwal, CMC Reviewer.

RECOMMENDATION:

From the Biopharmaceutics perspective, NDA 205435 for Tedizolid Phosphate Tablets, 200 mg, is recommended for APPROVAL.

Minerva Hughes, Ph.D.
Biopharmaceutics Reviewer
Office of New Drug Quality Assessment

Angelica Dorantes, Ph.D.
Biopharmaceutics Team Leader
Office of New Drug Quality Assessment

Reference ID: 3468713

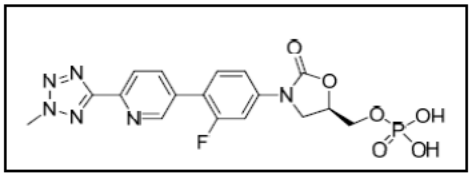
BIOPHARMACEUTICS ASSESSMENT
QUESTION BASED REVIEW - IR TABLET

I. GENERAL ATTRIBUTES

1. What are the highlights of the chemistry and physico-chemical properties of the drug substance (e.g. solubility) and formulation of the drug product?

Drug Substance:

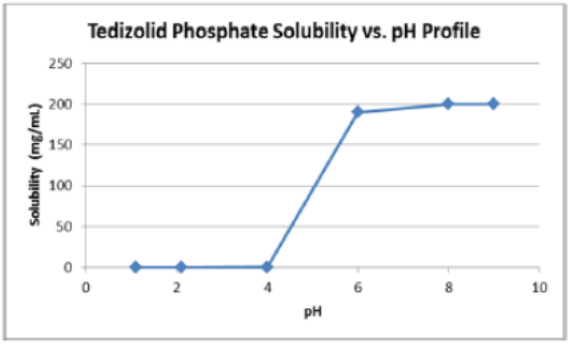
The drug substance is tedizolid phosphate (TR-701-FA), a novel oxazolidinone prodrug antibiotic under development for the treatment of acute bacterial skin and skin structure infections (ABSSSI) by both intravenous (IV) and oral routes of administration. This NDA is for the oral tablet formulation. TR-701 FA is rapidly converted by phosphatases to the microbiologically active moiety, tedizolid (TR-700). (b) (4) [redacted] The chemical structure is illustrated below.



TR-701 FA is an ionic species with pH dependant solubility. The first pKa of tedizolid phosphate drug substance is 1.8 (calculated). The second pKa is 6.5 (measured by titration). The aqueous solubility data are presented in the following table and figure.

Aqueous Solution	Solubility (mg/mL)
Water (pH 3.9)	0.1
0.1% Phosphoric Acid, pH 2.0	0.003
0.1 N HCl, pH 1.1	0.005
0.01 N HCl, pH 2.1	0.005
0.05 M Potassium Biphthalate, pH 4.0	0.5
0.05 M Potassium Phosphate, pH 6.0	>195
0.05 M Sodium Phosphate, pH 8.0	>200
0.1 M Potassium Borate, pH 9.0	>200

Source: Table 1, Section 3.2.S.1.3.



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Source: Figure 1, Section 3.2.S.1.3.

(b) (4)

Drug Product:

The proposed drug product is an immediate-release, film coated yellow oval-shaped tablet debossed with "TZD" on the obverse and "200" on the reverse side. Tedizolid phosphate tablets will be packaged in high density polyethylene (HDPE) bottles or as blister packs with (b) (4) film and aluminum foil backing. The qualitative and quantitative formulation of tedizolid phosphate tablets is presented in the following table.

Component	Quality Standard	Function	200 mg Tablet	
			Weight (mg/unit)	% (w/w)
Tedizolid Phosphate[a]	In-house	Active Ingredient	200	(b) (4)
Microcrystalline Cellulose	NF			(b) (4)
Mannitol[b]	USP			
Povidone	USP			
Crospovidone	NF			
	(b) (4)			
Magnesium Stearate[d]	NF			
	(b) (4			
Total Coated Tablet Weight				

Abbreviations: NF=National Formulary; USP=United States Pharmacopeia
-- not applicable

(b) (4)

Source: Table 1, Section 3.2.P.1.

The drug product manufacturing process employs (b) (4) operations. The Applicant states that the manufacturing process has not changed since the first clinical lot of tablets.

The tedizolid phosphate tablet is designe (b) (4)

Reviewer's Evaluation: *The Applicant provided an adequate overview of the drug substance physico-chemical properties and formulation development considerations to aid in understanding the biopharmaceutics program. The formulation includes excipients* (b) (4)

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(b) (4)

2. **Is there any information on BCS classification? What claim does the applicant make based on BCS classification? What data are available to support this claim?**

(b) (4)

Tedizolid phosphate is technically a Biopharmaceutical Classification System (BCS) Class IV compound, with poor solubility below pH 4 and poor permeability of the phosphate prodrug moiety. (b) (4)

Reviewer's Evaluation: (b) (4)

Thus, from this Reviewer's perspective, a BCS IV designation is more appropriately aligned with the Agency's guidelines for BCS classification based on the available data. Because a BCS 1 designation is not claimed, there is no further action warranted from a regulatory perspective. Only BCS 1 products are eligible for regulatory flexibility, owing to the BCS status, on the CFR requirement to submit bioavailability/bioequivalence data for certain formulation and process changes.

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II. DISSOLUTION INFORMATION

A. DISSOLUTION METHOD

1. What is the proposed dissolution method?

The proposed dissolution method is as follows.

Proposed Dissolution Method

Parameter	Value
Apparatus	USP Apparatus II (paddles)
Dissolution Media	pH 6.8 Phosphate Buffer, 0.05 M
Dissolution Volume	900 mL
Rotation Speed	60 rpm
Temperature	37°C ±0.5°C
Sample Analysis	HPLC/UV

Abbreviations: HPLC=high performance liquid chromatography; rpm=revolutions per minute; USP=United States Pharmacopeia; UV=ultraviolet
Source: Table 1, Dissolution Method Development Report, Section 3.2.P.5.6.

[redacted] (b) (4) *(Reviewer's Note:* [redacted] (b) (4))

2. What data are provided to support the adequacy of the proposed dissolution method (e.g. medium, apparatus selection, etc.)?

A dissolution method development report (TR701-001.0) was included in Section 3.2.P.5.6. The proposed method was developed with consideration for the drug substance solubility, apparatus type and agitation speed.

Dissolution Medium: [redacted] (b) (4) herefore, 0.05 M phosphate buffer, pH 6.8, was selected as the dissolution medium for the Tedizolid Phosphate Tablets, 200 mg.

Comparative dissolution profile data in pH [redacted] (b) (4) buffer and pH 6.8 phosphate buffer were provided to support the medium selection.

Apparatus Type: Paddles were selected over baskets [redacted] (b) (4) No comparative dissolution data were provided.

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Paddle Speed: (b) (4)

(b) (4)

Analytical: Dissolution samples are analyzed via HPLC with ultraviolet (UV) detection. (b) (4) The method parameters are tabulated below.

Dissolution Sample HPLC Parameters

Parameter	Condition/Setting
Column	(b) (4)
Detection	
Column Temperature	
Injection Volume	
Flow Rate	
Mobile Phase	
Run Time	
Retention Time	

Source: Table 9, Dissolution Method Development Report, Section 3.2.P.5.6.

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3. What data are available to support the discriminating power of the method?

(b) (4)

A summary of the process development variations is illustrated in the following table.

(b) (4)

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(b) (4)

The resulting mean dissolution profiles are presented in Figure 6 below.

(b) (4)

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(b) (4)

[redacted]

Reviewer's Evaluation (Dissolution Method Questions 1-3 above): *The proposed dissolution method was discussed at the 4 October 2011 End of Phase 2 meeting with the firm (Dr. Angelica Dorantes for Biopharmaceutics) and subsequently evaluated by Dr. Zedong Dong (DARRTS Quality Review dated 8 February 2013 RE: IND 77,872 – SN 175, 26 November 2012). The dissolution method development report submitted to this NDA (dated 20 November 2012) is the same dissolution method development report evaluated by Dr. Dong under IND 77872. As per Dr. Dong's review, and communicated to the Applicant on 8 March 2013, the proposed dissolution method was acceptable but the discriminating ability was not fully demonstrated. In*

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addition, the dissolution data supported an acceptance criterion of Q = (b) (4) in 15 minutes; however, a final determination on the acceptance criterion would be made under the NDA and the Applicant should submit complete multipoint dissolution profile data for the clinical and registration batches under the NDA.

This Reviewer agrees with the previous IND review findings regarding the adequacy of the proposed dissolution testing parameters. The selected apparatus (b) (4) (i.e., 60 rpm) to facilitate dissolution and a variety of testing conditions were explored.

(b) (4)

(b) (4)

Reference is made to the Quality Review by Dr. Rajiv Agarwal for full details on the drug product manufacturing process. However, from the Biopharmaceutics perspective, the sensitivity limitations of the dissolution method appear appropriately supplemented by (b) (4) the Applicant's overall quality system.

4. What information is available to support the robustness (e.g. linearity, accuracy, etc.) of the dissolution methodology?

The proposed dissolution method was validated for specificity for dissolution medium and placebo interference, linearity, method repeatability, intermediate precision, (b) (4) verification, sample and standard solution stability, and accuracy by recovery. Dissolution data were collected at 15-, 30-, 45-, and 60-minute time points. Summary results of the dissolution method validation are tabulated below.

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Data Element	Acceptance Criteria	Result
Specificity	(b) (4)	
Standard Linearity		
(b) (4) Recovery		
Repeatability		
Intermediate Precision -Analyst -Equipment		
Solution Stability 1, 3, 5, 7 days		
Accuracy (Recovery) 10%, 100%, 150%		

Abbreviations: NLT=not less than; NMT=not more than; RSD=relative standard deviation

Source: Table 10, Dissolution Method Development Report, Section 3.2.P.5.6.

The dissolution HPLC method's robustness was not evaluated.

Reviewer's Evaluation: *The dissolution HPLC method validation studies will be reviewed by the Dr. Rajiv Argawal. Reference is made the Dr. Argawal's CMC-Quality review for a final determination on the acceptability of the method validation data. It is noted that robustness was not evaluated for the dissolution HPLC method as recommended in ICH Q2(R1). In addition, there were no clearly defined system suitability parameters. These comments were communicated to the CMC Reviewer for consideration.*

5. Is the proposed dissolution method biorelavant? What data are available to support this claim?

No. The proposed dissolution method is for product quality control purposes only and is not predictive of in vivo performance. (b) (4)

6. Is the proposed method acceptable? If not, what are the deficiencies?

Yes, the proposed dissolution method is acceptable.

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B. ACCEPTANCE CRITERION

1. What is the proposed dissolution acceptance criterion for this product?

The proposed acceptance criterion is Q = (b) (4) at 20 minutes.

2. What data are available to support it?

Reference is made to the FDA feedback of 11 February 2013 that the proposed acceptance criterion of Q = (b) (4) at (b) (4) minutes (b) (4) from registration and clinical batches. (b) (4) he proposed acceptance criterion of NLT (b) (4) (Q) at 20 minutes will ensure a consistent systemic exposure from lot to lot and within the same lot therefore providing consistent safety and efficacy profiles.

(b) (4)

Tablet dissolution data for the primary stability batches were summarized as follows.

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		Range of % dissolved at selected dissolution times (after storage for 3, 6, 9 and 12 months at 25°C/60%RH)
Dissolution parameter	**Package Configuration**	(b) (4)
Mean (% dissolved)		(b) (4)
Minimum (% dissolved)		
Maximum (% dissolved)		

		Range of % dissolved at selected dissolution times (after storage for 3, 6, 9 and 12 months at 25°C/60%RH)
Dissolution parameter	**Package Configuration**	(b) (4)
Standard Deviation (% dissolved)		(b) (4)

The dissolution specification is being set based on a small dataset (only 5 drug product lots), and the Applicant does not consider dissolution to be a critical product attribute.

Reviewer's Evaluation: *In general, the mean dissolution profiles during development and for the registration stability lots is consistent with FDA's initial recommendation of Q=* (b) (4) *at* (b) (4) *minutes.* (b) (4)

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(b) (4)

(b) (4)

3. **Is the setting of the dissolution acceptance criterion based on data from clinical and registration batches?**

Yes. Three lots of tedizolid phosphate tablets, 200 mg were manufactured at (b) (4) site, and three lots of tedizolid phosphate tablets were manufactured at (b) (4) The 3 lots of tedizolid phosphate tablets manufactured at (b) (4) were made using the same formulation, manufacturing process, and class of equipment that was used to manufacture tedizolid phosphate tablets at (b) (4). Three (b) (4) lots were used for primary stability studies and one lot (GNFF) was used in the Phase 3 clinical trials. The (b) (4) lots were used for supportive stability studies and Phase 1, 2, and 3 clinical trials.

4. **Are mean (b) (4) dissolution profile data used for the setting of the acceptance criterion?**

No. The acceptance criterion is based on a mean dissolution of n=6 samples, with consideration for pooled data from stability studies.

5. **Is the acceptance criterion acceptable? If not, what is the recommended criterion?**

Yes, the proposed acceptance criterion of Q = (b) (4) at 20 minutes is acceptable.

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III. DRUG PRODUCT FORMULATION DEVELOPMENT and BRIDGING ACROSS PHASES

1. What is the composition of the formulation of the proposed product?

See Section I, Question 1.

2. What are the highlights of the drug product formulation development?

(b) (4)

[redacted]

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An overview of the early formulation use by clinical study is illustrated in the following table.

Table 1 TR-701/TR-701 FA Biopharmaceutics Program

Study	Prodrug Form	Route	Oral Formulation	Dose	Objective
TR701-103	TR-701	Oral	Capsule	600 mg	Food Effect
TR701-107	TR-701 FA	IV, Oral	Tablet	200 mg	Absolute Oral Bioavailability
Bayer 16101	TR-701 FA	IV, Oral	Tablet	200 mg	Absolute Oral Bioavailability
TR701-108	TR-701 TR-701 FA	Oral	Capsule	150 mg[a]	Relative Bioavailability

[a]expressed in terms of pharmacological activity (TR-700).
Source: Section 2.7.1.

Study TR701-108 investigated the relative oral bioavailability of the two prodrug forms, administered in capsules containing 150 mg of active moiety (200 mg for TR-701, 182 mg for TR-701 FA). A brief study synopsis is as follows.

STUDY TITLE: An Open-Label, Randomized, Single-Dose, 2-Treatment, 2-Sequence, Crossover, Exploratory Pharmacokinetic and Bioavailability Study of 2 Capsule Formulations of TR 701 (Torezolid Phosphate) in Normal Healthy Adults

STUDY DESIGN: This was an open-label, randomized, 2-treatment, 2-sequence crossover, PK study conducted in 12 healthy male and female subjects. Subjects were confined to the clinical research unit (CRU) from approximately 24 hours prior to first dose until approximately 72 hours after final dose (approximately 10 days). The planned study conduct duration was approximately 4 weeks.

SUBJECTS PLANNED AND ANALYZED: Twelve subjects were planned and twelve subjects were enrolled in the study. All subjects completed the study and were included in all analyses.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION: Subject was male or female; between 18 and 60 years of age (inclusive); with a body mass index (BMI) between 18.5 to 32.0 kg/m^2 (inclusive); a non-smoker; and in good health based upon results of medical history, physical examination, 12-lead electrocardiogram (ECG), and laboratory test results.

TEST PRODUCT, DOSE, ROUTE, LOT NUMBER: Subjects received each of the following treatments in a crossover design, according to a randomization schedule generated by a Covance Biostatistician:

• **Treatment A (Reference):** One 200 mg capsule of TR-701 di-sodium salt (equivalent to 150 mg TR-700) administered orally (PO) after at least an 8-hour fast with 240 mL room temperature water;

• **Treatment B (Test):** One 182 mg capsule of TR-701 FA (equivalent to 150 mg TR-700) administered PO after at least an 8-hour fast with 240 mL room temperature water;

There was at least a 5-day washout between dose administrations.

BIOANALYTICAL METHOD: Samples were analyzed using a validated LC/MS/MS method.

Analyte/ matrix	Specificity	Linear-Valid range (ng/mL)	Sensitivity (LLOQ) (ng/mL)	Accuracy (%) intra-/inter-run	Precision (%) intra-/inter-run	Recovery over range (%)	Stability[a] bench/ freeze-thaw/ long-term
TR-700/ plasma	No interference	Linear 5-1000	5.00	98.5-103.3/ 98.09-102.7	± 1.2-3.3/ ± 2.1-2.5	86.2-87.7	Acceptable

[a] Stability summary: Stock Solution: Room temperature (6 hours); 2°C to 8°C (89 days);
Working Standard Solution: 2°C to 8°C (50 days);
Plasma: matrix samples: Room temperature 24 hours; freeze-thaw 5 cyles; frozen (-60°C to -80°C; 195 days);
processed samples: 2°C to 8°C (75 hours)
collection (whole blood) samples: Room temperature (2 hours) and on ice (2 hours);

Reference: Validation Report 7978-102.

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PHARMACOKINETIC RESULTS: An overall mean relative bioavailability of 96% was observed

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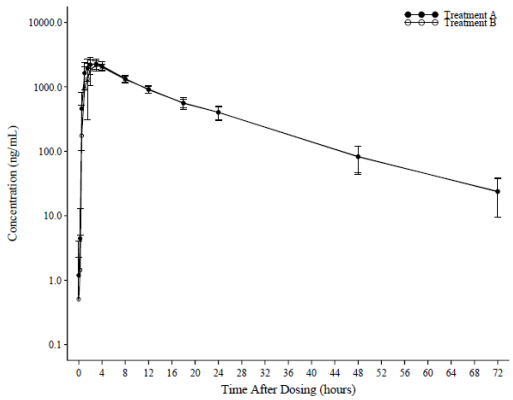
between the tested 182 mg TR-701 FA powder capsule (Treatment B) and the reference 200 mg TR-701 di-sodium salt formulated capsule (Treatment A). The 90% CIs for least squares (LS) mean Cmax and AUC values comparing the test Treatment B to the reference Treatment A were entirely contained within the 80% to 125% equivalence range, indicating that there are no significant differences in the rate and extent of TR-700 absorption between the TR-701 FA powder and the TR-701 di-sodium salt formulations. These analyses of the results indicate that the products meet the regulatory criteria for bioequivalence, thus the TR-701 FA powder capsule and the TR-701 di-sodium salt formulated capsule are deemed bioequivalent.

The TR-700 mean CL/F and Vz/F values were comparable between the two TR-701 formulations in Treatments A and B.

Figure 11-1 Mean (SD) Plasma Concentrations for TR 700



SAFETY RESULTS: Four (33.3%) of the 12 subjects enrolled in this study reported at least 1 AE after study drug administration; however, none of the AEs were judged treatment-related. The majority of AEs were mild and no severe AEs were reported. There were no deaths or SAEs during the study and no subjects discontinued from the study due to AEs. No specific AE was reported by more than 1 subject.

No clinically significant changes or findings were noted from clinical laboratory evaluations, vital sign measurements, physical examinations, or 12-lead ECGs for this study. Overall, the changes in the clinical safety assessments were unremarkable.

Reviewer's Evaluation: *Study TR701-108 satisfactorily demonstrates that the salt-form of the drug substance is bioequivalent to the free base form when administered as capsule formulations, and thus provides an appropriate scientific bridge to link the two API forms. However, there is no direct in vitro or in vivo comparison between the final TR-701 FA tablet drug product and TR-701 or TR-701 FA capsules.*

Further, the food effect study was not done using the TR-701 FA tablet formulation. Generally, a change in dosage form, as undertaken in this submission, requires bioequivalence data to scientifically bridge the two dosage forms. In vitro dissolution is not an acceptable surrogate for this type of product change; moreover, the Applicant does not provide any in vitro data. Because the proposed commercial formulation was used in the pivotal efficacy and safety studies, and bioavailability data are available on the final formulation, the impact of this deficiency is really limited to the adequacy of the food-effect study and does not impact the reliability of the clinical efficacy and safety data to support approval. The determination on whether an additional food-effect study is warranted in this case will be made by Clinical Pharmacology.

Reference ID: 3468713

Reference is made to the Clinical Pharmacology review by Dr. Zhixia Yan for additional information regarding food-effect.

3. **Are all the strengths evaluated in the pivotal clinical trials? If not, what data are available to support the approval of lower strengths?**

   Yes. One tablet strength is proposed for commercialization, 200 mg, which was used in the pivotal studies.

4. **Are there any manufacturing changes implemented to the clinical trial formulation? What information is available to support these changes?**

   There was a manufacturing facility change between the clinical and commercial products. The 3 lots of tedizolid phosphate tablets manufactured at (b) (4) (commercial site) were made using the same formulation, manufacturing process, and class of equipment that was used to manufacture tedizolid phosphate tablets at (b) (4) (clinical supplies). The three (b) (4) lots were used for primary stability studies and one lot (GNFF) was used in the Phase 3 clinical trials (TR701-113). The (b) (4) lots were used for supportive stability studies and Phase 1, 2, and 3 clinical trials.

   Because product manufactured at the (b) (4) site was used in clinical studies, additional dissolution profile data are not necessary. The comparative batch analysis data adequately support the new facility from the Biopharmaceutics perspective.

5. **Is the formulation of the clinical product the same formulation of the to-be-marketed product? If not, what information is available to support the formulation changes?**

   Yes. The formulation used in the pivotal clinical studies is the same at the to-be marketed product.

6. **Is the manufacturing site the same for the clinical and to-be-marketed products? If not, what information is available to support the new site?**

   See responses to # 4 above.

IV. DISSOLUTION APPLICATIONS

A. BIOWAIVERS

1. **Is there a waiver request for in vivo BA or BE data (Biowaiver)? If yes, what is/are the purpose/s of the biowaiver request/s? What data support the biowaiver request/s? Is the biowaiver request acceptable?**

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No. There is no request to waive in vivo BA or BE study data. The to-be-marketed formulation was used in the pivotal clinical studies.

2. **Is there any IVIVR or IVIVC information submitted? What is the regulatory application of the IVIVR/IVIVC in the submission? What data are provided to support the acceptability of the IVIVR or IVIVC model?**

   There is no IVIVR or IVIVC information in this NDA.

B. SURROGATES IN LIEU OF DISSOLUTION

1. **Are there any manufacturing parameters (e.g. disintegration, drug substance particle size, etc.) being proposed as surrogates in lieu of dissolution testing? What data are available to support the approval of the proposed surrogate test?**

   No.

C. DISSOLUTION AND QBD

1. **Does the application contain QbD elements? If yes, is dissolution identified as a CQA for defining design space?**

   No. The application does not contain any QbD elements.

2. **Was dissolution included in the DoE? What raw materials and process variables are identified as having an impact on dissolution? What is the risk assessment been performed to evaluate the criticality of dissolution?**

   Not applicable. See Question 1 above.

3. **What biopharmaceutics information is available to support the clinical relevance of the proposed design space?**

   Not applicable. See Question 1 above.

4. **Is there any dissolution model information submitted as part of QbD implementation? What is the regulatory application of the dissolution model in the submission? What data are provided to support the acceptability of the dissolution model?**

   Not applicable. See Question 1 above.

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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MINERVA HUGHES
03/11/2014

ANGELICA DORANTES
03/11/2014

CLINICAL PHARMACOLOGY FILING FORM/CHECKLIST FOR NDA/BLA SUBMISSIONS

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

NDA/BLA Number	NDA 205,435 & NDA 205,436	**Brand Name**	TBD
OCP Division (I, II, III, IV, V)	DCP4	**Generic Name**	Tedizolid Phosphate
Medical Division	DAIP	**Drug Class**	Oxazolidinone Prodrug
OCP Reviewer	Zhixia (Grace) Yan, Ph.D.	**Indication(s)**	Treatment of Acute Bacterial Skin and Skin Structure Infections (ABSSSI)
OCP Team Leader	Kimberly L. Bergman, Pharm.D.	**Dosage Form**	Oral Tablet/Intravenous Solution
Pharmacometrics Reviewer	TBD	**Dosing Regimen**	200 mg (oral/IV) once daily for 6 days
Date of Submission	October 18, 2013	**Route of Administration**	Oral/Intravenous
Estimated Due Date of OCP Review	TBD	**Sponsor**	Trius Therapeutics Inc
Medical Division Due Date	TBD	**Priority Classification**	Priority
PDUFA Due Date	June 21, 2014	**AC Meeting (if applicable)**	February 27, 2014

Clinical Pharmacology and Biopharmaceutics Information

	"X" if included at filing	**Number of studies submitted**	**Number of studies reviewed**	**Critical Comments If any**
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X	19		
I. Clinical Pharmacology				
Mass balance:	X	1		TR701- 106 (^{14}C TR701 ADME)
Isozyme characterization:	X	1		PDM-0701-07-028 (In Vitro Metabolic Stability of TR-701 and TR-700 in Liver and Plasma)
Blood/plasma ratio:	-			
Plasma protein binding:	X	2		PDM-13-0701-080 PDM-13-0701-081
Pharmacokinetics (e.g., Phase I) -	X	15		Three biopharmaceutical studies Twelve human PK studies
HEALTHY VOLUNTEERS -				
single dose:	X	5		TR701- 101 (Oral SAD/MAD) TR701-107 (IV SAD/MAD) TR701- 102 (Distribution- muscle and adipose) TR701- 106 (ADME) TR701- 119 (Pulmonary Disposition)
multiple dose:	X	2		TR701- 101 (Oral SAD/MAD) TR701-107 (IV SAD/MAD)
PATIENTS -				
single dose:	-			
multiple dose:	X	4		Two Phase 2 studies Two Phase 3 studies
Dose proportionality -				
fasting / non-fasting single dose:	X	2		TR701- 101 (Oral SAD/MAD) TR701-107 (IV SAD/MAD)

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fasting / non-fasting multiple dose:	X	2		TR701- 101 (Oral SAD/MAD) TR701-107 (IV SAD/MAD)
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:	X	2		TR701- 105 (MAO/Tyramine) TR701- 114 (MAO/Pseudoephedrine)
In-vitro:	X	11		*Drug Metabolizing Enzymes* PDM-07-0701-034 CPH-12-0701-050 PDM-10-0701-012 CPH-13-0701-090 *Membrane Transporters* CPH-10-0701-013 CPH-11-0701-022 CPH-12-0701-033 CPH-12-0701-046 CPH-12-0701-045 CPH-12-0701-064 CPH-12-0701-065
Subpopulation studies -				
ethnicity:	-			
gender:	-			
pediatrics:	X	1		TR701- 111 (adolescents, 12-17 years old)
geriatrics:	X	1		TR701- 109 (≥65 years)
renal impairment:	X	1		TR701- 123
hepatic impairment:	X	1		TR701- 124
PD -				
Phase 2:	X	2		TR701- 104 TR701- 126
Phase 3:	X	2		TR701- 112 TR701- 113
PK/PD -	X	1		CLN-13-0701-102
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:	X	1		CLN-13-0701-072
Data sparse:	X	1		CLN-10-0701-009
II. Biopharmaceutics				
Absolute bioavailability	X	1		TR701- 107
Relative bioavailability -	X	1		TR701- 108
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies	X	1		TR701- 103
Bio-waiver request based on BCS	-			
BCS class	-			
Dissolution study to evaluate alcohol induced dose-dumping	-			
III. Other CPB Studies				
Genotype/phenotype studies	-			
Chronopharmacokinetics	-			
Pediatric development plan	X			Request for deferral (0 to <18 years)
Literature References	-			
TOTAL NUMBER OF STUDIES		36		14 In vitro studies 3 Biopharmaceutical studies 12 Human PK studies 2 Phase 2 studies 2 Phase 3 studies 1 Exposure-Response study report 2 Pop PK study reports

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CLINICAL PHARMACOLOGY FILING FORM/CHECKLIST FOR NDA/BLA SUBMISSIONS

APPEARS THIS WAY ON ORIGINAL

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CLINICAL PHARMACOLOGY FILING FORM/CHECKLIST FOR NDA/BLA SUBMISSIONS

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?			X	The to-be-marketed tedizolid phosphate oral/IV formulations were used in pivotal Phase 3 trials.
2	Has the applicant provided metabolism and drug-drug interaction information?	X			
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	X			
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	X			
5	Has a rationale for dose selection been submitted?	X			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	X			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	X			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	X			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
	Data				
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	X			
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			X	
	Studies and Analyses				
11	Is the appropriate pharmacokinetic information submitted?	X			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?	X			
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?	X			
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?	X			
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			X	

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16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			X	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	X			
	General				
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	X			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			X	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

Yes.

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Not applicable.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None.

Zhixia (Grace) Yan, Ph.D. November 18, 2013
Reviewing Clinical Pharmacologist Date

Kimberly L. Bergman, Pharm.D. November 18, 2013
Team Leader/Supervisor Date

Reference ID: 3476548

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ZHIXIA YAN
03/24/2014

KIMBERLY L BERGMAN
03/24/2014