CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-821

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY & BIOPHARMACEUTICS REVIEW

NDA#	21-821
PRODUCT	Tigecycline (Tygacil™)
FORMULATION	Sterile Powder for injection
DOSAGE STRENGTH	50mg vial
SUBMISSION DATES	December 15, 2004; March 14, 2005; April 12, 2005; May 5, 2005; May 16, 2005; May 19, 2005; May 27, 2005
SUBMISSION TYPE	New Molecular Entity
SPONSOR	Wyeth Pharmaceuticals
OCPB DIVISION	Division of Pharmaceutical Evaluation III
MEDICAL DIVISION	Division of Anti-Infective Drug Products
REVIEWER	Jeffrey J. Tworzyanski, Pharm.D.
PM REVIEWER	Yaning Wang, Ph.D.
TEAM LEADER	Venkat R. Jarugula, Ph.D.
PM TEAM LEADER	Joga Gobburu, Ph.D.

1 EXECUTIVE SUMMARY

Wyeth Pharmaceuticals submitted a New Drug Application for Tygacil™ (Tigecycline for injection) on December 15, 2004. The FDA granted this submission a priority review based upon the sponsor's studies in the treatment of resistant organisms. Tigecycline represents a new class of antimicrobials known as glycylcyclines. The glycylcycline class of antimicrobial agents is a synthetic derivative of minocycline. The mechanism of action of the tetracyclines is by binding to the 30S ribosomal subunit at the A-site which blocks entry of amino-acyl transfer RNA molecules into the ribosome which prevents incorporation of amino acid residues into elongating peptide chains. The glycylcyclines (tigecycline) also inhibit the 30S ribosomal subunit but bind with substantially higher affinity than the tetracyclines. The glycylcyclines interact directly with another region of the A-site.

Wyeth is requesting approval for two indications for tigecycline. The first indication is complicated skin and skin structure infections caused by *Escherichia coli*, *Enterococcus faecalis* (vancomycin-susceptible strains only), *Staphylococcus aureus* (methicillin-susceptible and –resistant strains), *Streptococcus agalactiae*, *Streptococcus anginosus* grp. (includes *S. anginosis*, *S. intermedius*, and *S. constellatus*), *Streptococcus pyogenes* and *Bacteroides fragilis*. The second indication for tigecycline that Wyeth is seeking is complicated intra-abdominal infections caused by *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Enterococcus faecalis* (vancomycin-susceptible strains only), *Staphylococcus aureus* (methicillin-susceptible strains only), *Streptococcus anginosus* grp. (includes *S. anginosus*, *S. intermedius*, and *S. constellatus*), *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*, *Bacteroides vulgatus*, *Clostridium perfringens*, and *Peptostreptococcus micros*.

The proposed intravenous dosage regimen for tigecycline is an initial dose of 100mg, followed by 50mg every 12 hours. Intravenous infusions of tigecycline should be administered over approximately 30 to 60 minutes every 12 hours. The recommended duration of treatment with tigecycline for complicated skin and skin structure infections or for complicated intra-abdominal infections is 5-14 days depending upon severity and site of infection.

A total of 17 Phase 1 clinical pharmacology studies were conducted to investigate single dose and multiple dose pharmacokinetics, metabolic disposition, the effect of special populations (renal and hepatic impairment), drug-drug interactions (digoxin, warfarin), and the effect of race, age, and gender on the pharmacokinetics. Eleven phase 2/3 studies were submitted of which four Phase 3 and two Phase 2 studies were pertinent to the proposed indications. In addition the sponsor performed population PK analysis on the sparse sample data collected in Phase 2/3 studies. Thirteen out of 17 submitted Phase 1 studies were evaluated in this review. Four Phase 1 studies were not reviewed because they did not provide relevant or additional information. Sponsor has adequately characterized the pharmacokinetics and pharmacodynamics of tigecycline following intravenous infusion.

1.1 RECOMMENDATIONS

The Office of Clinical Pharmacology and Biopharmaceutics/Division of Pharmaceutical Evaluation III (OCPB/DPE III) has reviewed NDA 21-821. The submission is acceptable from a Clinical Pharmacology point of view. The labeling comments need to be communicated to the sponsor.

1.2 PHASE 4 COMMITMENTS

There are no phase 4 commitments.

1.3 SUMMARY OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FINDINGS

Pharmacokinetic characteristics:

Following intravenous infusion of tigecycline, the drug concentrations in serum declined in a polyphasic manner. The initial steep decline in serum concentrations at the end of infusion represents the distribution phase during which the drug is distributed out of the systemic circulation into various tissues. The terminal elimination phase represents the movement of the drug out of tissues into systemic circulation and the subsequent elimination from the body. The pharmacokinetics of tigecycline (Cmax and AUC) after single dose infusion over 60 minutes are linear over the dose range of 12.5 mg to 300 mg. The mean systemic clearance (CL) values were consistent among dose groups and ranged from 0.2 L/h/kg to 0.3 L/h/kg. Tigecycline was well distributed into various tissues as shown by the mean steady state volume of distribution (V_{ss}), which ranged from 7 to 14 L/kg. The estimated terminal phase half life varied among dosages (ranges from 18 hrs at 50 mg dose to 42 hrs at 200 mg dose) because the terminal phase was not adequately characterized at lower doses due to assay sensitivity. The steady state plasma

concentrations are achieved by Day 3 with the proposed dosage regimen of 100 mg loading dose followed by 50 mg q 12hrs. The mean terminal elimination half-life at steady state following the administration of the therapeutic regimen was approximately 40 hrs.

Distribution:
In vitro plasma protein binding of tigecycline is concentration dependent and ranges from 71% at 0.1 μg/ml to 89% at 1 μg/ml to 96% at 15 μ/ml. Following intravenous infusion of 100 mg dose, the concentration of tigecycline was 38 times higher in the gallbladder than in the serum, two-fold higher in the colon than in the serum and 8.6 times higher in the lung compared to serum. The degree of penetration of tigecycline into skin blister fluid as measured by the ratio of AUC in blister to AUC in serum was 74%.

Metabolism and Excretion:
Based on the results of in vitro metabolism studies, tigecycline is not metabolized by the cytochrome P450 (CYP 450) enzyme system and it does not inhibit the metabolism of the drugs that are metabolized by CYP 450 system. Therefore, tigecycline is not expected to cause CYP 450 based metabolic drug interactions.

Following administration of [^{14}C]tigecycline, approximately 33% of the administered dose is recovered in urine and 59% of the administered dose is recovered in feces, for a total recovery of 92% of the administered dose. Approximately 22% of the total dose is excreted as unchanged tigecycline in urine. The biliary excretion of tigecycline and its metabolites is the primary route of elimination. The secondary elimination pathways of tigecycline and its metabolites are renal excretion, glucuronidation, and amide hydrolysis followed by N-acetylation to form N-acetyl-9-aminominocycline; and these secondary pathways each account for 15% or less of the total elimination of tigecycline.

Special populations:
Renal Impairment:
In study 103-US (Protocol 3074A1) the sponsor evaluated the PK of tigecycline 100mg dose in 20 subjects. Approximately one third of the subjects were with normal renal function, one third subjects with CrCl < 30 ml/min, and one third subjects with end-stage renal disease (ESRD) who were receiving hemodialysis. The renal clearance of tigecycline in healthy subjects was similar to creatinine clearance (Cl_{Cr}), and it represented approximately 20% of the total systemic clearance of tigecycline. Consequently, the systemic clearance of tigecycline was reduced by approximately 20% in subjects with severe renal impairment (Cl_{Cr}<30 ml/min) or ESRD, and tigecycline area under the curve (AUC) increased by approximately 30% in these subjects. Based on the results of this study, tigecycline dose does not need to be adjusted in patients with renal impairment. Hemodialysis did not remove tigecycline from the systemic circulation of the subjects.

Hepatic Impairment:
In study 105-EU (Protocol 3074A1) the sponsor evaluated the effect of hepatic impairment on the pharmacokinetics of tigecycline following the administration of a

single dose of 100mg in patients with mild (Child-Pugh-A: score of 5 or 6, n=10), moderate (Child-Pugh-B: score of 7 to 9, n=10), and severe hepatic impairment (Child-Pugh-C: score of 10 to 13, n=5) in comparison to 23 healthy subjects matching the cirrhotic subjects for age, sex, weight, and smoking habit. The results of the study indicate that dosage adjustment is not warranted in patients with mild hepatic impairment (Child-Pugh A) because of no change in the clearance of drug compared to healthy subjects. In moderate and severe hepatic impairment (Child-Pugh B and Child-Pugh C) the clearance was approximately 25% and 50% lower, respectively, than the healthy subjects. Since the clearance of the drug in the severe hepatic impairment population was decreased by 50% and the AUC was increased by 105% these patients should receive an initial dose of 100mg followed by a reduced maintenance dose of 25 mg every 12 hours.

Drug Interactions:
Digoxin: Concomitant administration of the proposed tigecycline regimen (100 mg loading dose followed by 50 mg q 12hrs) did not significantly alter the pharmacokinetics of digoxin.

Warfarin: Coadministration of the therapeutic regimen of tigecycline significantly decreased the oral clearance of both R-warfarin and S-warfarin, thereby increasing the Cmax (38% and 43%, respectively) and AUC (68% and 29%, respectively) of both compounds. The pharmacodynamic endpoint, the mean INRmax was reduced by 10%. Sponsor recommended in the label that prothrombin time or other suitable coagulation parameters should be monitored if tigecycline is administered with warfarin.

Oral contraceptives: Sponsor did not conduct a drug interaction study with oral contraceptives. However, tetracyclines are reported to reduce the effectiveness of oral contraceptives. Tigecycline is eliminated predominantly through biliary excretion and is shown to reduce the intestinal microflora. Therefore, the sponsor stated in the label that concurrent use of antibiotics with oral contraceptives may render oral contraceptives less effective.

Exposure response:
Animal data:
Based on the dose fractionation studies in murine neuropenic thigh model, both AUC and the time of plasma concentration above MIC (T>MIC) appeared to be best correlated with antimicrobial efficacy of tigecycline.

Human data:
Efficacy:
Even though good exposure-response relationships were established in animal model (see 2.2.4.1), the limited exposure levels studied in patients and high success rate in clinical trials made the exposure-response analysis quite challenging in human studies. For both cSSSI and cIAI indications the exposure-response analysis showed that AUCss(0-24)/MIC was a borderline statistically significant predictor of microbiologic and clinical response. However, the relationship between exposure and efficacy is so shallow that

under the proposed dosage regimen (100mg loading dose and 50mg maintenance dose), the exposure change did not cause clinically relevant difference in efficacy.

Safety:
First occurrence of nausea and vomiting was selected as the endpoint for tigecycline safety evaluation. No exposure-response relationship was established within the observed exposure levels in Phase 2/3 studies for safety. The analysis based on phase 1 studies (84 subjects from Study 3074A1-100, 46 subjects from Study 3074A1-102, and 6 subjects from Study 3074A1-103) showed AUC0-inf and Cmax were significant predictors for both first nausea and first vomiting occurrence due to the wide range of doses studied. The lack of exposure-response relationship for the first nausea and first vomiting occurrence in phase 2/3 studies was due to the narrow range of exposure under the two doses, 25mg and 50mg.

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Jeffrey J. Tworzyanski, Pharm.D.
Office of Clinical Pharmacology and Biopharmaceutics
Division of Pharmaceutical Evaluation III

RD/FT Initialed by Venkat R. Jarugula, Ph.D.,_____
Team Leader

cc:
Division File: NDA 21-821
HFD-520 (CSO/Milstein)
HFD-520 (MO/Cooper)
HFD-520 (Microbiology/Marsik)
HFD-880 (Division File, Lazor, Selen, Jarugula, Tworzyanski)
CDR (Clin. Pharm./Biopharm.)

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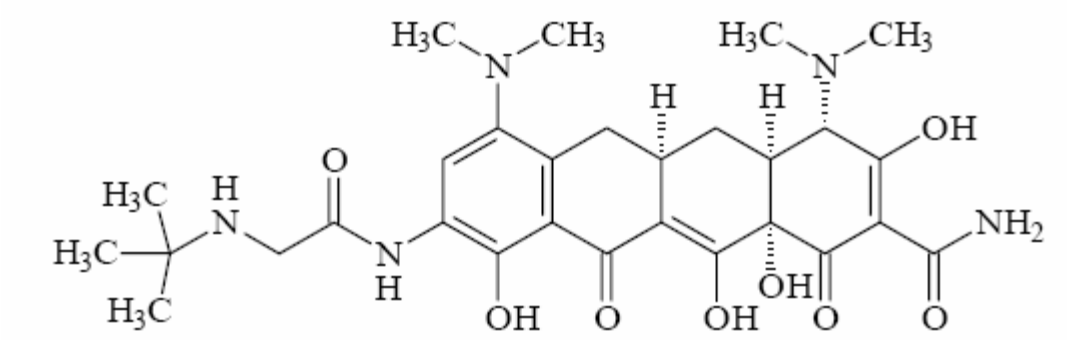
2 QUESTION BASED REVIEW

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 clinical pharmacology and biopharmaceutics review?

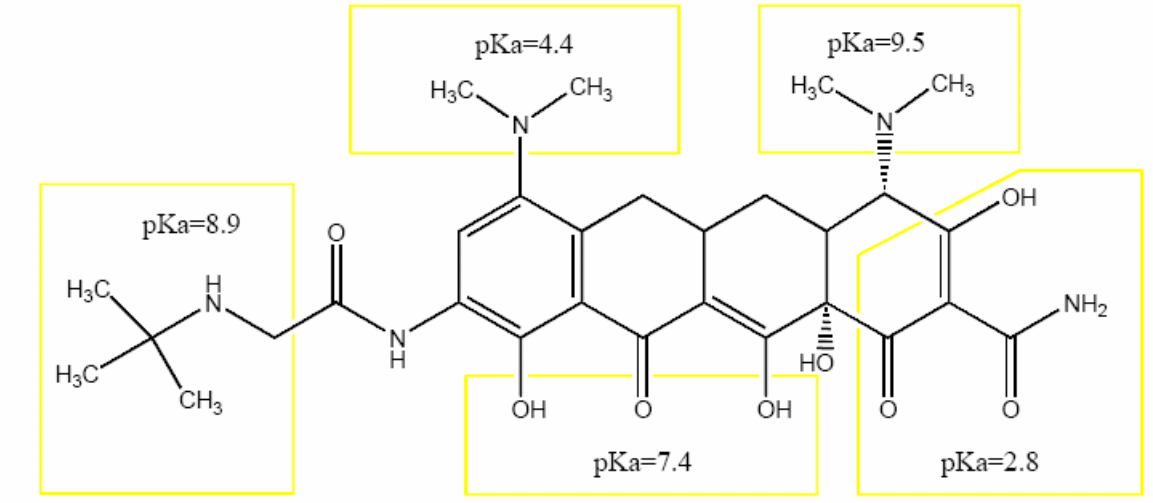
Tigecycline is a glycylcycline antibacterial for intravenous infusion. The chemical name of tigecycline is (4*S*, 4a*S*, 5a*R*, 12a*S*)-9-[2-(*tert*-butylamino)acetamido]-4,7-bis(dimethylamino)-1, 4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 10, 12, 12a-tetrahydoxyl-1, 11-dioxo-2-naphthacenecarboxamide. The empirical formula is $C_{29}H_{39}N_5O_8$ and the molecular weight is 585.65. The chemical structure of tigecycline is shown below:



Tigecycline for injection is supplied as an orange lypholized powder or cake. Each Tygacil vial contains 50mg lypholized powder for intravenous infusion. The product does not contain excipients or preservatives.

Tigecycline is freely soluble [redacted] when titrated with [redacted] in a wide pH range of [redacted] Tigecycline has five ionizable groups, three basic nitrogens and two acidic hydroxyl groups. The pKa's are expected to overlap and assignment of each pKa to its respective functional group is difficult. The NMR method was employed by the sponsor because it can determine the overlapping pKa values as well as allow the assignment of each pKa to its respective functional group. Figure 1 shows the chemical structure with pKa values determined by NMR.

Figure 1. Tigecycline pKa Values



Tigecycline's solubility is independent of pH because no changes in solubility were seen in a wide pH range.

2.1.2 What are the proposed mechanism(s) of action and therapeutic indication(s)?
The bacterial ribosome is the target for the tetracyclines. Binding to the 30S ribosomal subunit at the A-site blocks entry of amino-acyl transfer RNA molecules into the ribosome, preventing incorporation of amino acid residues into elongating peptide chains. Tigecycline has in its chemical structure a 9-glycyl amido residue substitution that allows for novel drug-amino acid interactions at binding sites. The sponsor's in vitro data suggest that tigecycline has a ribosomal binding site affinity that is at least 10-fold greater than tetracycline-class antibiotics. Ribosomal protection and efflux are the two major resistance mechanisms for tetracyclines. Tigecycline is active against strains with either mechanism of tetracycline resistance. In general, tigecycline is considered bacteriostatic.

The sponsor is seeking an indication for the treatment of complicated skin and skin structure infections caused by *Escherichia coli*, *Enterococcus faecalis* (vancomycin-susceptible strains only), *Staphylococcus aureus* (methicillin-susceptible and –resistant strains), *Streptococcus agalactiae*, *Streptococcus anginosus* grp. (includes *S. anginosus*, *S. intermedius*, and *S. constellatus*), *Streptococcus pyogenes* and *Bacteroides fragilis*. The sponsor is also seeking an indication for complicated intra-abdominal infections caused by *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterococcus faecalis* (vancomycin-susceptible strains only), *Staphylococcus aureus* (methicillin-susceptible strains only), *Streptococcus anginosus* grp. (includes *S. anginosus*, *S. intermedius*, and *S. constellatus*), *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*, *Bacteroides vulgatus*, *Clostridium perfringens*, and *Peptostreptococcus micros*.

2.1.3 What are the proposed dosage(s) and route(s) of administration?
The proposed dosage regimen for intravenous tigecycline is an initial dose of 100mg, followed by a dose of 50mg administered every 12 hours following the initial dose. These doses are administered over approximately 30 to 60 minutes. The proposed duration of treatment with tigecycline for complicated skin and skin structure infections or for complicated intra-abdominal infections is 5-14 days, depending upon severity and site of infection.

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?
The sponsor performed 17 Phase I studies to assess the single dose and multiple dose effects of safety, tolerability, pharmacokinetics, metabolic disposition, renally impaired patients, hepatically impaired patients, and drug interactions (digoxin, warfarin) with tigecycline.

The sponsor performed two Phase 2 studies (3074A1-200-Us and 3074A1-202-US) evaluating the safety and efficacy of tigecycline in subjects with cSSSI and cIAI.
In study 3074A1-200-US subjects were randomized to receive either an initial 100 mg IV tigecycline load dose, followed by 50 mg tigecycline IV q12h (n=54), or 50mg IV tigecycline load dose, followed by 25 mg tigecycline IV q12h (n=55) for the treatment of cSSSI for seven to 14 days. In study 3074A1-202-US subjects received an initial 100mg IV tigecycline load followed by 50 mg IV tigecycline q12h for the treatment of cIAI (n=66) for at least five days but not more than 14 days of therapy.

The sponsor performed four Phase 3 studies evaluating the safety and efficacy of tigecycline for the treatment of cSSSI and cIAI. Table 1 shows the features of these studies.

Table 1. Phase 3 Study Features

Study	Indications	Study Drug (number of subjects)	Comparator Drug (number of subjects)
3074A1-300-US/CA	Treatment of known or suspected diagnosis of cSSSI	Tigecycline 100mg load followed by 50mg IV q12h x 14days (n=292)	Vancomycin 1 g IV followed by Aztreonam 2g IV q12h (n=281)
3074A1-305-WW	Treatment of hospitalized subjects with cSSSI	Tigecycline 100mg load followed by 50mg IV q12h x 14days (n=281)	Vancomycin 1g IV followed byAztreonam 2 g IV q12h (n=269)
3074A1-301-WW	Treatment of cIAI	Tigecycline 100mg load followed by 50mg IV q12h x 14 days (n=413)	Imipenem/cilistatin IV q6h dose adjusted based upon weight, CrCl (n=412)
3074A1-306-WW	Treatment of cIAI	Tigecycline 100mg load followed by 50mg IV q12h x 14days (n=404)	Imipenem/cilistatin IV q6h dose adjusted upon weight, CrCl

			(n=413)

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?

cSSSI:
The primary efficacy endpoint was the assessment of clinical response at the test of cure (TOC) visit. The TOC visit was at least 12 but not more than 92 days after the last dose of study drug. Clinical response was measured according to predefined criteria. The secondary efficacy endpoints were microbiological response from specimens obtained from 2 sets of blood cultures, aerobic and anaerobic cultures from the primary site of infection.

cIAI:
The primary efficacy endpoint was the TOC assessment. The TOC visit was at least 12 but not more than 44 days after the last dose of study drug. Clinical response was measured according to predefined criteria. Microbiologic efficacy was evaluated by two sets of blood cultures, aerobic and anaerobic cultures from the primary intra-abdominal site of infection at baseline. The outcome of the microbiologic response at the subject level and pathogen level was described according to predefined criteria.

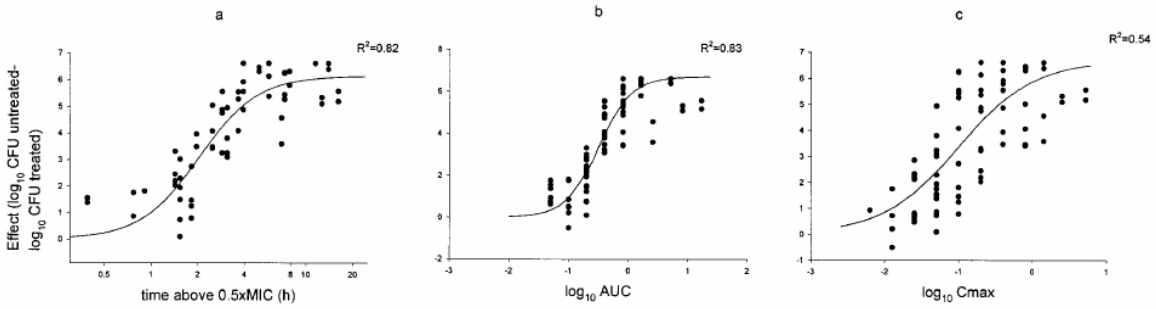
2.2.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?
Yes, refer to 2.6, Analytical Section

2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy?

Animal studies:
The exposure-response relationship of tigecycline has been evaluated using in vivo animal models of infection. A murine thigh model study was performed by (b) (4). Six week old, specific-pathogen-free female ICR/Swiss mice (weight 23 to 25 g) from Sprague-Dawley were used. The mice were rendered neutropenic (<100 neutrophils/µl) by injecting a dose of cyclophosphamide intraperitoneally at four days (150mg/kg) and one day (100 mg/kg) before the infection experiment. After a 1:10 dilution of pneumococci in fresh broth, 0.1ml (~10^6 CFU) was injected into the thighs of ether-anesthetized mice. Mice were treated for 24 hours with total doses in the range of 0.19 to 24 mg/kg body weight/day for the experiments with the strains of *S. pneumoniae.* Antibiotics were administered subcutaneously in 0.2ml volumes beginning 2 hours after thigh inoculation. Mouse thighs were removed and homogenized and cultured quantitatively. The level of detection of this assay was (b) (4)/thigh. Single-dose pharmacokinetic studies were performed with sera from thigh-infected mice with the following doses of tigecycline: 3, 12, and 48 mg/kg. The pharmacokinetics appeared to

be nonlinear, resulting in a higher elimination t½ at a dose of 48mg/kg. The results of the pharmacokinetic-pharmacodynamic parameters for tigecycline are shown in figure 2.

Figure 2. Relationship between pharmacokinetic-pharmacodynamic parameters and therapeutic efficacy of tigecycline (free drug) against S. pneumnoniae 1199 in the neutropenic mouse thigh muscle infection model



(R^2 = 0.82, 0.83, and 0.54 for panels a, b, and c, respectively)
(a) time above the 0.5 x MIC versus effect
(b) Log AUC versus effect
(c) Log C_{max} versus effect

The pharmacokinetic parameter that correlated best with efficacy was the time above a certain factor times the MIC for five of the six organism-drug combinations studies. The magnitude of this factor varied from 0.5 to 4. The only exception to this observation was the combination of tigecycline and *S. pneumoniae* 1199, for which both AUC and time above MIC were important in predicting outcome (R^2 = 0.83 and 0.82, respectively). The results of the study indicated that tigecycline exhibited time-dependent antimicrobial activity in vivo. However, due to the relatively long t½ and the long post-antibiotic effect (PAE), the AUC was also reasonably predictive, with slightly lower R^2 values.

A murine intraperitoneal model was used for tigecycline to determine PK in CD-1 mice after 0.15, 1, 4, 10 and 20mg/kg doses. Tigecycline concentrations in blood were assayed by HPLC methods at various times after dosing. The pharmacokinetic analysis indicates that AUC is linear over the dose range tested. Table 2 presents the range of AUCs and AUC/MIC from the murine intraperitoneal test performed at Wyeth (MIRACL-26501; MIRACL-26884; GTR-28013; MIRACL-25770).

Table 2. Range of AUCs and AUC/MICs in the mouse Intraperitoneal Infection Model

Organism [N]	MIC Ranges (μg/mL)	ED_{50} Ranges (mg/kg)	AUC Ranges μg x hr/ml	AUC/MIC Ranges
S. aureus [15]	0.06 – 0.25	0.39 - 4.0	1 - 4	8 - 67
E. coli [6]	0.06 – 0.12	1.50 - 3.9	2 – 4	17 – 33
E. faecalis [1]	0.06	1.0	2	23
S. pneumoniae [3]	0.015	0.54 – 1.7	1 – 2.0	67 - 133

a. MIRACL-26501; MIRACL-26884; GTR-28013; MIRACL-25770.

Human studies

Dose selection:

Animal studies have shown that both AUC and the T>MIC ratio as important parameters for efficacy and tigecycline was efficacious in animals at AUC exposures comparable to those observed in humans with a 50 to 100mg total daily dose. Based on this data, doses of 25mg and 50mg every 12 hours were studied in Phase 2. A Phase 2 study for cSSSI showed that the cure rate was higher in the 50mg q12h group (75%) than the 25mg q12h dose group (67%). Phase 1 studies showed tigecycline was tolerable up to 50mg q12 and 75mg q12 was not well tolerated because of high incidence of nausea and vomiting. Therefore, the sponsor selected the current dose regimen of 100mg load followed by 50mg q12h for Phase 3 studies.

Efficacy:

Even though good exposure-response relationships were established in animal model (see 2.2.4.1), the limited exposure levels studied in patients and high success rate in clinical trials made the exposure-response analysis quite challenging in human studies. For cIAI microbiological efficacy, 1 phase 2 study (3074A1-202: 19 patients, 32 observations) and 2 phase 3 studies (3074A1-301: 12 patients, 17 observations; 3074A1-306: 40 patients, 57 observations) were combined for exposure-response analysis at the pathogen level (Table 3). Formal statistical analysis, however, could not be applied unless cohorts 1, 2 and 3 are combined due to the low failure rate in each single cohort. The sponsor found that AUCss(0-24)/MIC, as a continuous covariate, was a borderline statistically significant predictor of microbiologic response for cohorts 1, 2 and 3 combined. Under the proposed dosage regimen (100mg loading dose and 50mg maintenance dose), the exposure change did not cause clinically relevant difference on microbiological efficacy as indicated by Figure 3.

Table 3: Baseline Pathogen Classification Used in Exposure-Response Analysis for cIAI Efficacy

Cohort	Baseline Pathogen(s) Included
1	Monomicrobial *E. coli*infections
2	Other monomicrobial or polymicrobial Gram-negative infections (*Klebsiella*spp., *Enterobacter*spp. and/or *Citrobacter*spp. plus or minus *E. coli*)
3	Infections with at least one Gram-negative pathogen plus at least one anaerobic pathogen

4	Infections with at least one Gram-negative pathogen plus at least one Gram-positive pathogen
5	All other monomicrobial or polymicrobial infections

For cIAI clinical efficacy, similar results were obtained. Both baseline APACHE II score and AUCss(0-24)/MIC were identified as statistically significant predictors of clinical response based on univariate logistic analysis. Under the proposed dosage regimen (100mg loading dose and 50mg maintenance dose), the exposure change did not cause clinically relevant difference on clinical efficacy as indicated by Figure 4.

Figure 3. Exposure-Response Analysis of cIAI Efficacy - Final Logistic Regression Model of Microbiological Response versus AUCSS(0-24)/MIC Ratio with Histogram of Observed Data (71 patients with 106 observations.)

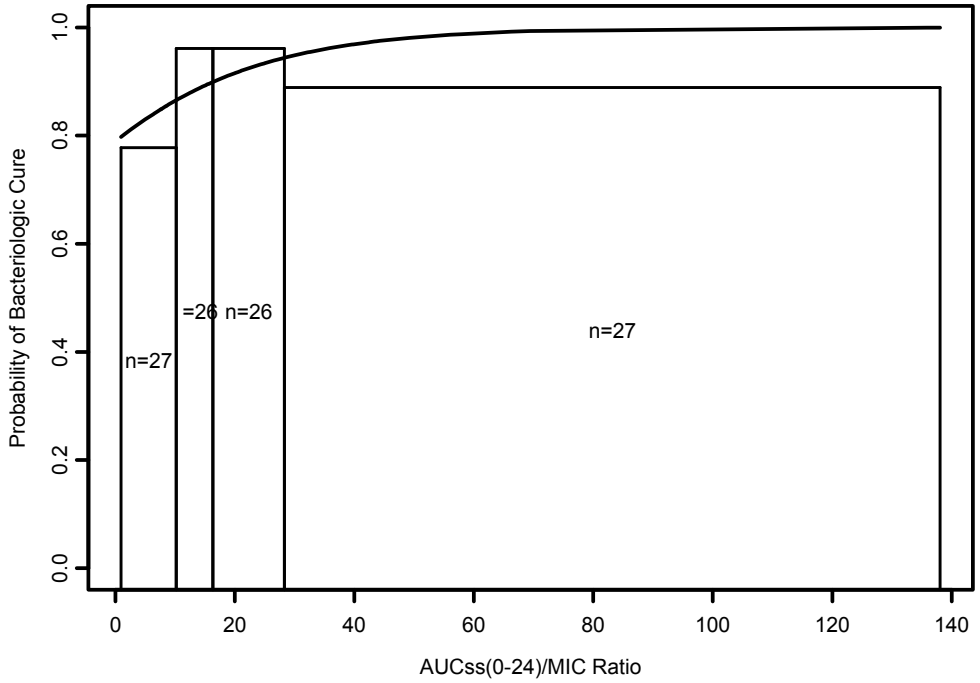
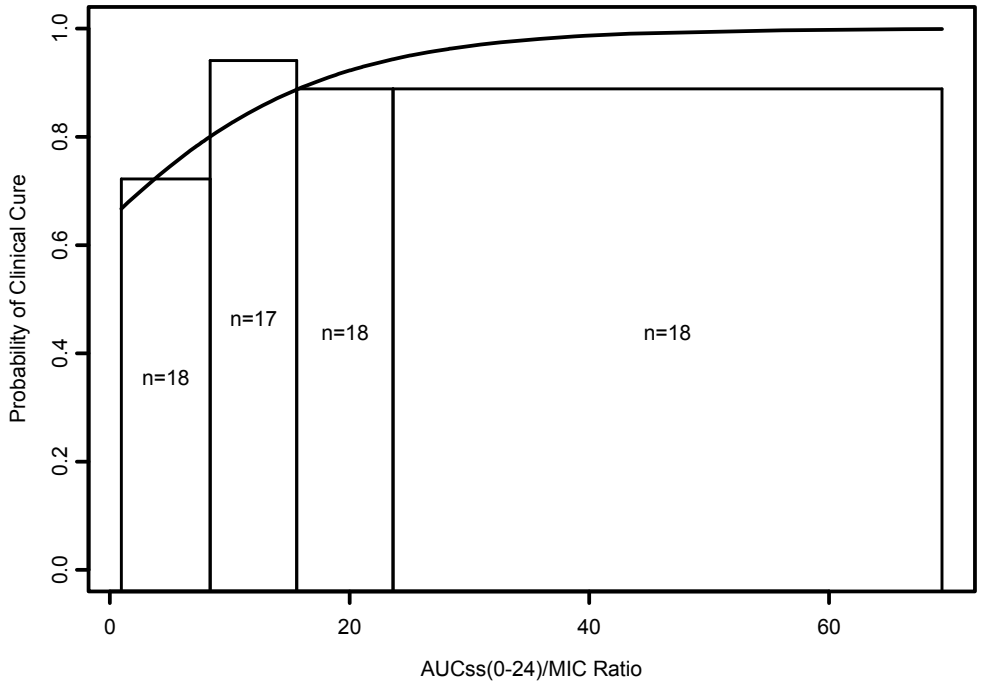


Figure 4. Exposure-Response Analysis of cIAI Efficacy - Final Logistic Regression Model of Clinical Response versus AUCSS(0-24)/MIC with Histogram of Observed Data (71 patients)



For cSSSI microbiological efficacy, 1 phase 2 study (3074A1-200: 25 patients) and 2 phase 3 studies (3074A1-300: 2 patients; 3074A1-305: 9 patients) were combined for exposure-response analysis at the patient level (Table 4). Formal statistical analysis, however, could not be applied unless cohorts 2 and 3 are combined due to the low failure rate in each single cohort. The sponsor found that AUCss(0-24)/MIC, as a continuous covariate, was a borderline statistically significant (p=0.113) predictor of microbiologic response for cohorts 2 and 3 combined. Under the proposed dosage regimen (100mg loading dose and 50mg maintenance dose), the exposure change did not cause clinically relevant difference on microbiological efficacy as indicated by Figure5.

Table 4: Baseline Pathogen Classification Used in Exposure-Response Analysis for cSSSI Efficacy

Cohort	Baseline Pathogen(s) Included
1	Monomicrobial *S. aureus*
2	Monomicrobial *S. aureus* or *Streptococcus* spp.
3	Polymicrobial *S. aureus* plus *Streptococcus* spp. or two *Strep.* spp.
4	Other polymicrobial Gram-positive and/or Gram-negative pathogens
5	Gram-negative or anaerobic monomicrobial pathogens

For cSSSI clinical efficacy, similar results were obtained. Both AUCss(0-24) (p=0.065) and AUCss(0-24)/MIC (p=0.1723) were identified as marginally significant predictors of clinical response based on univariate logistic analysis. Under the proposed dosage regimen (100mg loading dose and 50mg maintenance dose), the exposure change did not cause clinically relevant difference on clinical efficacy as indicated by Figure 6.

Figure 5. Exposure-Response Analysis of cSSSI Efficacy - Final Logistic Regression Model of Microbiological Response versus AUCSS(0-24)/MIC Ratio with Histogram of Observed Data in Cohorts 2 and 3 and AUCSS(0-24)/MIC Ratio Range under Each Dose (36 subjects)

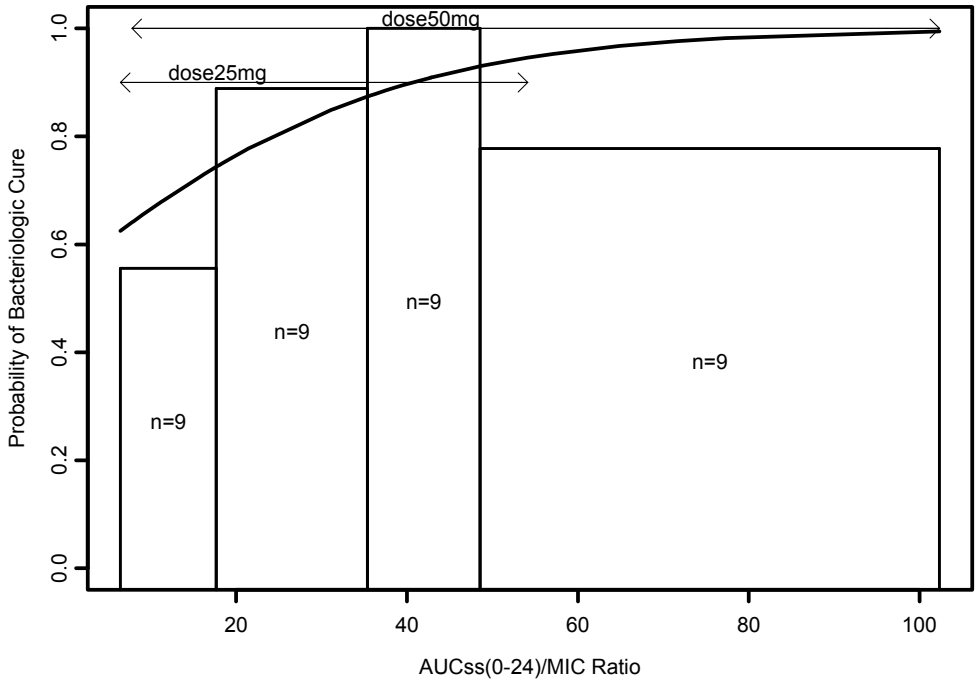
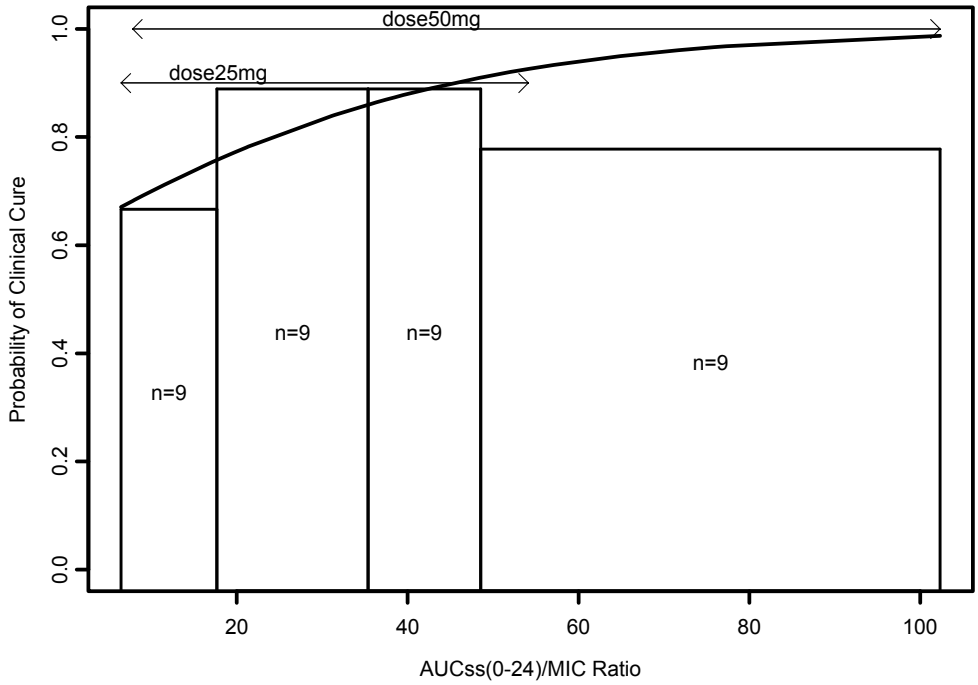


Figure 6. Exposure-Response Analysis of cSSSI Efficacy - Final Logistic Regression Model of Clinical Response versus $AUC_{SS(0-24)}$/MIC Ratio with Histogram of Observed Data in Cohorts 2 and 3 and $AUC_{SS(0-24)}$/MIC Ratio Range under Each Dose (36 subjects)



Human Studies:

Safety:

First occurrence of nausea and vomiting was selected as the endpoint for tigecycline safety evaluation. For cIAI, only phase 2 and 3 studies were included (153 subjects from Study 3074A1-202, 513 subjects from Study 3074A1-301, and 464 subjects from Study 3074A1-306). No exposure-response relationship was established within the observed exposure levels in these studies for safety. The analysis based on phase 1 studies (84 subjects from Study 3074A1-100, 46 subjects from Study 3074A1-102, and 6 subjects from Study 3074A1-103) showed AUC0-inf and Cmax were significant predictors for both first nausea and first vomiting occurrence due to the wide range of doses studied (Figures 7 and 8). The lack of exposure-response relationship for the first nausea and first vomiting occurrence in phase 2/3 studies was due to the narrow range of exposure under the two doses, 25mg and 50mg (Figure 9) and the further shrinkage of the exposure range based on empirical Bayesian estimates.

Even though an exposure-risk relationship was observed in healthy subjects, special populations, such as those with renal or hepatic impairment, did not show increased risk of nausea or vomiting despite the increased exposure of tigecycline (Tables 15 and 16). In fact, no nausea or vomiting was reported in any of the hepatically impaired patients. However, these special population studies included small sample size (n=5-10). Dose adjustment may not be necessary based on the risk/benefit for this indication except that the patients with severe hepatic impairment (Child-Pugh C) should receive half of the standard maintenance dose from PK considerations.

Figure 7. Exposure-Response Analyses of Phase 1 Safety - Final Logistic Regression Model of First Nausea Occurrence versus AUC(0-∞) with Histogram of Observed Data and AUC(0-∞) Range under Each Dose (N=135)

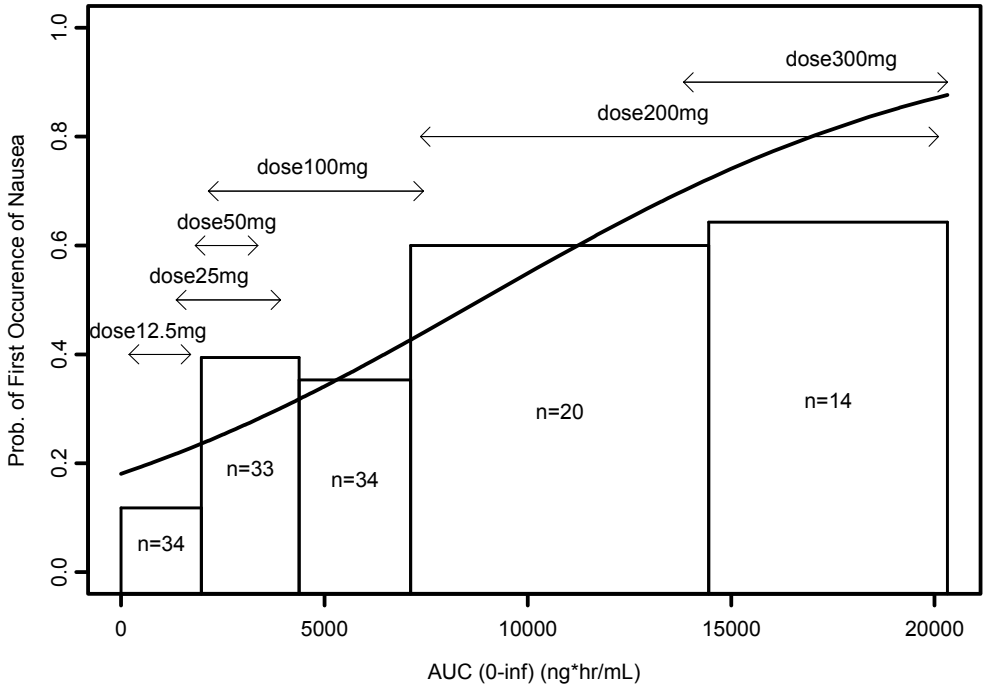


Figure 8. Exposure-Response Analyses of Phase 1 Safety - Final Logistic Regression Model of First Vomiting Occurrence versus AUC(0-∞) with Histogram of Observed Data and AUC(0-∞) Range under Each Dose (N=135)

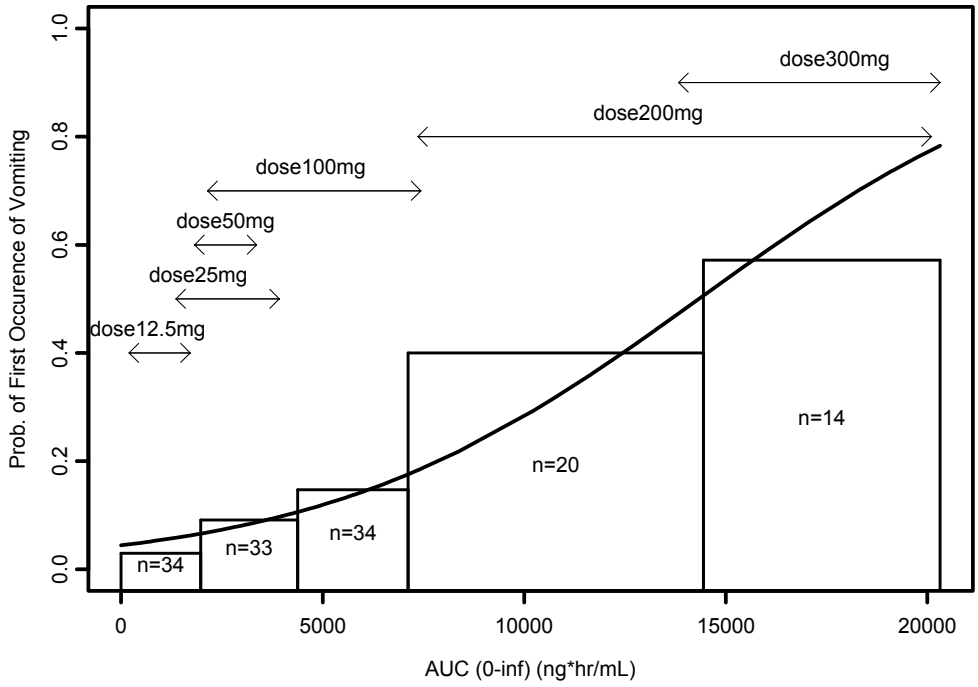
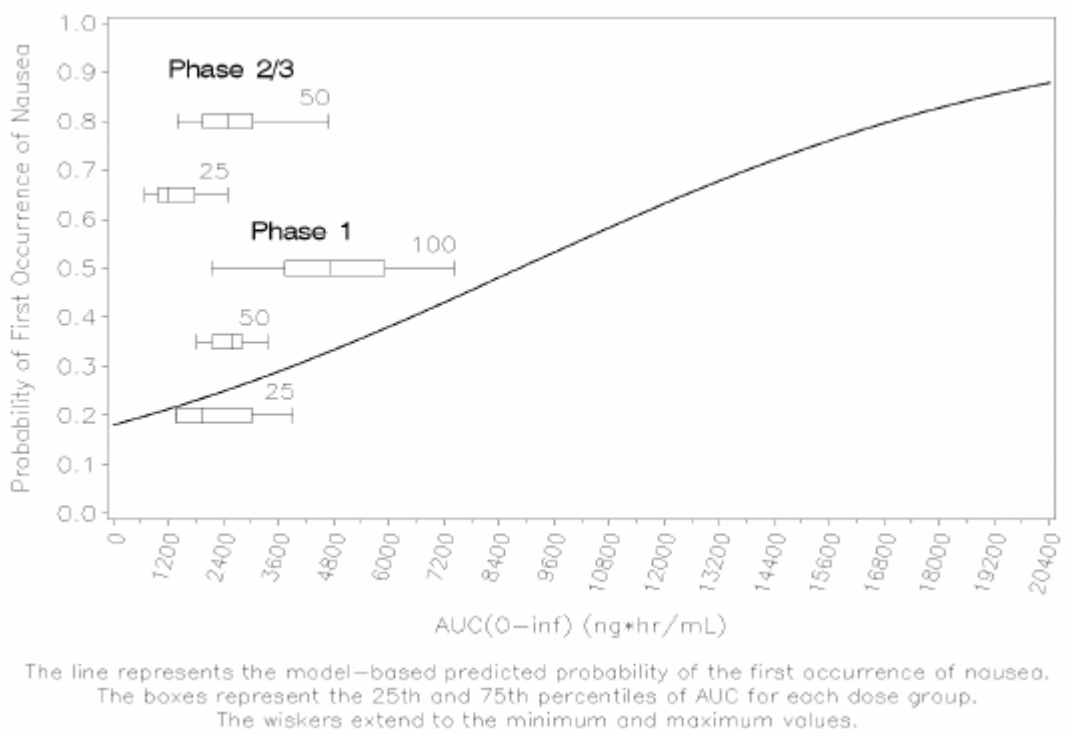


Figure 9. Exposure-Response Analyses of Phase 1 Safety - Final Logistic Regression Model of First Nausea Occurence versus AUC(0-∞) with Boxplots of AUC Values for Phases 1 and 2/3



2.2.4.3 Does this drug prolong the QT or QTc interval?

Tigecycline is a synthetic derivative of minocycline which belongs to tetracycline class of antibiotics. Clinically, tetracycline class is not known to have problems related to QT prolongation effect. Thus, the sponsor did not conduct a positive-controlled, thorough QT study. Sponsor proposed to evaluate ECGs measured in phase 1, 2 and 3 studies in addition to preclinical studies.

In preclinical studies, dogs were administered tigecycline by a single 30-minute IV infusion at dosages of 0, 2, 5, and 12 mg/kg using an escalating-dose design, yielding exposures (AUC) up to approximately six times higher than the proposed clinical dose. Arterial blood pressure (systolic, diastolic, and mean), heart rate, electrocardiogram (ECG), and spontaneous gross motor activity were monitored by telemetry every 30 minutes for 24 hours before and after dosing. There were no tigecycline related effects on ECG, including QT interval, at any dosage.

In Phase I clinical pharmacology studies, the majority of the ECG interval data were collected from investigator read ECG's, however for some studies [100 101, 102, 103, 105 (except for 18 subjects), and 109] the ECG's were evaluated by a central ECG lab (b) (4). These are listed as "centrally read" ECG's in the following tables. If a study had both investigator and centrally read ECG's, only the centrally read ECG data are used in the analysis. All ECG data were examined to identify individual subjects who had changes of potential clinical importance (PCI). ECG changes that met the pre-determined

PCI's were identified and the medical monitor assessed the clinical importance of these findings. A total of 12 of 205 (5.9%) tigecycline treated subjects and 1 of 38 (2.6%) placebo-treated subjects had centrally read ECG's of potential clinical importance. A categorical analyses of Phase I/clinical pharmacology ECG results were performed and the results are displayed in Tables 5 and 6.

The sponsor has not done an analysis of central tendencies on phase 1 studies due to the small sample size of subjects, different study designs and infrequent sampling of ECG's.

Table 5. Summary of Change in QTc from Baseline in Phase I/Clinical Pharmacology Studies: Number/Number of Subjects Tested in Safety Population

	Healthy Subjects				
				Interaction Studies	
Category[a] Test[b]	Total Placebo (n = 55)	Single Dose (n = 141)	Multiple Dose (n = 112)	Tigecycline Treated[c] (n = 44)	Warfarin/Digoxin Only[d] (n = 49)
ECG					
QTc Interval					
0 ≤ Increase < 30 msec	12/ 16 (75.0)	34/ 40 (85.0)	36/ 67 (53.7)	32/ 44 (72.7)	35/ 49 (71.4)
30 ≤ Increase < 60 msec	0/ 16	0/ 40	3/ 67 (4.5)	7/ 44 (15.9)	3/ 49 (6.1)
Increase ≥ 60 msec	0/ 16	1/ 40 (2.5)	1/ 67 (1.5)	0/ 44	1/ 49 (2.0)
Centrally ECG				-	-
QTc (B)	35/ 38 (92.1)	86/101 (85.1)	39/ 41 (95.1)		
0 ≤ Increase < 30 msec	25/ 38 (65.8)	58/101 (57.4)	18/ 41 (43.9)	-	-
30 ≤ Increase < 60 msec	5/ 38 (13.2)	22/101 (21.8)	5/ 41 (12.2)	-	-
Increase ≥ 60 msec	0/ 38	0/101	0/ 41	-	-
QTc (F)					
0 ≤ Increase < 30 msec	32/ 38 (84.2)	80/101 (79.2)	32/ 41 (78.0)	-	-
30 ≤ Increase < 60 msec	1/ 38 (2.6)	5/101 (5.0)	7/ 41 (17.1)	-	-
Increase ≥ 60 msec	0/ 38	0/101	0/ 41	-	-

Abbreviations: QTc (B) = Bazett's correction; QTc (F) = Fridericia's correction.
a. If a manual reading was done for a parameter, it was used for this analysis. Otherwise, the electronic reading was used.
b. Only the tests performed between the time of first dose and last dose + 5 days were included in this analysis.
c. Events presented in this column occurred during the study periods where subjects were on tigecycline only, or in tigecycline in combination with either warfarin or digoxin.
d. Events presented in this column occurred during the study periods where subjects were on warfarin only, or digoxin only.
Protocols included in this analysis: 100, 101, 102, 103, 104, 105, 106, 109, 111, 112, 113, 115 and 116.

Table 6. Summary of Change in QTc from Baseline in Phase I/Clinical Pharmacology Studies: Safety Population

Category[a] Test[b]	Special Population: Renally Impaired (n = 14)	Hepatically Impaired (n = 25)	Healthy Subjects Age > 55 yrs (n = 34)	Total Tigecycline Treated[c] (n = 424)
ECG	1/ 1 (100)	5/ 8 (62.5)	1/ 1 (100)	121/161 (75.2)
QTC Interval				
0 ≤ Increase < 30 msec	1/ 1 (100)	5/ 8 (62.5)	1/ 1 (100)	109/161 (67.7)
30 ≤ Increase < 60 msec	0/ 1	0/ 8	0/ 1	10/161 (6.2)
Increase ≥ 60 msec	0/ 1	0/ 8	0/ 1	2/161 (1.2)
Centrally Read ECG	8/ 13 (61.5)	14/ 17 (82.4)	24/ 33 (72.7)	171/205 (83.4)
QTc (B)				
0 ≤ Increase < 30 msec	5/ 13 (38.5)	11/ 17 (64.7)	22/ 33 (66.7)	114/205 (55.6)
30 ≤ Increase < 60 msec	2/ 13 (15.4)	1/ 17 (5.9)	1/ 33 (3.0)	31/205 (15.1)
Increase ≥ 60 msec	0/ 13	0/ 17	0/ 33	0/205
QTc (F)				
0 ≤ Increase < 30 msec	6/ 13 (46.2)	14/ 17 (82.4)	19/ 33 (57.6)	151/205 (73.7)
30 ≤ Increase < 60 msec	2/ 13 (15.4)	0/ 17	0/ 33	14/205 (6.8)
Increase ≥ 60 msec	0/ 13	0/ 17	0/ 33	0/205

Abbreviations: QTc (B) = Bazett's correction; QTc (F) = Fridericia's correction.
a. If a manual reading was done for a parameter, it is used for this analysis. Otherwise, the electronic reading is used.
b. Only the tests performed between the time of first dose and last dose + 5 days are included in this analysis.
c. This column presents the combined number of healthy subjects and special populations who were treated with tigecycline in all phase 1/clinical pharmacology studies included in part a and part b of this table. Phase 1/clinical pharmacology studies included in this analysis: 100, 101, 102, 103, 104, 105, 106, 109, 111, 112, 113, 115 and 116.

From the centrally read ECG's, there were no tigecycline-treated subjects who had a ≥ 60 msec increase in QTc, and the frequency of QTc increase of 30 ≤ and < 60 was similar between the tigecycline and then placebo-treated subjects (15.1% vs 13.2%, respectively. From the investigator read ECG's, there were two subjects with a ≥ 60 msec increase in QTc. One of these increases was actually the result of data entry error for the baseline QTc interval for subject 106-001-0037. This subject's baseline was entered as 286msec, with the actual value being 386 msec. With the correct baseline, the maximum increase in QTc for this subject is 49 msec. the other subject, 112-001-0029, was a 29 year old male in the multiple dose intrapulmonary PK study. His pre-dose (baseline) QTc was 393 msec, and increased to 461 msec on Day 4 (an apparent increase of 68 msec). At screening however, his QTc was 431 msec. No subjects with renal impairment, hepatic impairment, or subjects older than 55 years had elevated QTc ≥ 60 msec.

In a Phase II trial, the results of the analysis of ECG parameters at the time of peak tigecycline plasma concentrations, including the analysis of the interval by using Bazett's correction, Fridericia's correction, and the Linear Model correction are presented in Table 7.

Table 7. Analysis of ECG Parameters at Peak Time (One hour after the start of the Morning Dose)

ECG Parameter	n	Mean Change	SEM	SD	Median Change	95% CI for Median	Within-Group p-Value
ECG Heart Rate (bpm)	88	-14.4	1.8	17.1	-15.0	-18.0, -11.0	<0.001
PR Interval (msec)	85	1.4	1.6	14.3	3.0	-1.0, 6.0	0.184
QRS Interval (msec)	88	1.3	0.8	7.1	1.0	0.0, 4.0	0.044
RR Interval (msec)	88	108.6	13.7	128.1	107.0	78.0, 127.0	<0.001
QT Interval (msec)	88	24.0	3.8	36.0	21.5	14.0, 34.0	<0.001
QTc(B) Interval (msec)	88	-2.0	2.9	27.6	-3.0	-10.0, 4.0	0.396
QTc(F) Interval (msec)	88	7.9	3.0	27.7	8.5	-2.0, 14.0	0.007
QTc(L) Interval (msec)	88	-3.3	2.6	24.0	-4.9	-8.8, 0.6	0.180

Note: Subjects received an initial loading dose of 100 mg tigecycline followed by 50 mg every 12 hours.
Abbreviations: SEM = standard error of the mean; SD = standard deviation; CI = confidence interval; QTc(B) = Bazett's Correction; QTc(F) = Fridericia's Correction; QTc(L) = Linear Model Correction.

A mean change in QTc of 7.9 msec (95% CI for median -2.0, 14.0) from baseline was reported based on Fridericia's correction method. However, with other methods (Bazett and Linear) the mean change in QTc was negative. The difference in results between different methods of correction could be due to the observed effect of tigecycline on heart rate (a mean change of -14.4 bpm).

Changes from the baseline for the corrected QTc values were categorized as <30 msec, 30 to 60 msec, >60 msec. By using the Bazett's, Fridericia's, and the Linear Model Corrections, respectively, 2.3%, 6.8%, and 1.1% of the subjects had increases from baseline in QTc values > 60 msec, as shown in Table 8.

Table 8. Frequency Tabulation for the Peak of the Day 3 Morning Dose versus Baseline: Subjects with increase in QTc Values > 60 msec

Correction Formula	Number (%) of Subjects With Increase in QTc Value > 60 msec From Baseline
Bazett	2/88 (2.3)
Fridericia	6/88 (6.8)
Linear	1/88 (1.1)

Note: Subjects received an initial loading dose of 100 mg tigecycline followed by 50 mg every 12 hours.

Due to the limited sample size and lack of a control group in this trial, the clinical importance of these changes is not clear.

An assessment of QTc was performed in the Phase III studies at baseline and on study day 3 or 4 (at 3hrs after the start of infusion), using the Bazett, Fridericia and log-linear

(L) correction methods. The results of the pooled four Phase III studies are shown in Table 9.

Table 9. Changes in Median Baseline QTc Intervals (ms) within 3 hours after dosing on days 3, 4, or 5 using Non-Parametric Analysis of Studies 300-US/CA, 305-WW, 301-WW, and 306-WW: Safety Population

	Tigecycline				Comparator[a]				
Parameter (units) Time period	n[c]	Median	Median Change	Within Group 95% CI	n[c]	Median	Median Change	Within Group 95% CI	Between Group 95% CI[b]
QTc (L), msec									
Screening/day 1	773	396.2			788	394.5			
Day 3/4 or 5	773	398.9	3.3	2, 5	788	397.4	0.6	-1, 3	0.4, 4.9
Test-of-cure	665	394.2	-2.5	-5, -1	697	395.1	-0.8	-3, 1	-4.2, 0.9
QT (B), msec									
Screening/day 1	773	413.0			788	413.0			
Day 3/4 or 5	773	410.0	-2.0	-4, 0	788	408.0	-4.0	-7, -2	-1.0, 4.0
Test-of-cure	665	403.0	-9.0	-11, -7	697	406.0	-7.0	-9, -5	-5.0, 1.0
QT (F), msec									
Screening/day 1	773	389.0			788	388.0			
Day 3/4 or 5	773	396.0	6.0	4, 7	788	392.5	3.0	2, 5	1.0, 5.0
Test-of-cure	665	391.0	1.0	-2, 3	697	391.0	2.0	0, 4	-4.0, 1.0

Note: QTc (B) = Bazett's correction; QTc (F) = Fridericia's correction; QTc(L) = log linear regression correction; CI = confidence interval.

a. Comparator refers to vancomycin/aztreonam for studies 300-US/CA and 305-WW, imipenem/cilastatin for studies 301-WW and 306-WW.

b. Analysis of variance is based on the change from baseline to day 3, 4, or 5, 3 hours after the dosing.

c. Subjects who had ECG evaluation at the 3 visits (screening, day 3, 4, or 5 and the test-of-cure).

Source: Biostat ecg, 13 Oct 2004

The results of the pooled four Phase 3 studies (300/305 and 301/306), across both indications, showed that a significantly greater median QTc (L) change occurred in the tigecycline (3.3 msec; 95% CI: 2.0, 5.0 msec) compared to the comparator group (0.6 msec; 95% CI: -1.0, 3.0 msec; 95% CI of the difference: 0.4, 4.9). With Fridericia correction method, the median change in QTc from baseline was greater (6.0 msec: 95% CI 4,7). More tigecycline than comparator subjects had increases of 30 to 60 msec and > 60 msec as well as values over the normal limit in men of 450 msec. No subjects were discontinued because of QTc prolongation. Although an effect on cardiac repolarization from tigecycline can not be definitely excluded from these phase 3 data, the magnitude of the median observed effect was small (around 3-6 milliseconds).

It should be noted that the above analysis on phase 3 data was based on ECGs measured at 3 hrs after dosing on day 3 and ECGs were not measured at the end of infusion, when the maximum plasma concentrations of tigecylcine are expected. However, the ECG's were done on day 3 and the drug extensively distributes into the tissues and has a long half life which slows elimination.

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?

Table 10 shows the PK parameters of tigecycline when administered as single dose in study (3074A1-100-EU).

Table 10. Pharmacokinetic Parameters of GAR-936 After Various Single Intravenous (IV) doses of GAR-936

	Dose Groups Receiving GAR-936 (mg)[b]											
	1-h IV Infusion									4-h IV Infusion		
PK Parameter	I 12.5 (fasting)	II 25 (fasting)	III 50 (fasting)	IV 75 (fasting)	V 100 (fasting)	VI 200 (fasting)	VIII 200 (fed)	XI 200 (fasting)[c]	IX 300 (fed)	VII 200 (fasting)	X 300 (fed)	p-Value
C_{max} (ng/mL)	108.5 (10)	252 (25)	383 (17)	566 (14)	911 (29)	1643 (18)	1528 (22)	2189 (30)	2817 (17)	680 (22)	960 (10)	0.001
AUC (ng•h/mL)	753 (68)	2255 (45)	2558 (21)	3658 (27)	6396 (10)	12426 (23)	11719 (19)	14462 (17)	17856 (10)	14237 (22)	16732 (16)	0.06
CL (L/h/kg)	0.3 (67)	0.2 (50)	0.3 (14)	0.3 (16)	0.2 (13)	0.2 (22)	0.2 (12)	0.2 (19)	0.2 (11)	0.2 (11)	0.3 (13)	0.27
V_{ss} (L/kg)	2.8 (34)	6.4 (20)	6.4 (31)	7.5 (10)	8.6 (18)	11 (25)	13 (18)	12 (43)	12 (20)	14 (11)	12 (24)	0.001
$t_{1/2}$ (h)	11 (84)	32 (64)	18 (21)	21 (25)	38 (14)	42 (28)	54 (28)	53 (20)	46 (13)	58 (14)	42 (23)	0.001
CL_R (L/h)	ND[d]	ND	ND	ND	2.6 (26)	3 (50)	2.2 (22)	1.8 (24)	2.7 (23)	NA[e]	2 (28)	0.13
f_e (%)	2.1 (113)	6.5 (39)	1.9 (72)	8 (37)	11 (25)	12 (31)	7.8 (25)	8.5 (38)	10.4 (22)	NA	8 (39)	0.001

a: Data presented as mean (CV%).
b: Dose groups are not presented in the order in which the doses were administered.
c: This group also received ondansetron (Zofran).
d: ND - Could not be determined accurately due to low concentrations in urine and serum.
e: NA – Not available.

Figure 10. Mean Serum Concentration-Time Profiles in Healthy Subjects in the Fasting State after Various Single Doses of GAR-936 Given as Intravenous Infusions

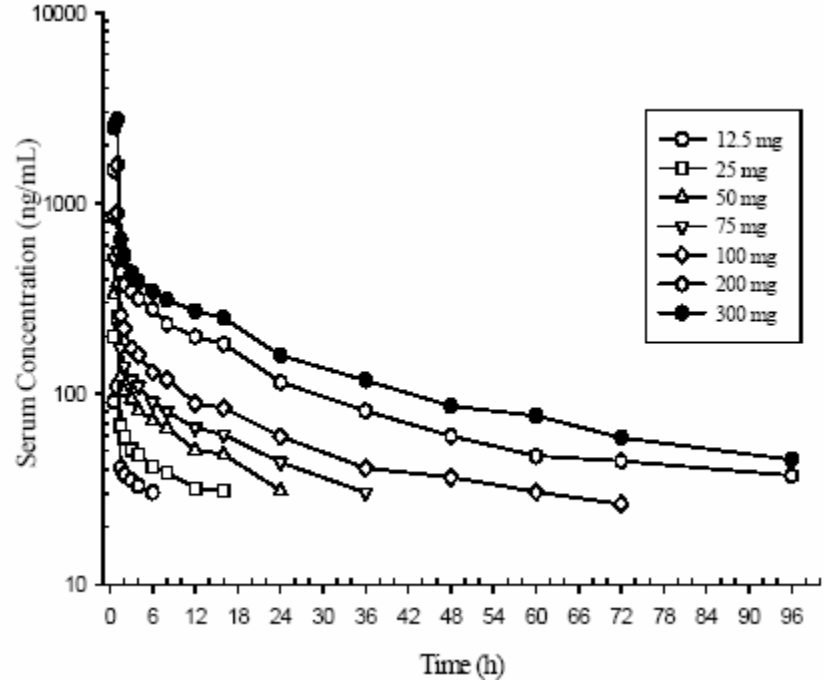


Figure 10 shows the mean serum concentration time profile of tigecycline at various dosage levels. The decline in drug concentrations after the end of the tigecycline infusion

follows a polyphasic pattern. The steep decrease in serum tigecycline concentrations at the end of infusion represents the distribution phase during which the drug is distributed out of the central compartment into various tissues. The terminal elimination phase is characterized by the movement of tigecycline from the tissues into the vascular compartment followed by subsequent elimination of tigecycline. The pharmacokinetics of tigecycline (Cmax and AUC) after single dose infusion over 60 minutes are linear over the dose range of 12.5 mg to 300 mg. The mean systemic clearance (CL) values were consistent among dose groups and ranged from 0.2 L/h/kg to 0.3 L/h/kg. Tigecycline was well distributed into various tissues as shown by the mean steady state volume of distribution (Vss), which ranged from 7 to 14 L/kg. The estimated terminal phase half life varied among dosages (ranges from 18 hrs at 50 mg dose to 42 hrs at 200 mg dose) because the terminal phase was not adequately characterized at lower doses due to assay sensitivity.

Table 11 shows the PK data from a multiple dose study (3074A1-101-US).

Table 11. Mean (CV%) Pharmacokinetic Parameters of GAR-936 after various intravenous doses infused over a 1-hour period once every 12 hours

	--------Day 1--------		----------Day 10[a]----------					
GAR-936 Dose Group (mg)	C_{max} (ng/mL)	$AUC_{0-\tau}$ (ng•h/mL)	C_{max} (ng/mL)	AUC_{ss} (ng•h/mL)	$T_{1/2}$ (h)	CL (L/h/Kg)	V_{ss} (L/Kg)	R
25	261 (14)	796 (8)	324 (17)	1482 (18)	49.3 (72)	0.2 (17)	8.6 (23)	1.3 (18)
50	487 (17)	1440 (14)	621 (15)	3069 (12)	36.9 (32)	0.2 (9)	7.2 (7)	1.3 (10)
100	816 (15)	2389 (13)	1173 (15)	4980 (19)	66.5 (34)	0.2 (20)	9.1 (32)	1.3 (18)
75	766 (16)	2149 (10)	NA[b]	NA	NA	NA	NA	NA

a: Day 10 values for the 100 mg GAR-936 dose group are actually day 9 values.
b: Not available because subjects in the 75 mg dose group withdrew from the study by day 7.

Following multiple dose administration, steady state was achieved at seven days. The observed accumulation ratio for C_{max} was 1.3 and for AUC it was 2.1. The volume of distribution at steady state for a 100mg dose was 9.1 (L/kg). The clearance remains the same (0.2 L/h/kg) at 100mg for both the single and multiple dose studies. When tigecycline is given as multiple dose administration, the serum concentration curves often show a secondary peak occurring 12 to 16 hours after administration. The secondary peaks are occasionally seen after single dose administration, but they are much less pronounced than for multiple administration. The sponsor believes that this may be due to a redistribution of tigecycline from the various tissues, including colon, which exhibits tissue concentrations approximately 2.1-fold higher than the concentrations in serum.

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

Table 12 shows the Pharmacokinetic parameters in the 307 healthy subjects in clinical pharmacology studies and 276 patients in Phase 2 and Phase 3 efficacy trials. Before calculating the summary statistics, all data were normalized to a common 100mg single dose or a 100 mg daily dose (50mg q12h), and multiple observations within a subject

were averaged together so that each subject contributed only one observation to the pooled means in the table.

Table 12. Tigecycline Pharmacokinetic Parameters From Pooled Analyses

Parameter (units)	Pooled Clinical Pharmacology Studies Single-Dose 100 mg (n=224[a])	Pooled Clinical Pharmacology Studies Steady-State 50 mg q12h (n=103)	Pooled Efficacy Studies Steady-State 50 mg q12h (n=276[b])
C_{max} (ng/mL) – 30-minute infusion	1454 ± 321 (22.1%)	866 ± 233 (26.9%)	798 ± 461 (57.7%)
C_{max} (ng/mL) – 60-minute infusion	902 ± 266 (29.5%)	634 ± 97 (15.2%)	488 ± 284 (58.1%)
C_{min} (ng/mL)	- -	132 ± 77 (58.5%)	155 ± 91 (58.6%)
$AUC_{0-\infty}$ (ng • h/mL)	5192 ± 1855 (35.7%)	- -	- -
AUC_{0-24h} (ng • h/mL)[c]	- -	4697 ± 1699 (36.2%)	5848 ± 2479 (42.4%)
$t_{½}$ (h)	27.1 ± 14.3 (52.7%)	42.4 ± 35.3 (83.4%)	- -
Cl (L/h)	21.8 ± 8.9 (40.1%)	23.8 ± 7.8 (33.0%)	19.9 ± 8.1 (40.7%)
Cl (L/h/kg)	0.31 ± 0.12 (38.5%)	0.31 ± 0.09 (31.0%)	0.25 ± 0.10 (39.6%)
Cl_r (L/h)	2.3 ± 1.9 (81.5%)	3.1 ± 1.8 (58.1%)	- -
Cl_r (mL/min)	38 ± 31 (81.5%)	51 ± 30 (58.1%)	- -
V_{ss} (L)	568 ± 244 (43.0%)	639 ± 307 (48.1%)	- -
V_{ss} (L/kg)	7.9 ± 3.5 (43.6%)	8.2 ± 3.9 (48.1%)	- -
A_e (%-dose)	9.1 ± 4.6 (51.0%)	15.7 ± 7.7 (49.0%)	- -

Abbreviations: A_e=amount of drug excreted in urine; $AUC_{0-\infty}$ area under the single-dose serum concentration-time curve from 0 to infinity; AUC_{0-24}=area under the steady-state serum concentration-time over a full day; Cl=clearance; Cl_r=renal clearance; C_{max}=peak concentration; CV=coefficient of variation; SD=standard deviation;$t_{1/2}$=terminal elimination half-life; V_{ss}=apparent steady-state volume of distribution. Values are presented as mean ± SD (CV%).
a: Excluding renally-impaired and hepatically impaired subjects.
b: Patient pharmacokinetics from sparse sampling (0, end-of-infusion, 3, and 6 hours after administration).
c: AUC_{0-24h} was calculated as 2 x AUC_{0-12h}.

In the phase 2 and 3 efficacy studies, the end of infusion samples were used to define the peak concentration (C_{max}) values, and according to the protocol, these samples were either collected at the end of the infusion or within five minutes after the end of infusion. The mean steady-state C_{max} values in the phase 2 and phase 3 subjects were 8% to 23% lower than the mean values from the pooled clinical pharmacology studies. If the end of infusion samples were collected after the infusion completed, while the tigecycline serum concentrations are dropping rapidly, the observed steady-state serum C_{max} values may be slightly underestimating the true C_{max} in this population. The mean steady-state area under the concentration-time curve (AUC) in the phase 2 and phase 3 subjects is approximately 25% higher than the mean value from the pooled clinical pharmacology studies. This difference between the healthy and the phase 2 and phase 3 subjects may be an artifact of the sparse sampling scheme (pre-dose, end of infusion, three hours, and six hours) used to evaluate the AUC in the clinical efficacy studies.

2.2.5.3 What are the characteristics of drug absorption?

Not Applicable as the proposed route of administration is intravenous.

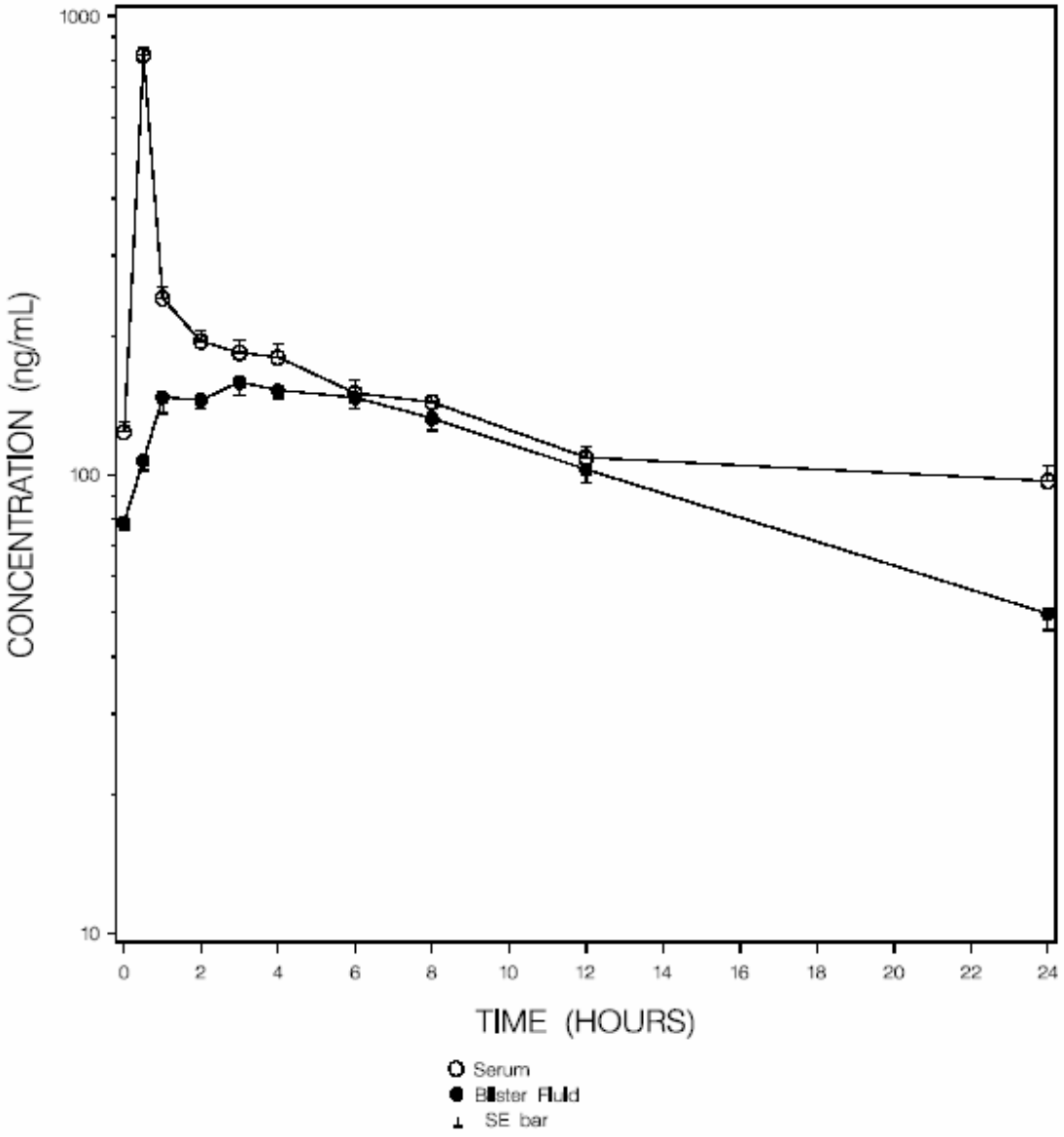
2.2.5.4 What are the characteristics of drug distribution?

Protein Binding: The in-vitro binding of [^{14}C] tigecycline in human plasma was assessed by ultrafiltration at concentrations of 0.1, 1, and 15µg/ml. An assessment of non-specific

binding of [^{14}C] tigecycline at a concentration of 10µg/ml showed an average percent binding of 3.87 ±
0.92 in buffer and 4.20 ± 0.83 in protein-free plasma filtrate. Radioactive [^{14}C] tigecycline was stable in human plasma for at least 2 hours at 37ºC. In human plasma at concentrations of 0.1, 1.0, and 15.0µg/ml, the mean (±SD) percent of unbound [^{14}C] tigecycline was 28.9 ± 1.35, 13.0 ± 0.78, and 4.70 ± 0.89%, respectively.
Another study was conducted to assess whether the concentration-dependent plasma protein binding of [^{14}C] tigecycline may be associated with minor degradants whose formation is concentration and temperature dependent. The stability of [^{14}C] tigecycline in plasma was assessed at 37ºC and [^{14}C] tigecycline protein binding in human plasma was assessed at 25ºC and 37ºC using ultracentrifugation. After incubation at 37ºC for four hours in human plasma at 0.1 and 15µg/ml, there was less than a 5% decrease in the amount of radioactivity associated with [^{14}C] tigecycline. The mean percentages of bound [^{14}C] tigecycline in human plasma determined at 25ºC were 57.8%, 74.7% and 90%, respectively; and at 37ºC were 78.7%, 88.6%, and 96.3% at nominal total tigecycline concentrations of 0.1, 1.0, and 15µg/ml, respectively.

Skin Blister:
The degree of penetration into blister fluid as measured by the ratio of $AUC_{0\text{-}12h,\ Blister}/AUC_{0\text{-}12h,\ Serum}$ was 74%. Figure 11 shows the mean tigecycline concentrations in serum and skin blister fluid following multiple dose administration in study 3074A1-113-US.

Figure 11. Mean Tigecycline Concentrations in Serum and Blister Fluid



The tigecycline partitions slowly into skin blister fluid with a mean time of peak concentration (t_{max}) of 2.8 hours after administration. The levels of the drug are the same at six hours for both serum and blister fluid.

Selected Tissue Concentration:
After administration of a 100mg dose, the concentration of tigecycline was 38 times higher in the gallbladder than in the serum, two-fold higher concentration in the colon than in the serum, and 8.6 times higher in the lung than in the serum.

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

The results of the mass balance study suggest that hepatic is the major route of elimination. In study-3074A1-104-US that was conducted in twelve healthy men, radiolabeled intravenous tigecycline was administered to six subjects to determine metabolic disposition and mass balance. Intravenous [^{14}C]tigecycline was administered on day four following multiple doses of tigecycline. After the administration of the single 53µCi, intravenous dose of [^{14}C]tigecycline, the mass balance results of the excreta over 10 days showed 33% urine recovery and 59% fecal recovery. Thus, hepatic metabolism of biliary excretion of tigecycline and its metabolites appears to be the primary route of elimination. The sponsor measured the unchanged tigecycline in urine for 10 days and the amount was 22% of the total dose. The sponsor did not measure the amount of unchanged tigecycline in the feces samples at 10 days, but did measure the amount at 48 hours which was 9.9%. The secondary elimination pathways of tigecycline are renal excretion, glucuronidation, and amide hydrolysis followed by N-acetylation (to form N-acetyl-9-aminominocycline), each contributing up to 15% of the total elimination. Although a moderate amount of tigecycline epimer was measured in urine and fecal samples, the majority of the measured epimer was likely to be produced during the assay extraction process. Table 13 summarizes the recovery of tigecycline metabolites in urine and feces during the first 48 hours after administration of the [^{14}C]tigecycline dose.

Table 13. Percentage of the [^{14}C]Tigecycline Dose Excreted as Tigecycline-Related Compounds

Matrix	Collection Interval	Tigecycline	Tigecycline Epimer	t-Butylamino-acetic Acid	Tigecycline Glucuronide	Tigecycline Epimer Glucuronide
Urine	0-4	7.9±0.9	0.5±0.2	1.4±0.5	0.4±0.2	ND
	4-8	2.4±1.1	0.3±0.1	1.2±0.2	1.0±0.3	ND
	8-24	3.3±1.1	0.6±0.2	2.4±0.6	1.9±0.5	ND
	24-48[a]	1.7±0.7	0.6±0.2	1.6±0.3	0.8±0.4	ND
	Total: 0-48	14.8±2.9	2.0±0.3	6.3±0.9	4.1±1.4	ND
Feces	0-48	9.9±7.7	5.5±4.7[b]	1.5±1.0	4.0±3.2	1.4±1.0
Total	0-48	24.7±8.7	7.5±4.9	7.8±0.7	8.1±4.2	1.4±1.0

Abbreviation: ND=metabolite was not detected.

a: Subject 104-001-0006 withdrew from the study 24 hours after administration, and data from only 5 subjects were included in the means for the 24-48 hour urine, total feces, and total urine plus feces.

b: A significant amount of the tigecycline epimer observed in the fecal homogenate extracts was likely produced by the assay extraction process.

Following the single dose IV administration of [^{14}C]tigecycline, the total radioactivity in serum decreased rapidly. The sponsor reported that only 1-, 4-, and 8-hour serum samples had sufficient radioactivity to determine the metabolite profiles. Tigecycline was the major component of serum accounting for 46% to 63% of the radioactivity. Tigecycline epimer, tigecycline glucuronide, and the glucuronide of tigecycline epimer were accounted for 5% to 8%, up to 12%, and up to 3% of the serum radioactivity, respectively.

2.2.5.6 What are the characteristics of drug metabolism?

The sponsor investigated the in vitro metabolism of tigecycline using liver microsomes from humans, cryopreserved human hepatocytes, and human liver slices. Incubations with microsomes and cryopreserved human hepatocytes were performed at 20, 50, 100, and 200µM tigecycline. Minocycline which is a structurally similar tetracycline, was incubated with microsome and hepatocyte samples to compare with tigecycline. Hepatocyte and microsome samples were analyzed by HPLC with UV detection and by LC/MS. The in vitro incubations with human liver slices were conducted with 77µM [^{14}C]tigecycline at 37ºC in a 95% O_2 and 5% CO_2 atmosphere for 6 or 24 hours. Samples from the liver slice experiments were analyzed by HPLC with radioisotope and UV detectors, selected samples analyzed by LC/MS. Tigecycline was not significantly metabolized in any of these systems.

2.2.5.7 What are the characteristics of drug excretion?

The primary route of elimination of tigecycline is biliary excretion, predominantly as unchanged drug. Approximately 59% of a radioactive dose is recovered in feces over 10 days. Approximately 33% is recovered in urine. The terminal elimination half-life of tigecycline is approximately 27 hours following 100mg single dose and approximately 42 hours following multiple dose administration of 50mg.

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

Data from Phase 1 study 3074A1-100-EU shows that a linear relationship exists between C_{max} and dose (r^2 =0.99) as shown in Figure 12. Data from the same study shows a linear relationship exists between AUC and dose (r^2 = 0.99) as shown in Figure 13.

Figure 12. Relationship between C_{max} and dose of GAR-936 after various GAR-936 doses to healthy subjects

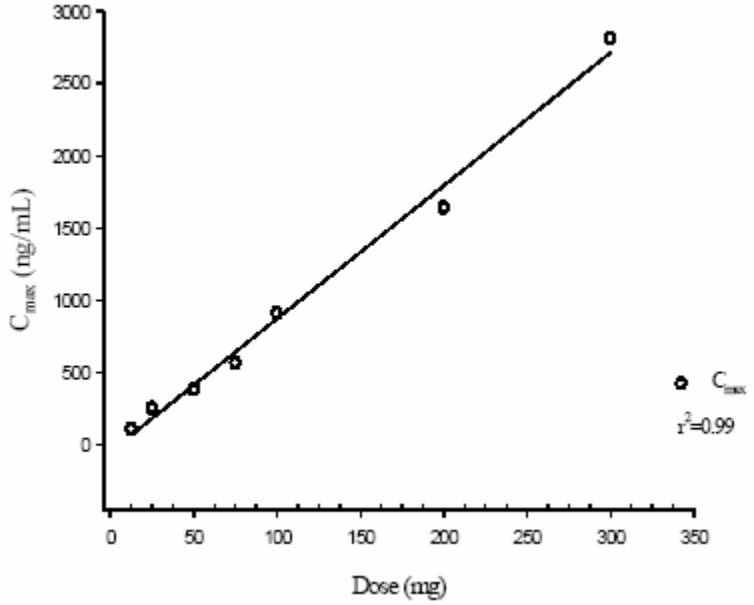
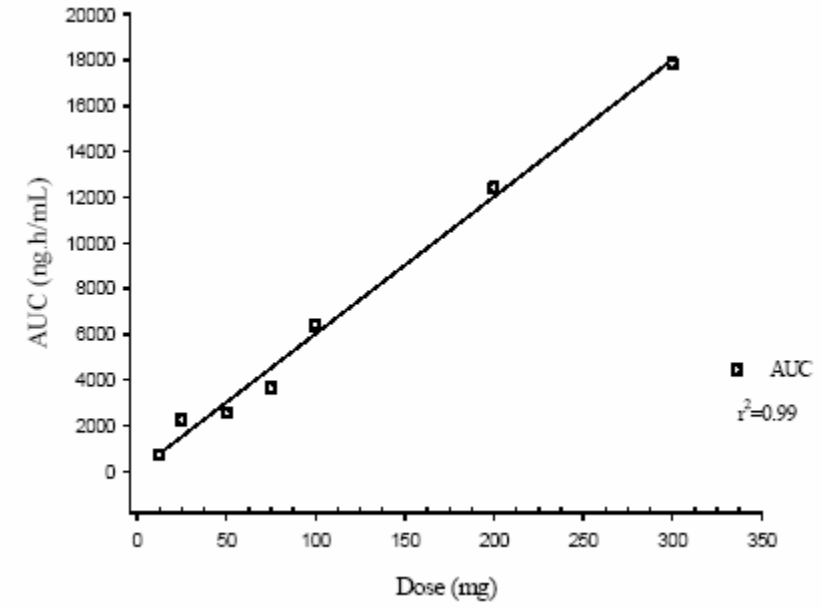


Figure 13. Relationship between AUC and dose of GAR-936 after various GAR-936 doses to healthy subjects



2.2.5.9 How do the PK parameters change with time following chronic dosing?
The time to steady state following the administration of the proposed dose regimen (100mg load followed by 50mg q12h) is three days. The mean clearance with chronic dosing remained the same at 0.2 L/h/kg. The mean AUC increased from 1440 to 3069 ng/h/ml. The mean C_{max} also increased from 487 to 621 ng/ml. Thus, the drug accumulates over time with chronic dosing.

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?
The inter-subject variability was estimated to be 30% to 40% for clearance (Cl) and 40% to 50% for the volume of distribution (Table 12). The intra-subject variability was not estimated.

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?

Renal impairment:
In study 103-US (Protocol 3074A1) the sponsor evaluated the PK of tigecycline 100mg dose in 20 subjects. Approximately one third of the subjects were with normal renal function, one third subjects with CrCl < 30 ml/min, and one third subjects with end-stage renal disease (ESRD) who were receiving hemodialysis. The study showed that the administration of the 100mg tigecycline dose is safe and well tolerated. Based on the

pharmacokinetics, the dose does not need to be adjusted. Table 14 summarizes the PK profile of tigecycline.

Table 14. Tigecycline Pharmacokinetic Parameters

Group	C_{max}[a] (ng/mL)	t_{max}[a] (h)	$t_{1/2}$[a] (h)	AUC[a] (ng·h/mL)	Cl[a] (L/h)	Cl_r[a] (L/h)
Healthy subjects	604±243	0.7±0.3	27.3±5.2	3330±709	31.1±5.9	6.6±3.0
Severe renal impairment	605±166	1.0±0.0	26.8±7.0	4758±1811	23.4±7.6	1.4±0.6
ESRD (predialysis)	982±161	0.9±0.3	17.8±3.6	4152±458	24.3±2.8	- -
ESRD (postdialysis)	940±342	1.0±0.0	31.8±19.2	3929±1023	26.9±7.8	- -
P-value[b]	0.02	0.02	0.19	0.24	0.24	0.001

a: Mean ± standard deviation (SD).
b: 1-Factor ANOVA of log-transformed data for all 4 groups.

The renal clearance of tigecycline in healthy subjects was similar to creatinine clearance (Cl_{Cr}), and it represented approximately 20% of the total systemic clearance of tigecycline. Consequently, the systemic clearance of tigecycline was reduced by approximately 20% in subjects with severe renal impairment (Cl_{Cr}<30 ml/min) or ESRD, and tigecycline area under the curve (AUC) increased by approximately 30% in these subjects. Hemodialysis did not remove tigecycline from the systemic circulation of the subjects.

Hepatic impairment:
In study 105-EU (Protocol 3074A1) the sponsor evaluated 48 subjects at two study sites following the administration of a single dose of 100mg tigecycline. Ten of the subjects had compensated cirrhosis (Child-Pugh-A: score of 5 or 6), ten subjects had mild to moderately decompensated cirrhosis (Child-Pugh-B: score of 7 to 9), and five subjects had severely decompensated cirrhosis (Child-Pugh-C: score of 10 to 13) without severe encephalopathy. Twenty-three healthy subjects matching the cirrhotic subjects for age, sex, weight, and smoking habit were enrolled in the study. Table 15 shows the PK results.

Table 15. Tigecycline Pharmacokinetic Parameters

Group	C_{max}[a] (ng/mL)	t_{max}[a] (h)	$t_{1/2}$[a] (h)	AUC[a] (ng·h/mL)	Cl[a] (L/h)	V_{ss}[a] (L)
Healthy (n=23)	981±536	0.9±0.2	18.7±7.2	3749±1315	29.8±11.3	524±157
Child-Pugh-A (n=10)	865±382	0.7±0.3	19.1±5.4	3835±1814	31.2±13.9	617±234
Child-Pugh-B (n=10)	914±551	1.0±0.0	23.0±5.0	5636±3419	22.1±9.3	542±246
Child-Pugh-C (n=5)	1207±414	1.0±0.0	26.8±6.1	7656±1534	13.5±2.7	378±107
P-value[b]	0.40	0.001	0.03	0.001	0.001	0.07

a: Mean ± standard deviation (SD).
b: 1-Factor ANOVA of log-transformed data.

The hepatic mechanism of biliary excretion of unchanged tigecycline is the primary elimination pathway of tigecycline, and glucuronidation (Phase II metabolism) and renal excretion of unchanged tigecycline are secondary pathways. The clearance of tigecycline in the Child-Pugh A subjects was similar to that of the healthy subjects, and the Cl in the Child-Pugh B and Child-Pugh C subjects was approximately 25% and 55% lower, respectively, than it was in the healthy subjects. The mean tigecycline AUC was 50% higher in the Child-Pugh B subjects and 105% higher in the Child-Pugh C subjects.

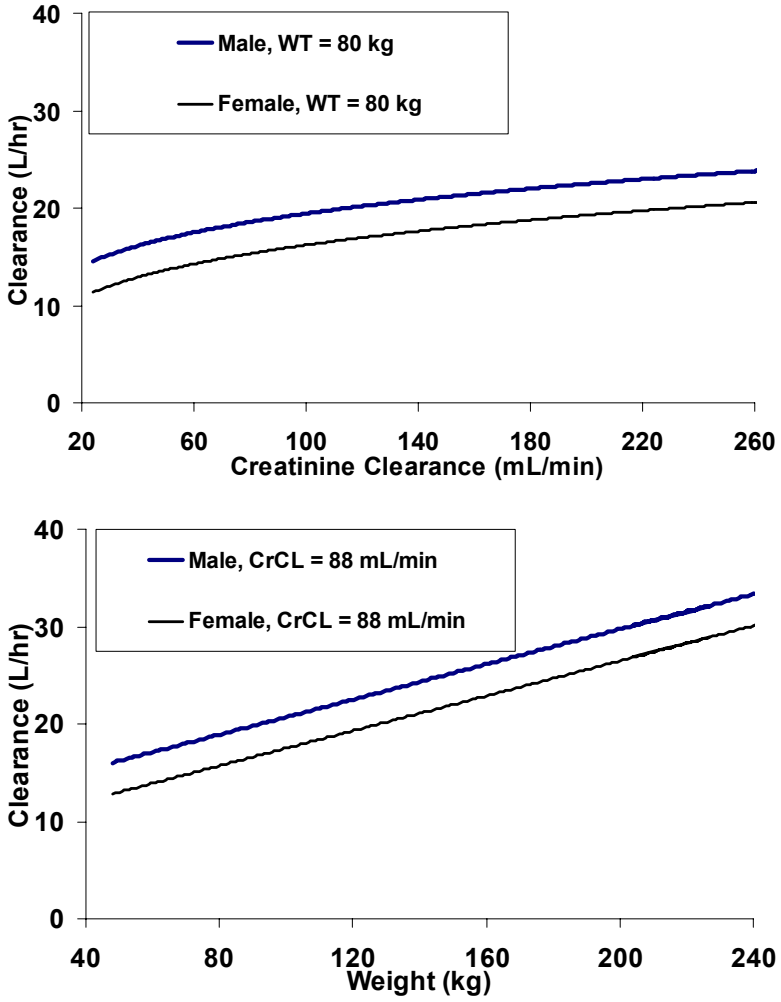
Population PK

Creatinine clearance (CrCLj), body weight (WTj) and gender (SEXj=1 for male and 0 for female) are the only factors found to influence the drug clearance (CLj) through the following equation:

$$CL_j(L/hr) = 15.7 \cdot (\frac{CrCL_j}{88.3})^{0.25} + 0.09 \cdot (WT_j - 80) + 3.23 \cdot SEX_j$$

Figure 14 shows the typical value of clearance based on this equation. The between subject variability for clearance is 35% after correction for these covariates.

Figure 14. Plots of the Typical Value of Clearance Extrapolated Over the Studied Range of the Statistically Significant Patient Covariates in the Final Model



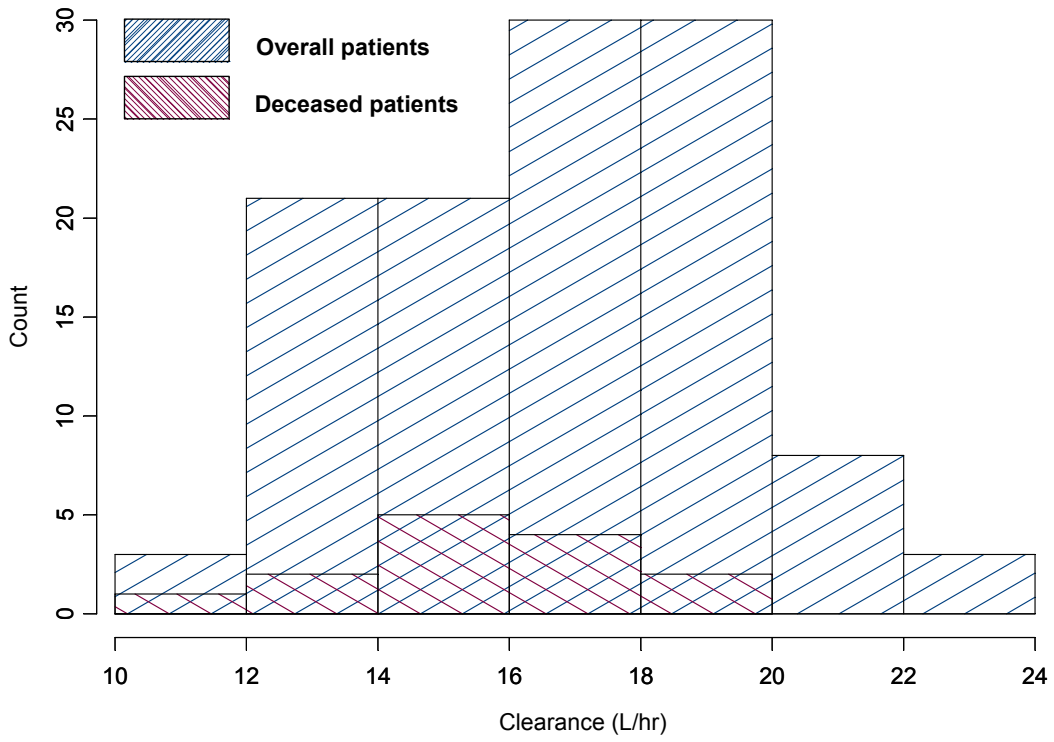
The unexplained variability in clearance, after adjusting for the covariates is 35%. Table 16 listed the range of drug clearance (L/hr) based on these covariates in the studies included for exposure-response analysis for cIAI and cSSSI (2 phase 2 studies, 4 phase 3 studies).

Table 16. The Range of Drug Clearance (L/hr) for Exposure-Response Analysis

Indication	Minimum	25% Percentile	Median	75% Percentile	Maximum
cIAI	(b) (4)	14.5	17.1	18.6	(b) (4)
cSSSI		16.1	18.2	19.2	

Based on the exposure-benefit/risk analysis result, the exposure difference due to these covariates was not clinically significant and dose adjustment based on these covariates may not be necessary under the proposed dosage regimen (100 mg loading dose and 50 mg BID maintenance dose). The identified covariates were applied to patients who died during the trials to explore the possibility that those patients might have had systematic higher drug clearance, leading to lower drug exposure. The result (Figure 15) indicated that the clearance distribution of these patients was consistent with the overall population in the trials.

Figure 15: The Distribution of Clearance for Overall Patients and Deceased Patients



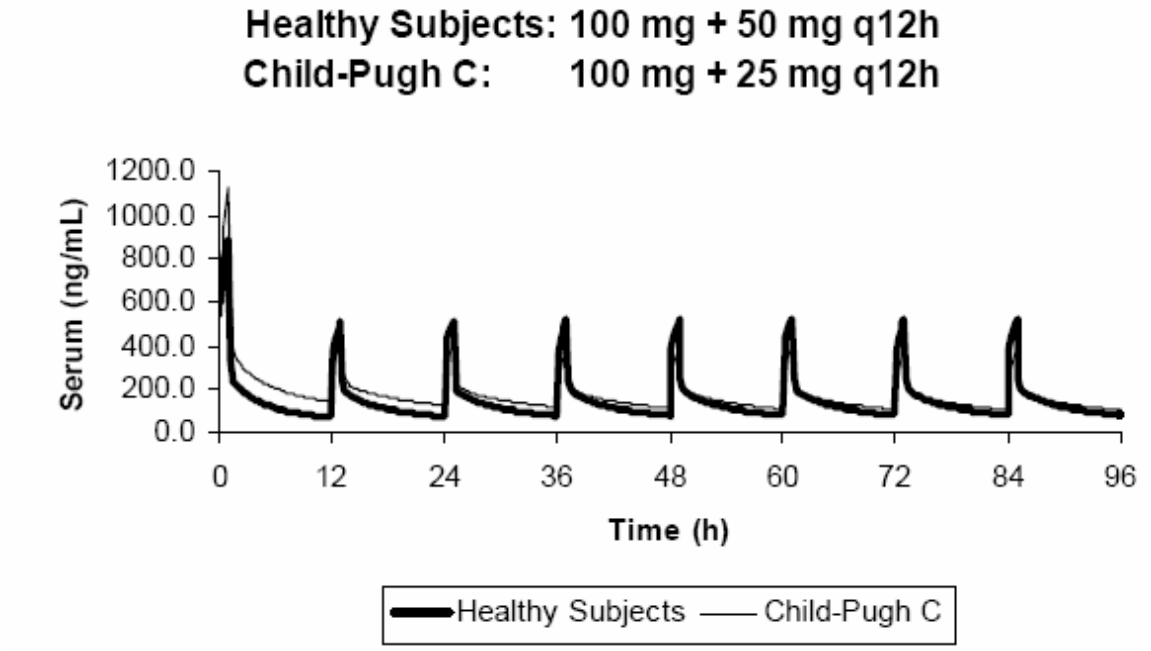
2.3.2 Based upon what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations, what dosage regimen adjustments, if any, are recommended for each of these groups? If dosage regimen adjustments are not based upon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

2.3.2.5 Renal Impairment:
Tigecycline dose does not need to be adjusted in subjects with renal impairment, including subjects with end-stage renal disease. There is no significant difference between the clearance for the healthy subjects and the severely renally impaired subjects. Also, treatment with tigecycline does not need to be delayed until after hemodialysis because tigecycline is not dialyzable.

2.3.2.6 Hepatic Impairment:
Dosage adjustment is not warranted in patients with mild hepatic impairment (Child-Pugh A) because of no change in the clearance of drug compared to healthy subjects. In moderate and severe hepatic impairment (Child-Pugh B and Child-Pugh C) the clearance was approximately 25% and 50% lower, respectively, than the healthy subjects. According to the exposure response analysis this may result in an increased rate of nausea and vomiting in the severe hepatic impairment subjects. Since the clearance of the drug in the severe hepatic impairment population was decreased by 50% and the AUC was increased by 105%, these patients should receive an initial dose of 100mg followed by a reduced maintenance dose of 25 mg every 12 hours. The rationale for this dose adjustment is provided below.

The tigecycline single dose C_{max} was approximately 25% higher in patients with severe hepatic impairment. Consequently, patients with severe hepatic impairment receiving the standard therapeutic regimen initially would be exposed to only slightly higher serum concentrations of tigecycline over the first few hours compared to patients with normal hepatic function. However, upon multiple dose administration, patients with severe hepatic impairment (Child-Pugh-C) receiving the standard therapeutic regimen would be exposed to approximately twice the steady-state serum concentrations of tigecycline as the patients in the clinical efficacy trials (or the equivalent dose of 100 mg q12h). The amount of safety information in healthy subjects and infected subjects at this exposure level is very limited. Simulated steady-state serum concentrations of tigecycline in healthy subjects receiving the therapeutic regimen of 100 mg followed by 50 mg q12h and subjects with severe hepatic impairment (Child-Pugh-C) receiving a 50% reduction in the maintenance dose (25 mg q12h) are shown in Figure 16. Reduction in maintenance dose by 50% as expected provides similar exposure at steady state in subjects with severe hepatic impairment compared to subjects with normal hepatic function.

Figure 16. Simulation of tigecycline concentrations



2.4 Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or –response and what is the impact of any differences in exposure on response?

The sponsor did not submit any information or data for review to determine the interaction of tigecycline with herbal products, smoking, alcohol etc…

2.4.2 Drug-drug interactions

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

In vitro interaction studies conducted at a tigecycline concentration of 100µM (58.56 µg/ml) indicated that tigecycline did not inhibit CYP3A4, CYP2D6, CYP2C19, CYP 2C9, CYP2C8, or CYP1A2. The median inhibition concentration (IC_{50}) was assessed as greater than 100µM. Human serum C_{max} values are at least 40-fold lower than the IC_{50} for metabolic inhibition.

2.4.2.4 Is the drug a substrate and/or inhibitor of P-glycoprotein transport processes?

In vitro studies to evaluate whether tigecycline is a substrate or an inhibitor of Pgp were not conducted. However, based on a drug interaction study with oral digoxin, tigecycline appears to be not an inhibitor of Pgp.

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

Not evaluated.

2.4.2.6 Does the label specify co-administration of another drug (e.g., combination therapy in oncology) and, has the interaction potential between these drugs been evaluated?

Not evaluated

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

In the Phase 3 trials the patients received concomitant medications including other antiinfectives (eg. aminoglycosides, macrolides, beta-lactams, topicals), antidepressants, beta blocking agents, diuretics etc… The sponsor did not mention any interactions that altered safety or efficacy in these trials. It is not expected that tigecycline would produce any serious interactions with other medications since it is not a substrate or inhibitor of the CYP-450 isoenzyme pathway.

2.4.2.8 Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure response relationships are different when drugs are co-administered?

Digoxin:

There is no significant pharmacokinetic drug interaction between tigecycline and digoxin. The sponsor studied the effect of tigecycline on the pharmacokinetics of digoxin in an open-label, single-sequence, 3-period, multiple-dose drug interaction study (3074A1-111-US) in 30 subjects of whom 20 completed the trial. On day one of period one (days one to five), each subject received a single infusion of 100 mg tigecycline. During period two (days six to 14), each subject received 0.5mg of digoxin orally on day seven followed by 0.25 mg of digoxin on subsequent days. During period three (days 15 to 23), each subject continued receiving 0.25 mg of digoxin daily through day 19; each subject also received 100 mg of tigecycline as the first dose on day 15 followed by 50 mg of tigecycline every 12 hours beginning with the second dose on day 15 and ending with the first dose on day 19. Table 17 summarizes the PK parameters and GLS mean ratios and 90% CIs for plasma and urine digoxin PK parameters.

Table 17. Statistical Comparison of Plasma and Urine Digoxin Pharmacokinetic Parameters (n=20)

Parameter (units)	Digoxin Alone[a] (mean ± SD)	Digoxin With Tigecycline[a] (mean ± SD)	GLS Mean Ratio (%)[b] (90% CI)
Plasma			
C_{max} (ng/mL)	1.19 ± 0.20	1.09 ± 0.46	87 (77-98)
t_{max}	1.35 ± 0.56	1.48 ± 0.55	111 (91-135)
AUC_{0-24h} (ng•h/mL)	11.7 ± 2.3	11.2 ± 2.7	95 (88-103)
CL/F (mL/h/kg)	4.54 ± 1.08	4.79 ± 1.21	105 (97-113)
Urine			
$A_{e,\%}$	41.3 ± 9.0	37.8 ± 9.4	91 (80-102)
CL_r	1.82 ± 0.37	1.75 ± 0.40	95 (87-104)

Abbreviations: GLS = geometric least squares; SD = standard deviation; CI = confidence interval; C_{max} = peak concentration; t_{max} = time to peak concentration; AUC_{0-24h} = area under the concentration time curve during a dose interval; CL/F = oral-dose clearance; $A_{e,\%}$ = percentage of digoxin excreted in urine; CL_r = renal clearance.

a: Digoxin pharmacokinetic parameters for both treatments are based on daily 0.25-mg doses of digoxin.

b: Ratio of (digoxin + tigecycline)/(digoxin alone).

The 90% CIs for the plasma digoxin AUC_{0-24h} and CL/F were both within the 80 to 125% bioequivalence window, but the 90% CIs for C_{max} (CI= 77-98%) and t_{max} (CI= 91-135%) were not. Thus, tigecycline did not affect digoxin total exposure (AUC) or oral-dose clearance (CL/F); but the digoxin absorption rate was slightly decreased.

Warfarin:
The sponsor studied the effect of tigecycline on the pharmacokinetics and pharmacodynamics of warfarin in an open-label, nonrandomized, inpatient/outpatient, 2-period, 2-treatment study (3074A1-115-US) in 19 healthy subjects of whom eight completed the study. On day one (period one), each subject received a single dose of warfarin 25mg followed by a 12 day washout period. Then on day one (period two) until day eight, each patient received tigecycline 100mg IV load followed by tigecycline 50 mg q12h. A single dose of warfarin 25 mg was given on day five of period two. Eleven subjects withdrew from the study, five because of adverse events (eg. nausea and vomiting) and six for other reasons. Table 18 summarizes the PK profile with coadministration of tigecycline and warfarin.

Table 18. Tigecycline Steady-State Pharmacokinetic Parameters (Mean±SD)

Treatment	C_{max} (ng/mL)	t_{max} (h)	C_{12h} (ng/mL)	AUC_{0-12h} (ng·h/mL)	Cl (L/h/kg)
Tigecycline	886±202	0.50±0.00	109±28	2097±514	0.310±0.072
Tigecycline + warfarin	898±192	0.50±0.00	114±32	2192±494	0.296±0.057
p-value[a]	0.646	- -	0.255	0.004	0.004
Geo. Mean Ratio[b]	102%	- -	108%	107%	93%
90% CL[b]	94–112%	- -	96-123%	104-111%	90-96%

Abbreviations: AUC_{0-12h} = area under the concentration time curve during the dose interval 0 to 12 hours; C_{12h} = concentration at hour 12; C_{max} = peak concentration; CL = confidence limits; Cl = intravenous clearance; t_{max} = time to peak concentration
a: Treatment p-value from a 2-factor ANOVA of log-transformed data.
b: Geometric mean ratio and 90% confidence limits.

Following coadministration of warfarin, the mean steady-state tigecycline systemic clearance and exposure (C_{max}, C_{12h}, and AUC) were within 10% of the mean values for tigecycline monotherapy. The 90% confidence limits of the geometric mean parameter ratio were all within the 80-125%. Thus, coadministration of warfarin does not alter the PK profile of tigecycline. Table 19 summarizes the pharmacokinetic profile of R-warfarin and S-warfarin. Table 20 summarizes the INR pharmacodynamic profile of warfarin.

Table 19. R-Warfarin and S-Warfarin Single-Dose Pharmacokinetic Parameters (Mean±SD)

	R-warfarin			S-warfarin		
Treatment	C_{max} (ng/mL)	AUC (µg·h/mL)	Cl/F (mL/h/kg)	C_{max} (ng/mL)	AUC (µg·h/mL)	Cl/F (mL/h/kg)
Warfarin	1158±259	73±18	4.3±0.8	1104±282	47±18	7.0±1.9
Tigecycline + Warfarin	1584±259	122±28	2.5±0.5	1555±236	59±17	5.4±1.2
p-value[a]	0.001	0.001	0.001	0.001	0.001	0.001
Geo. Mean Ratio[b]	138%	168%	60%	143%	129%	77%
90% CL[b]	126-151%	150-187%	54-67%	129-158%	119-140%	71-84%

Abbreviations: AUC = area under the time concentration curve; Cl/F = oral dose clearance; C_{max} = peak concentration.
a: Treatment p-value from a 2-factor ANOVA of log-transformed data.
b: Geometric mean ratio and 90% confidence limits.

Coadministration of the therapeutic regimen of tigecycline significantly decreased the oral clearance of both R-warfarin and S-warfarin, thereby increasing the C_{max} and AUC of both compounds.

Table 20. INR Pharmacodynamic Parameters (Mean±SD)

Treatment	INR_{max}	t_{max} (h)	$AUC_{0\text{-}168h}$ (h)
Warfarin	1.8±0.3	30±9	216±14
Tigecycline + Warfarin	1.6±0.2	39±6	220±13
P-value[a]	0.036	0.068	0.564
Geo. Mean Ratio[b]	90%	136%	102%
90% CL[b]	84-107%	104-179%	97-107%

Abbreviations: $AUC_{0\text{-}168h}$ = area under the time concentration curve for the interval 0 to 168 hours; INR = international normalized ratio; t_{max} = time to maximum INR value.
a: Treatment p-value from a 2-factor ANOVA of log-transformed data.
b: Geometric mean ratio and 90% confidence limits.

Figure 17. Stick plots demonstrating the INRmax(ratio) and INR AUC 0-168 (ratio*h) for subjects receiving warfarin alone (25 mg) and warfarin (25mg) with concurrent multiple IV doses of tigecycline 50mg q12h

The R-warfarin and S-warfarin plasma concentrations were higher following tigecycline coadministration, but the mean INR_{max} values were 10% lower following administration of the combination treatment and the mean INR AUC_{0-168h} values were unchanged. There were 19 subjects who participated in the warfarin study. The sponsors obtained INRmax and INR AUC_{0-168} information on eight of them. There were six subjects who had a decrease, one subject who had an increase, and one subject who remained the same in the INRmax group (Range of individual ratios: (b) (4)). There were three subjects who had a decrease and five subjects who had an increase in INR AUC_{0-168} (Range of individual ratios: (b) (4)). The plots in figure 17 represent individual subject data for the INR max (ratio) and INR AUC_{0-168}.

The sponsor recommended in the label that patients who are receiving concomitant warfarin and tigecycline therapy monitor their prothrombin time or other coagulation tests.

2.5 General Biopharmaceutics
Does not apply

2.6.1 How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?
The tigecycline was measured by HPLC and LCMS in the studies.

2.6.4.2 What are the lower and upper limits of quantification (LLOQ/ULOQ)?
The lower limit of quantification is (b) (4) ml and the upper limit of quantification is (b) (4) /ml.

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

Table 21, 22, 23, and 24 summarizes for each study the accuracy (% bias) and the precision (% coefficient of variation) of the assay quality control (QC) samples of the serum, plasma, urine, and tissue assays, respectively.

Table 21. Summary of Accuracy (% Bias) and Precision (%CV) of the QC Samples in the Serum Assays

			Low QC			Middle QCs			High QC		
Protocol Number	Analyte	Linear Range	Target Conc	Accuracy (% Bias)	Precision (% CV)	Target Conc	Accuracy (% Bias)	Precision (% CV)	Target Conc	Accuracy (% Bias)	Precision (% CV)
3074A1-100	Tigecycline	(b) (4)									
3074A1-101	Tigecycline										
3074A1-102	Tigecycline										
3074A1-103	Tigecycline										
3074A1-104	Tigecycline										
3074A1-105	Tigecycline										
3074A1-106	Tigecycline										
3074A1-109	Tigecycline										
3074A1-111	Digoxin										
3074A1-111	Tigecycline										
3074A1-112	Tigecycline										
3074A1-113	Tigecycline										
3074A1-115	Tigecycline										
3074A1-116	Tigecycline										
3074A1-117	Tigecycline										
3074A1-200	Tigecycline										
3074A1-202	Tigecycline										
3074A1-204	Tigecycline										
3074A1-300	Tigecycline										
3074A1-301	Tigecycline										
3074A1-305	Tigecycline										
3074A1-306	Tigecycline										

Abbreviations: CV=coefficient of variation; QC=quality control.

Table 22. Summary of Accuracy (% Bias) and Precision (% CV) of the QC Samples in the Plasma Assays

			Low QCs			Middle QCs			High QCs		
Protocol Number	Analyte	Linear Range	Target Conc	Accuracy (% Bias)	Precision (% CV)	Target Conc	Accuracy (% Bias)	Precision (% CV)	Target Conc	Accuracy (% Bias)	Precision (% CV)
3074A1-111	Digoxin	0.1 – 8.0 µg/mL	0.45	-0.9	(b) (4)	2.0	-4.8	12.8	4.0	+1.7	5.2

Abbreviations: CV=coefficient of variation; QC=quality control

Table 23. Summary of Accuracy (% Bias) and Precision (%CV) of the QC Samples in the Urine Assays

			Low QCs			Middle QCs			High QCs		
Protocol Number	Analyte	Linear Range	Target Conc	Accuracy (% Bias)	Precision (% CV)	Target Conc	Accuracy (% Bias)	Precision (% CV)	Target Conc	Accuracy (% Bias)	Precision (% CV)
3074A1-100	Tigecycline	(b) (4)									
3074A1-101	Tigecycline										
3074A1-102	Tigecycline										
3074A1-102	Tigecycline										
3074A1-103	Tigecycline										
3074A1-104	Tigecycline										
3074A1-105	Tigecycline										
3074A1-106	Tigecycline										
3074A1-111	Digoxin										
3074A1-204	Tigecycline										

Abbreviations: CV=coefficient of variation; QC=quality control.

Table 24. Summary of Accuracy (% Bias) and Precision (% CV) of the QC Samples in the Tigecycline Assays in Tissues

Protocol Number	Tissue	Linear Range	Low QCs: Target Conc	Low QCs: Accuracy (% Bias)	Low QCs: Precision (% CV)	Middle QCs: Target Conc	Middle QCs: Accuracy (% Bias)	Middle QCs: Precision (% CV)	High QCs: Target Conc	High QCs: Accuracy (% Bias)	High QCs: Precision (% CV)
3074A1-112	Alveolar cells										
3074A1-112	Bronchial Lavage fluid										
3074A1-113	Skin blister Fluid										
3074A1-117	Bone										
3074A1-117	Colon										
3074A1-117	Gallbladder										
3074A1-117	Lung										
3074A1-117	Synovial fluid										

(b) (4)

Abbreviations: CV=coefficient of variation; QC=quality control.

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A metabolic disposition and mass balance study of [^{14}C]-labeled intravenous tigecycline in healthy men(Protocol 3074A1-104-US)

Dates: July 7, 2003 to July 26, 2003
Clinical site: (b) (4)
Analytical sites:

1. Serum and urine samples for tigecycline determination were analyzed at (b) (4)
2. Materials for radioactivity determination including serum, whole blood, RBCs, urine, feces, toilet tissue, and emesis samples, plus IV tubing, pre-dose samples, and IV bags were analyzed at (b) (4)
3. Serum samples for metabolites were analyzed at Wyeth Research, 500 Arcola Road/ S-1142, Collegeville, PA 19426

OBJECTIVES:
The primary objective of the study was to characterize the metabolism and excretion of [^{14}C]tigecycline in healthy men. A secondary objective was to provide the pharmacokinetic (PK) profile of [^{14}C]tigecycline in healthy men.

FORMULATION:
Sterile tigecycline powder for injection was supplied by Wyeth Research in 5ml, flint glass vials, each containing 53 mg lyophilized tigecycline free base. The tigecycline batch numbers were 2001B0022 and 2000B0392. The batch number for the [^{14}C]tigecycline was 7981703. The contents of the vial were reconstituted with sterile normal saline (0.9% NaCl for Injection, USP).

STUDY DESIGN:
This study was an open-label, nonrandomized, inpatient, multiple-dose tigecycline, a single-dose [^{14}C]tigecycline study in healthy men at one investigational site. The study enrolled 12 healthy men (age 18 to 45 years) and, six of the twelve subjects received [^{14}C]tigecycline. Each subject received a loading dose of 100 mg IV unlabeled tigecycline infused over 30 minutes, followed by five doses (q12h) of 50mg of IV unlabeled tigecycline infused over 30 minutes. On the morning of study day four, six subjects were given 50 mg of IV ^{14}C-tigecycline (53 μCi) infused over 30 minutes. All doses were administered approximately one hour after a high fiber meal.

Whole blood samples and blood samples for determination of tigecycline serum concentrations were collected on day four at predose and at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, and 240 hours after the start of [^{14}C]tigecycline administration. Blood samples for metabolite analysis were collected at predose on day one and day four and 1, 4, 8, 24, and 48 hours after the start of [^{14}C]tigecycline administration.

Complete urine output was collected for determination of tigecycline, radioactivity, and metabolite profiles beginning on day four, at predose and at time intervals 0 to 4, 4 to 8, 8

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, and 216 to 240 hours after the start of [^{14}C]tigecycline administration. In addition, toilet tissue, feces, and emesis (if available) were collected daily for determination of radioactivity and metabolic profiles.

TIGECYCLINE ASSAY METHODOLOGY:
Concentrations of tigecycline in serum and urine were determined by sensitive and specific liquid chromatography methods with mass spectrometer detection (LC/MS/MS).

Table 1. Assay Range and Sensitivity

Standard Curve	Tigecycline/Serum (ng/mL)	Tigecycline/Urine (µg/mL)
Linear range	(b) (4)	
Sensitivity	(b) (4)	

Table 2. Summary of Assay Performance for Serum Assays

	---Low QC (b) (4)			---Middle QC (b) (4)			--High QC (b) (4)		
Analyte	Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
Tigecycline	(b) (4)								

Abbreviations: CV=coefficient of variation; QC=quality control.

Table 3. Summary of Assay Performance for Urine Assays

	---Low QC (b) (4)			---Middle QC (b) (4)			--High QC (b) (4)		
Analyte	Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
Tigecycline	(b) (4)								

Abbreviations: CV=coefficient of variation; QC=quality control.

The radioactivity in serum and urine was measured by direct liquid scintillation counting (LSC), and the radioactivity in whole blood, RBC's, and fecal homogenates were measured by (b) (4) followed by LSC. To identify or quantify the metabolites of tigecycline in serum, urine, and fecal homogenates, these samples were analyzed by high performance liquid chromatography (HPLC) radiochromatography, with selected samples also analyzed by LC/MS.

DATA ANALYSIS:
Standard noncompartmental PK methods were used to analyze the tigecycline serum concentration and urinary recovery data. The statistical analysis was limited to descriptive statistics (ie., means and frequency tables). The radioactive concentration data (expressed in nanogram-equivalents of tigecycline per milliliter, ng-Eq/ml) in whole blood, RBC's and serum and the tigecycline serum concentration data for each subject

were analyzed by using model-independent PK methods. The peak concentration (C_{max}) and the time to peak concentration (t_{max}) were taken directly from the observed data. The apparent terminal-phase disposition rate constant (λ_z) was estimated by a log-linear regression of the last 2 to 5 observed concentrations that were determined to be in the log-linear elimination phase by visual inspection. The apparent terminal-phase disposition half-life ($t_{½}$) was calculated as $t_{½}=0.693/\lambda_z$. Only 1 dose of [^{14}C]tigecycline was administered , and the radioactivity concentration data in whole blood, RBC's, andserum were analyzed as single-dose data. The single-dose area under the concentration-time curve (AUC_T) to the last observable concentration (C_T) at time T was calculated by using the log-trapezoidal rule for decreasing concentrations and the linear-trapezoidal rule for increasing concentrations. The total single-dose AUC was estimated by $AUC=AUC_T + C_T/\lambda_z$. the single-dose mean residence time (MRT) was calculated as $AUC/AUMC-T_{inf}/2$, where AUMC is the total area under the first moment curve and T_{inf} is the duration of infusion (0.5 hour).

The unlabeled tigecycline serum concentration data represents multiple-dose administration, and the steady-state AUC over 1 dose interval ($AUC_{0-\tau}$, where τ=12 hours) was calculated by using the log-trapezoidal rule for decreasing concentrations and the linear-trapezoidal rule for increasing concentrations. The steady-state MRT was calculated as $AUC_{0-\tau}/ (AUMC_{0-\tau} + \tau \cdot AUC\ \tau-\infty)-T_{inf}/2$. The systemic clearance (CL) was calculated as dose/AUC, and the apparent steady-state volume of distribution (V_{ss}) was estimated as CL·MRT.

STATISTICAL ANALYSIS:
The radioactivity concentrations in whole blood, RBC's, and serum, the tigecycline serum concentrations, and the pharmacokinetic parameters were analyzed by descriptive statistics (eg, mean, standard deviation, percent coefficient of variation). No formal statistical comparisons were made in this study.

RESULTS:
Of the six subjects who received [^{14}C]tigecycline, subject # 0006 discontinued approximately 30 hours after administration, and the limited urine and fecal recovery data for this subject were not analyzed. Subjects 0001 and 0004 provided incomplete collections and the urine and fecal recovery data were analyzed both including and excluding the data from these two subjects. For the three subjects with complete fecal recovery, 33% of the administered radioactive material was recovered in urine, and 59% of the radioactive material was recovered in feces. Thus, 92% of the radioactive dose was recovered. Approximately 22% of the urine recovery occurred on the first day, an additional 5% recovered on day two, and 2.5% recovered on day three. Conversely, very little fecal recovery occurred on day one (0.03%). The majority of fecal recovery occurred on days 2, 3, 4, and 5 (33%, 11%, 5%, and 16%, respectively). Table four summarizes the recovery of tigecycline and its metabolite in urine and feces during the first 48 hours after administration of the [^{14}C]tigecycline dose.

Table 4. Percentage of the [^{14}C]Tigecycline Dose Excreted as Tigecycline-Related Compounds

Matrix	Collection Interval	Tigecycline	Tigecycline Epimer	t-Butylamino-acetic Acid	Tigecycline Glucuronide	Tigecycline Epimer Glucuronide
Urine	0-4	7.9±0.9	0.5±0.2	1.4±0.5	0.4±0.2	ND
	4-8	2.4±1.1	0.3±0.1	1.2±0.2	1.0±0.3	ND
	8-24	3.3±1.1	0.6±0.2	2.4±0.6	1.9±0.5	ND
	24-48[a]	1.7±0.7	0.6±0.2	1.6±0.3	0.8±0.4	ND
	Total: 0-48	14.8±2.9	2.0±0.3	6.3±0.9	4.1±1.4	ND
Feces	0-48	9.9±7.7	5.5±4.7[b]	1.5±1.0	4.0±3.2	1.4±1.0
Total	0-48	24.7±8.7	7.5±4.9	7.8±0.7	8.1±4.2	1.4±1.0

Abbreviation: ND=metabolite was not detected.

a: Subject 104-001-0006 withdrew from the study 24 hours after administration, and data from only 5 subjects were included in the means for the 24-48 hour urine, total feces, and total urine plus feces.

b: A significant amount of the tigecycline epimer observed in the fecal homogenate extracts was likely produced by the assay extraction process.

Table five summarizes the steady-state PK profile of unlabeled tigecycline in serum and the single-dose PK profile of radioactivity in whole blood, RBC's, and serum.

Table 5. Pharmacokinetic Parameters for Steady-State Tigecycline and Single –Dose Radioactivity

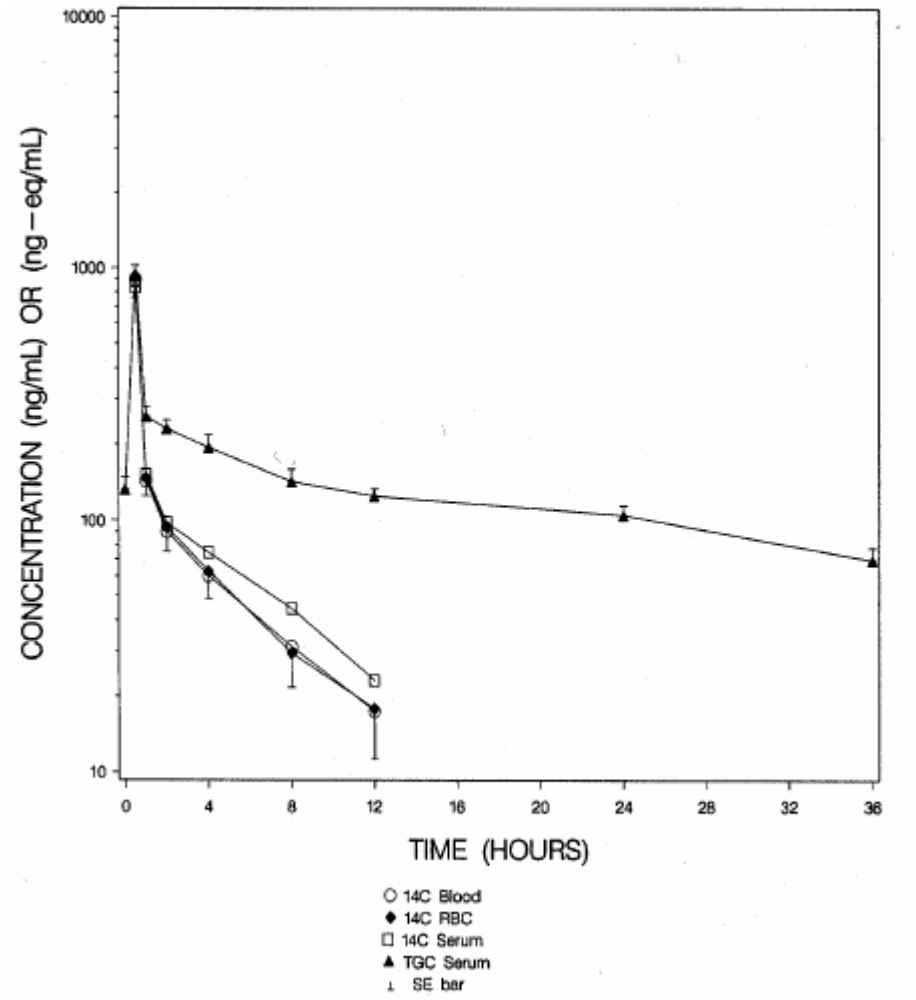
Analyte Matrix	C_{max} (ng-Eq/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC (ng-Eq·h/mL)	CL (L/h)	V_{ss} (L)
Tigecycline (Steady-State)						
Serum	940±201	0.5±0.0	55.8±7.3	2394±583	21.8±4.7	1574±1619
Radioactivity (Single-Dose)						
Whole blood	901±213	0.5±0.0	4.5±1.1	1095±499	53.8±23.4	197±62
Red blood cells	927±186	0.5±0.0	4.5±1.9	1132±414	48.7±15.3	201±41
Serum	839±191	0.5±0.0	6.9±3.6	1330±458	40.7±11.3	255±116

Abbreviations: AUC= total area under the concentration-time curve; CL=systemic clearance; C_{max}=peak concentration; $t_{1/2}$= terminal-phase elimination half-life; t_{max}=time peak concentration occurs; and V_{ss}= apparent steady-state volume of distribution.

Following a single-dose administration of [^{14}C] tigecycline, the overall PK profile of radioactive material was similar in whole blood, RBC's, and serum, indicating that tigecycline and its metabolites were taken up into RBC's in approximately equal concentrations to the serum. However, the concentrations of radioactive material in serum exhibited a much smaller volume of distribution and higher clearance than the unlabeled tigecycline in serum, which produced a shorter half-life for radioactivity than for tigecycline. The sponsor believes that this difference may have been caused by a significant amount of unlabeled tigecycline binding to the tissues prior to administration of [^{14}C]tigecycline, which may have blocked some of the tissue uptake of the [^{14}C]tigecycline. The sponsor refers to this profile as the "last-in", "first-out"

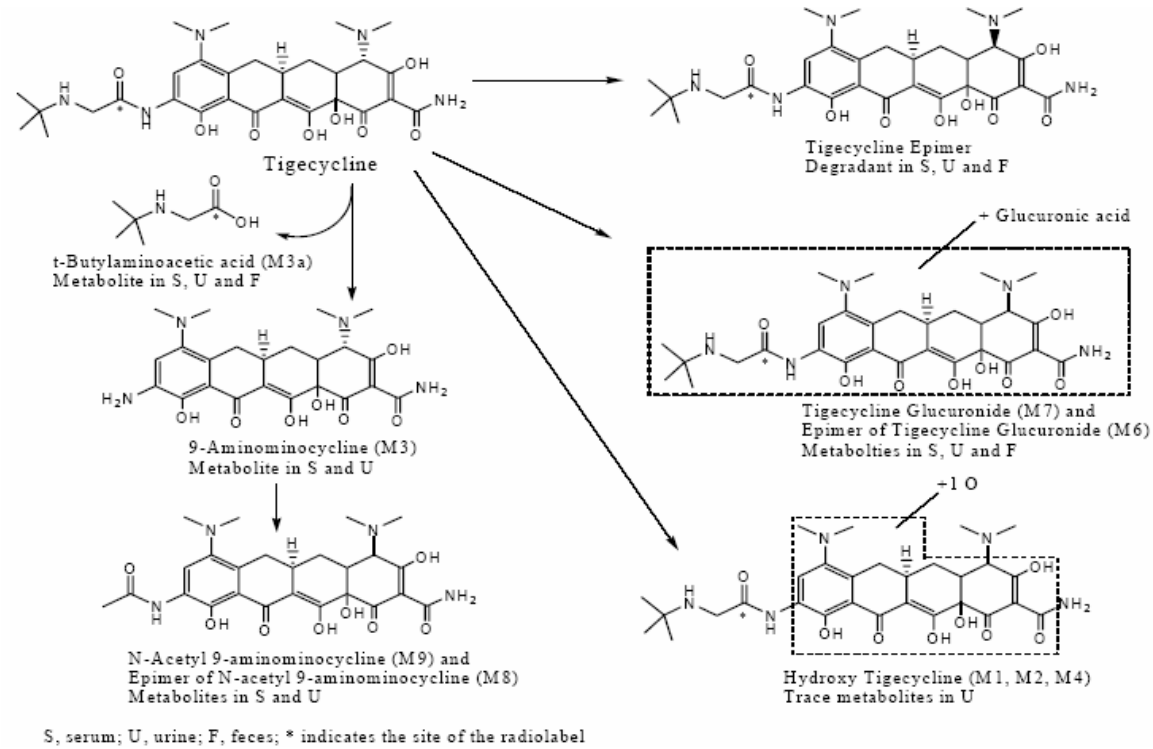
phenomenon. Figure 1 illustrates the concentration over time of radiolabeld tigecycline versus plain tigecycline in serum.

Figure 1. Mean 14C Tigecycline and Tigecycline Concentrations in Healthy Subjects after IV Doses of 50 UCI 14C Tigecycline and 50mg Tigecycline



This study was designed deliberately to limit the uptake of [^{14}C]tigecycline into bone, bone marrow, and other tissues that exhibit high affinity for tigecycline. The metabolite profile analysis indicated that tigecycline is the major drug-related component in serum, urine, and feces. Figure 2 illustrates the various metabolites that were observed tigecycline.

Figure 2. Tigecycline-Related Compounds Detected in Human Serum, Urine and Feces



In both serum and feces, the next highest drug-related compounds are tigecycline epimer and tigecycline glucuronide. However, in urine the next highest drug-related compounds are tigecycline glucuronide and N-acetyl-9-aminominocycline. The tigecycline epimer is present in serum following tigecycline administration, but it is also formed, to a large degree, during the extraction process. The majority of the measured tigecycline epimer was produced from tigecycline during the assay extraction.

CONCLUSION:

Following administration of [^{14}C]tigecycline, approximately 33% of the administered dose is recovered in urine and 59% of the administered dose is recovered in feces, for a total recovery of 92% of the administered dose. In this study, several doses of unlabeled tigecycline were administered to subjects prior to administration of [^{14}C]tigecycline, and the PK profile of the radioactivity exhibited what the sponsor referred to as the "first-in", "last-out" phenonmenon. The unlabeled drug has saturated the tissue binding sites and the [^{14}C]tigecycline from the last dose administered does not penetrate significantly into the tissues. The hepatic mechanism of biliary excretion of unchanged tigecycline is the primary route of elimination of tigecycline, and the secondary elimination pathways of tigecycline are renal excretion of unchanged tigecycline, glucuronidation, and amide hydolysis followed by N-acetylation to form N-acetyl-9-aminominocycline; and these secondary pathways each account for 15% or less of the total elimination of tigecycline.

A single ascending dose study of the safety, tolerance, and pharmacokinetics of GAR-936 in healthy male subjects (Protocol 3074A1-100-EU)

Dates: March 1998 to July 1998
Clinical site: (b) (4)
Analytical site: (b) (4)

OBJECTIVES:
The objectives of this study were 1) to assess the safety of GAR-936 and the tolerance of human subjects to single IV doses, and 2) to assess the preliminary PK of single, ascending IV doses of GAR-936.

FORMULATION:
GAR-936 100 mg/5 ml lyophilized powder Formulation # 0930919J (Batch # 1997B0186)
0.9% Sodium Chloride Injection USP (Batch # 1998P0991)
Ondansetron 2mg/ml vial for injection (Batch # PSG2063-010Q)

STUDY DESIGN:
This study was a single center, double-blind, randomized, placebo-controlled, sequential-group, single ascending dose study in 90 enrolled healthy male subjects in eleven dose groups. The study originally enrolled 90 subjects and 88 subjects completed the study (2 withdrew). Six subjects in each dose group were randomly assigned to receive GAR-936 and two to receive placebo (0.9% normal saline). Seven dose levels of GAR-936 (12.5, 25, 50, 75, 100, 200, and 300 mg) were administered to the subjects in the eleven dose groups. Since this was an ascending dose study the next higher dose of GAR-936 was not administered to the next group of subjects until the subjects from the previous dose level had completed their examinations. If significant drug-related toxicity occurred at any dose level, dose escalation was not to continue with the planned progression. In such a case, the next dose could be reduced to a level intermediate between the current dose and the previously administered dose, the same dose would be repeated, a dose lower than the next dose previously planned could be given, or the rate of infusion could be decreased. The dose groups differ from each other with respect to the dose of GAR-936 administered, the duration of the infusion, the subjects feeding status, and whether ondansetron was also administered. Table 1 shows the differences between dose groups.

Table 1. Summary of Dose Groups

Group[b]	Dose of GAR-936 (mg)	Length of Infusion (h)	Subjects' Feeding Status
I	12.5	1	Fasting
II	25	1	Fasting
III	50	1	Fasting
IV	75	1	Fasting
V	100	1	Fasting
VI	200	1	Fasting
VII	200	4	Fasting
VIII	200	1	Breakfast
IX	300	1	Breakfast
X	300	4	Breakfast and yogurt
XI	200	1	Fasting[c]

a: 6 subjects per group were randomly assigned to received GAR-936 and 2 to receive placebo.
b: Dose groups are numbered in the sequence in which the doses were administered.
c: Subjects in group XI received ondansetron (b) (4) 32 mg administered IV over 30 minutes, before receiving GAR-936 200 mg.

Gar-936 or placebo was administered as a constant-rate infusion over a 1- or 4-hour period. The patient in the one hour infusion group received meals after dose administration, three times daily (breakfast, lunch, and dinner) until the subjects left the center. In addition, subjects in group VIII, IX, and X received breakfast two hours before dose administration. The patients in the four hour infusion group received meals three times daily (breakfast, lunch and dinner) until the subjects left the center. Approximately 230g of whole milk or whole milk yogurt was consumed by the subjects in Group X.

Blood sampling for PK for subjects receiving a one hour infusion was to be done at 0h (within 2 hours before initiation of the infusion), 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12, 16, 24, 36, 48, 60, 72, and 96 hours. Blood sampling for PK for subjects receiving a four hour infusion was to be done at 0h (within 2 hours before initiation of the infusion), 2.0, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 10, 12, 16, 24, 36, 48, 60, 72, and 96 hours.

Urine samples for the one hour infusion group were collected on Day 1 at baseline (0 hrs), 1 to 4, 6 to 8, 12 to 16, and Day 2 to 3 at 24 to 48 hrs. Urine samples for the four hour infusion group were collected on Day 1 at 0-4, 4 to 8, 8 to 12, and 12 to 24 hrs, and Day 2 at 24 to 48 hrs. Urine was collected in a separate receptacle for each defined period of time.

ASSAY METHODOLOGY:
Serum GAR-936 concentrations were quantified by using a validated high performance liquid chromatography (HPLC) method with calibrators in the range of (b) (4) ng/ml. The lower limit of quantification was (b) (4)/ml. Intra-and inter-batch accuracy (% bias) and precision (CV%) of the GAR-936 assay were evaluated at the lower limit of quantification (LLOQ, (b) (4)/ml) and at three other concentration levels (b) (4) and (b) (4) ng/ml). The mean measured concentrations obtained at all concentration levels except the LLOQ were less than 10% different from the theoretical concentration (bias<10%); the bias at the LLOQ was (b) (4).
Gar-936 in human urine samples was quantified by using a validated HPLC/UV method of determination with a LLOQ of 2µg/ml. A 2-tier calibration curve (b) (4) was used. The intraday CV and bias at the low QC (b) (4) were used (b) (4) respectively. The interday CV and bias values a (b) (4) . the interday (n=25) precision (CV) values for the mid-QC samples (b) (4) (b) (4)) and the high QC (b) (4)) were between (b) (4) and the interday bias ranged from (b) (4) The precision (CV) and accuracy (expressed as bias) of GAR-936 calibration standards in the lower tier curve were between (b) (4) and between (b) (4) respectively. The precision (CV) and accuracy (expressed as bias) of GAR-936 calibration standards in the upper tier calibration curve were between (b) (4) and between (b) (4) repectively.

DATA ANALYSIS:
The pharmacokinetic parameters of GAR-936 were estimated using noncompartmental analysis. The parameters included peak concentration (C_{max}), area under the curve (AUC), mean residence time (MRT), systemic clearance (CL), renal clearance (CL_R), volume of distribution at steady state (V_{ss}), elimination half-life ($t_{½}$) and percent of drug excreted unchanged in urine (f_e[%]).

STATISTICAL ANALYSIS:
The pharmacokinetic parameters and arithmetic means are reported with standard deviations (SD) and CVs. Potential differences among dose groups in plasma concentrations and pharmacokinetic parameters of GAR-936 were assessed by using a 1-way analysis of variance (ANOVA).

RESULTS:
The mean age of the study participants ranged from 22 years in the 75 and 300 mg (4-hour infusion group to 32 years in the 300 mg (1-hour infusion) dose groups. The mean weight of the subjects ranged from 61.5 kg in the 200mg group (4-hour infusion) to 82.2 kg in the 12.5 mf dose group. Figures 1-3 shows the mean serum concentrations of GAR-936 at the various dosage levels

Figure 1. Mean Serum Concentration-Time Profiles in Healthy Subjects in the Fasting State after Various Single Doses of GAR-936 Given as Intravenous Infusions

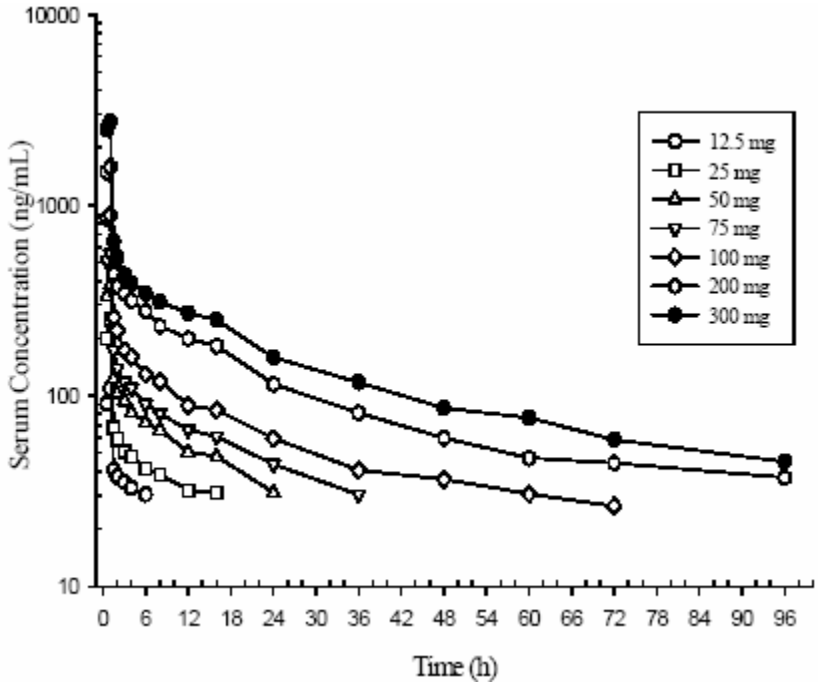


Figure 2. Mean GAR-936 SerumConcentration-Time Profiles in Healthy Subjects Receiving 200 mg Single Doses of GAR-936 as 1-Hour Intravenous Infusions

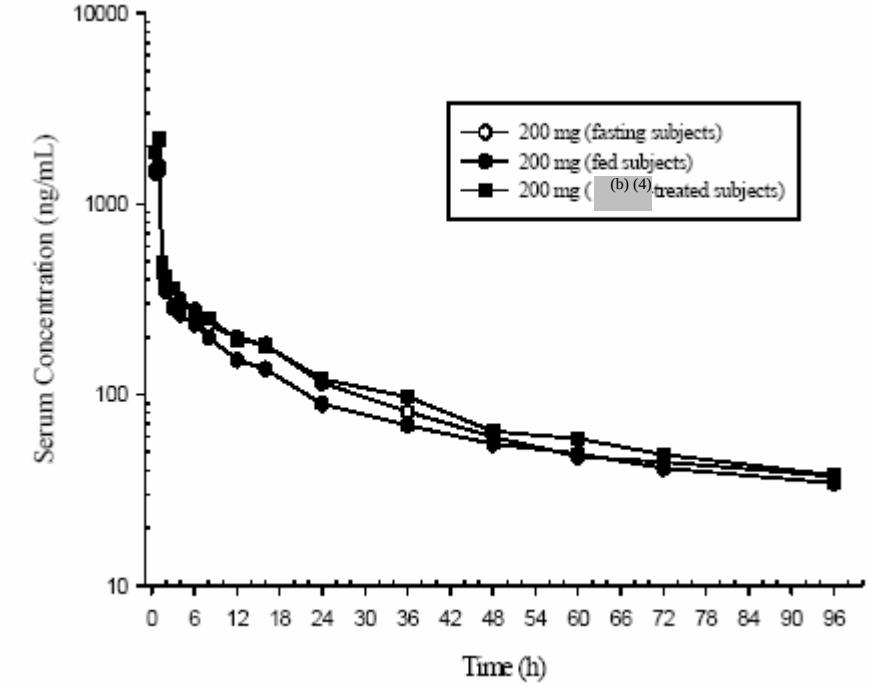
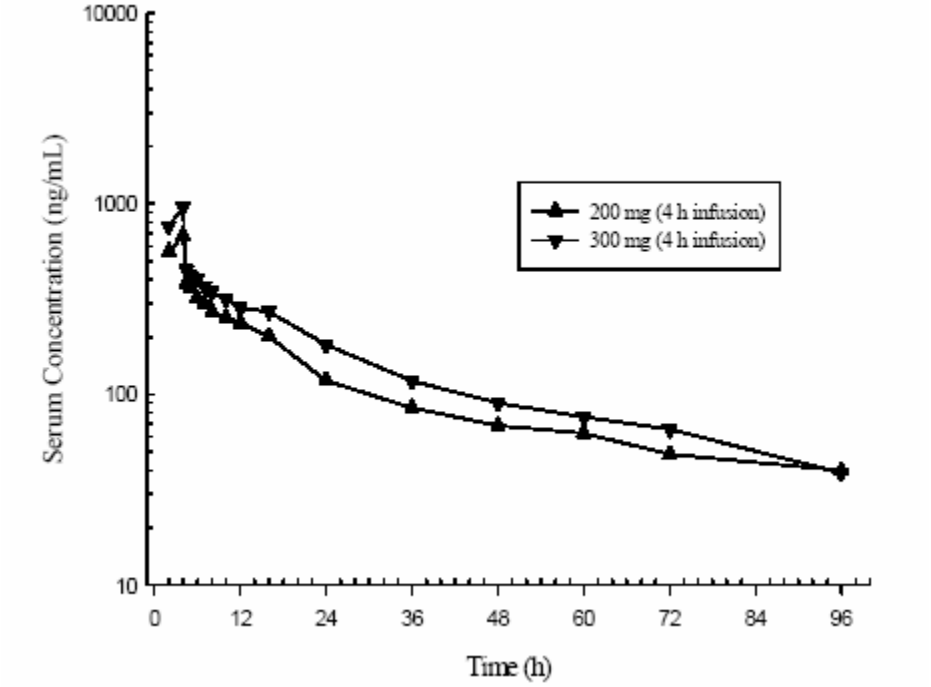


Figure 3. Mean GAR-936 Serum Concentration-Time Profiles in Healthy Subjects After Single 200 and 300mg Doses of GAR-936 Given as 4-H intravenous Infusions



The C_{max} of GAR-936 in subjects who received 1-hour infusions ranged from 108.5 ng/ml in the 12.5 mg dose group, to 2,817 ng/ml in the 300 mg dose group. The mean serum Cmax values in the 200 mg (680 ng/ml) and 300 mg (960 ng/ml) dose groups in which the length of the infusion was 4 hours were lower than the values seen after a 1-hour infusion of similar doses. Mean values for C_{max} obtained in the fed (1,528 ng/ml) and fasting (1,643 ng/ml) groups receiving 200 mg doses of GAR-936 (1-hour infusion) were not markedly different. However, the mean Cmax values observed in the 200 mg dose group in which subjects received ondansetron were slightly higher (2,189 ng/ml) than these values. Table 2 shows the mean PK parameters of GAR-936

Table 2. Pharmacokinetic Parameters of GAR-936 After Various Single Intravenous (IV) doses of GAR-936

	Dose Groups Receiving GAR-936 (mg)[b]											
	1-h IV Infusion									4-h IV Infusion		
PK Parameter	I 12.5 (fasting)	II 25 (fasting)	III 50 (fasting)	IV 75 (fasting)	V 100 (fasting)	VI 200 (fasting)	VIII 200 (fed)	XI 200 (fasting)[c]	IX 300 (fed)	VII 200 (fasting)	X 300 (fed)	p-Value
C_{max} (ng/mL)	108.5 (10)	252 (25)	383 (17)	566 (14)	911 (29)	1643 (18)	1528 (22)	2189 (30)	2817 (17)	680 (22)	960 (10)	0.001
AUC (ng•h/mL)	753 (68)	2255 (45)	2558 (21)	3658 (27)	6396 (10)	12426 (23)	11719 (19)	14462 (17)	17856 (10)	14237 (22)	16732 (16)	0.06
CL (L/h/kg)	0.3 (67)	0.2 (50)	0.3 (14)	0.3 (16)	0.2 (13)	0.2 (22)	0.2 (12)	0.2 (19)	0.2 (11)	0.2 (11)	0.3 (13)	0.27
V_{ss} (L/kg)	2.8 (34)	6.4 (20)	6.4 (31)	7.5 (10)	8.6 (18)	11 (25)	13 (18)	12 (43)	12 (20)	14 (11)	12 (24)	0.001
$t_{1/2}$ (h)	11 (84)	32 (64)	18 (21)	21 (25)	38 (14)	42 (28)	54 (28)	53 (20)	46 (13)	58 (14)	42 (23)	0.001
CL_R (L/h)	ND[d]	ND	ND	ND	2.6 (26)	3 (50)	2.2 (22)	1.8 (24)	2.7 (23)	NA[e]	2 (28)	0.13
f_e (%)	2.1 (113)	6.5 (39)	1.9 (72)	8 (37)	11 (25)	12 (31)	7.8 (25)	8.5 (38)	10.4 (22)	NA	8 (39)	0.001

a: Data presented as mean (CV%).
b: Dose groups are not presented in the order in which the doses were administered.
c: This group also received ondansetron (Zofran).
d: ND - Could not be determined accurately due to low concentrations in urine and serum.
e: NA – Not available.

The mean serum AUC's for GAR-936 ranged from 753 ng·h/ml in the 12.5 mg dose group to 17,856 ng·h/ml in the 300 mg dose group (1-hour infusion). The mean CL values of GAR-936 were consistent among dose groups and ranged from 0.2 L/h/kg to 0.3 L/h/kg. Gar-936 CL values were not significantly different among the various dose groups. The mean renal clearance values, as calculated from subjects receiving doses of GAR-936 greater than 75 mg, were low and ranged between 1.8 and 3.90 L/h. The amount of GAR-936 excreted unchanged in the urine could not be accurately determined because of the limitations of the assay in the 12.5 to 50mg dose group. However, based on the data gathered from subjects in the 75 to 300 mg dose groups, approximately 8% to 12% of GAR-936 was excreted in urine as unchanged drug. Gar-936 had an elimination t½ of approximately 40 to 60 hours as seen in groups receiving doses higher than 200mg. It was well distributed into various tissues as shown by the mean V_{ss}, which ranged from 7 to 14 L/kg. No significant differences in GAR-936 PK were seen between subjects who received Gar-936 in the fasting state and those who were given GAR-936 in the fed state.

A linear relationship exists between C_{max} and dose (r^2=0.99) as is shown in Figure 4, and also between AUC and dose (r^2=0.99) as is shown in Figure 5.

Figure 4. Relationship Between C_{max} and Dose of GAR-936 After Various GAR-936 Doses to Healthy Subjects

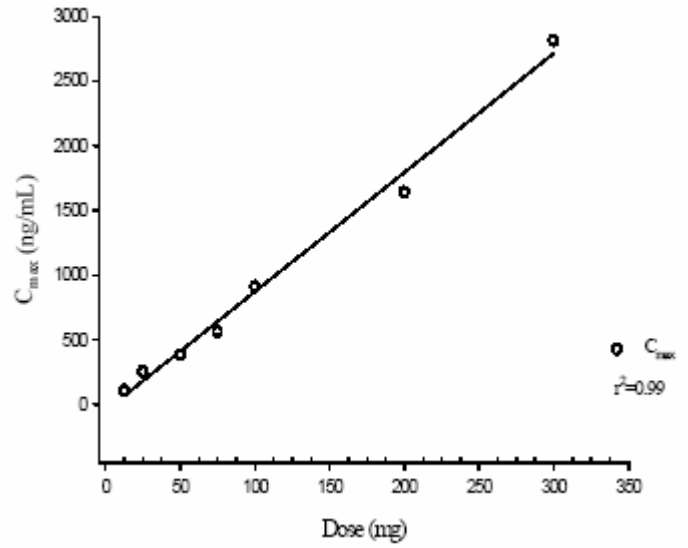
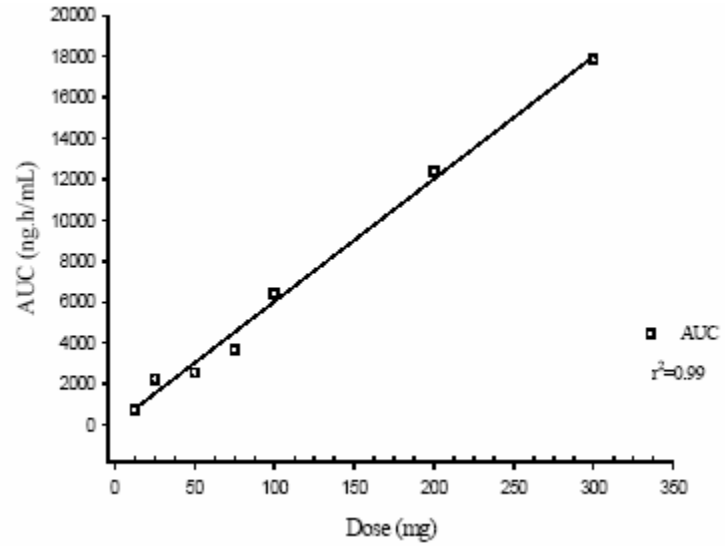


Figure 5. Relationship Between AUC and Dose of GAR-936 After Various GAR-936 Doses to Healthy Subjects



CONCLUSIONS:

The serum concentration-time profile of GAR-936 shows that the decline in drug concentrations after the end of GAR-936 infusion follows a polyphasic pattern. The steep decrease in serum GAR-936 concentration at the end of the infusion represents the distribution phase during which the drug is distributed out of the central compartment into various tissues. The terminal elimination phase is characterized by the movement of GAR-936 from the tissues into the vascular compartment followed by the subsequent elimination of GAR-936. Gar-936 exhibited linear PK in the dose range studied (12.5 to

300mg). It is well distributed into tissues and has a long t½. Food does not have any significant effect on the PK of GAR-936.

A double-blind, randomized, placebo-controlled, ascending multiple-dose study to assess the safety, tolerability, and pharmacokinetics of GAR-936 in healthy male subjects (Protocol 3074A1-101-US)

Dates: August 28, 1998 to November 17, 1998
Clinical Site: Clinical Pharmacology Unit, Wyeth-Ayerst Research, 1300 Wolf Street, Philadelphia, PA 19148
Analytical Sites:

Serum: (b) (4)

Urine: Drug Metabolism Division, Wyeth-Ayerst Research, 401 N. Middletown Road, Pearl River, NY 10965

OBJECTIVES:
The objectives of this study were to 1) assess the safety and tolerability of multiple intravenous (IV) doses of GAR-936 in healthy men (19 doses, 1 dose administered every 12 hours), and 2) to determine the pharmacokinetic profile of GAR-936 after multiple-dose administration.

FORMULATION:
GAR-936 100mg/5ml lyophilized powder Formulation # 0930919J (Batch # 1997B0186)
0.9% Sodium Chloride Injection USP (Batch# 1997B0186)

STUDY DESIGN:
This study was a single center, randomized, double-blind, placebo-controlled, ascending multiple-dose study in 32 enrolled subjects in four groups. Each of the four dosage groups was to have eight subjects; six randomly assigned to receive GAR-936 and two randomly assigned to receive placebo. Each subject received in IV dose of GAR-936 or placebo, twice a day (q12h) for nine consecutive days, followed by a single dose on the morning of day ten. The doses to be tested were 25, 50, 100 (1-hour infusions), and 150 mg (a 2-hour infusion) every 12 hours, which corresponds to a total daily dose of 50, 100, 200, and 300 mg, respectively. The first treatment was to be administered 30 minutes after breakfast and the second 12 hours later (30 minutes after dinner). The next higher dose of GAR-936 was not to be administered to the next group of subjects until the investigator reviewed the blinded data and consulted with the W-AR medical monitor.

Blood sampling for PK was taken less than 2 hours before the dose on Day1 and at 0.5, 1.0, 1.5, 2, 3, 4, 6, 8hrs on Day 1. Also a blood sample was taken on Day 1 at 12hr (before the evening dose). A blood sample was taken at 0 hrs on Day 7, 8, and 9. A blood sample was taken starting on Day 10 at 0, 0.5, 1, 1.5, 2.0, 2.5, 3, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, 96, 120, 144hrs until Day 16.

Urine samples for PK was collected on Day 1 before drug infusion (baseline sample) and on Day 10 in a separate receptacle for each defined period of time of 0-4, 4-8, 8-12, 12-24, 24-36, and 36-48 hrs after test article administration.

ASSAY METHODOLOGY:
Serum GAR-936 concentrations were quantified by using a validated high performance liquid chromatography (HPLC) method with calibrators in the range of (b) (4) ng/ml. The lower limit of quantification was (b) (4) /ml. Investigation of the intrabatch and interbatch accuracy showed that the coefficients of variation were less than (b) (4) at all concentrations studied. The corresponding mean measured concentration was less than 10% different from the theoretical concentration for concentration levels above the limit of quantification (b) (4) at LOQ).
Gar-936 in human urine samples was quantified by using a validated HPLC/UV method of determination with a lower limit of quantification of (b) (4) ml. A –tier calibration curve (b) (4) was to be used. However, only the lower tier curve was used for regression because all study sample concentrations were (b) (4) /ml. As seen during the validation, the intraday coefficient of variation (CV) and bias at the low QC (b) (4) were (b) (4) respectively. The inter-day CV and bias values at (b) (4) were (b) (4) The inter-day (n=25) precision (CV) values for the mid QC samples (b) (4) and the high QC (b) (4) were between (b) (4) and inter-day bias ranged from (b) (4) The precision (%CV) and accuracy (expressed as bias) of GAR-936 calibration standards in the lower tier curve were between (b) (4) respectively. The precision (%CV) and accuracy (expressed as bias) of GAR-936 calibration standards in the upper tier calibration curve were between (b) (4) respectively.

DATA ANALYSIS:
Pharmacokinetic parameters based on serum data for GAR-936 were estimated using the noncompartmental methods. The values for peak concentration (C_{max}) were obtained directly from the observed data on Day 1 and Day 10. The terminal elimination half life ($t_{½}$) was estimated on day 10 by dividing ln2 (0.693) by the elimination rate constant (λ_z). Area under the plasma concentration-time curve (AUC) during a dose interval ($AUC_{0-\tau}$) was determined by a log-linear trapezoidal rule from 0 to the time of the observed concentration at 12 hours after the first dose of the day on days 1 and 10. AUC at steady-state (AUC_{ss}) was obtained by determining $AUC_{0-\tau}$ on day 10. Systemic GAR-936 clearance was obtained by dividing the dose administered by the $AUC_{0-\tau}$ on day 10. Urine was collected from each subject at specific interval on day 10. The concentrations of GAR-936 were determined in the aliquots of urine obtained during each collection interval (0 to 4, 4 to 8, 8 to 12, and 12 to 24 hrs).

STATISTICAL ANALYSIS:
The pharmacokinetic parameters and the arithmetic means are reported with standard deviations (SD) and CV. Serum GAR-936 concentrations obtained at each collection time point on days 1 and 10 were compared using a one-way analysis of variance (ANOVA). All groups were normalized to the 100 mg dose group before ANOVA was performed. Pharmacokinetic parameters obtained on days 1 and 10 were compared among dose groups using a one-way ANOVA. Values for C_{max} (days 1 and 10), $AUC_{0-\tau}$ (day 1), and AUC_{ss} (day10) were dose normalized to the 100-mg dose group before ANOVA was performed.

RESULTS:
The planned dose administration schedule was modified because of GI intolerability, which was dose dependent in severity, frequency, and duration. The dose administration schedule was well tolerated by the subjects in the 25 and 50 mg dose groups. However, GI intolerance occurred in all GAR-936 treated subjects in the 100 mg dose group (nausea in six subjects, vomiting in four). Treatment of the 100 mg dose group was discontinued after nine days (total of 17 doses). An intermediate dose (75 mg) was then initiated (also administered at a flow rate of 200 ml per hour over a 1-hour period), and was also discontinued early (day 5) because of GI intolerance (nausea and vomiting occurred in all six subjects treated with GAR-936). The study was discontinued after the 75 mg dose group. Of the original 32 enrolled subjects, 20 completed the study. For pharmacokinetics, all but 2 subjects had day 1 data and 13 subjects were included in the day 10 analysis.

Subjects excluded from Pharmacokinetic Analysis:
Subject 10147-0007 (GAR-936 25 mg dose group was withdrawn after 12 doses because of an adverse reaction (rash; discussed in sections 7.3, 9.2.1, and 9.3.2). Although serum samples were collected on days 7,8, and 9 and the GAR-936 levels were determined in those samples, this subject was excluded from the PK analysis because he had not received GAR-936 on days 7, 8, and 9.

Subject 10147-0016 (GAR-936 50 mg dose group) was withdrawn after receiving 6 doses because of an adverse reaction (vomiting). This subject's day 10 PK data are not available.

Subject 10147-0017, -0019, and -0020 (GAR-936 100 mg dose group) were withdrawn after 9, 7, and 9 doses respectively, because of nausea, vomiting, or both. Therefore the day 10 PK parameters were not obtained for these subjects. Concentrations of GAR-936 tabulated under days 6, 7, and 8 were not included in the PK analysis because the subjects did not receive GAR-936 on those days.

None of the subjects in the 75 mg dose group continued beyond five days because of vomiting. Therefore, only day 1 PK results are available for these subjects.

Table 1 displays the demographics of the study participants. All of the subjects were healthy male volunteers.

Table 1 Demographic and Baseline Characteristics

	----------Dose (mg) of GAR-936----------					
Attribute	25 (n = 6)	50 (n = 6)	100 (n = 6)	75 (n = 6)	Placebo (n = 8)	Total (n = 32)
Age (years), n	6	6	6	6	8	32
Mean	33.2	36.0	40.0	37.0	34.1	35.9
SD	3.1	5.1	4.2	5.9	5.2	5.1
Minimum	30	30	32	30	26	26
Maximum	39	42	43	45	42	45
Ethnic origin, n (%)						
White	4 (67)	3 (50)	4 (67)	2 (33)	4 (50)	17 (53)
Black	2 (33)	3 (50)	1 (17)	2 (33)	3 (38)	11 (34)
Hispanic			1 (17)	2 (33)	1 (13)	4 (13)
Height (cm), n	6	6	6	6	8	32
Mean	176.6	179.9	181.7	181.3	176.9	179.1
SD	6.2	7.0	6.7	9.2	8.6	7.5
Minimum	166	168	174	172	167	166
Maximum	181	189	192	195	192	195
Weight (kg), n	6	6	6	6	8	32
Mean	83.3	80.7	82.7	83.0	80.7	82.0
SD	4.3	8.8	10.2	12.1	10.5	9.1
Minimum	76	74	68	73	67	67
Maximum	89	98	96	106	96	106

The mean age of the study subjects ranged from 33 years in the GAR-936 25 mg dose group to 40 years in the GAR-936 100 mg dose group. The mean weight of the subjects ranged from 81 kg in the placebo and GAR-936 50 mg dose groups to 83 kg in the other dose groups.

Table 2 displays the concomitant medications that the subjects received during this study. Twenty-two of the 32 subjects received one or more concomitant medications during the study. Three of six subjects in the 25 mg dose group, four of six in the 50 mg dose group, six of six in the 100mg dose group, six of six in the 75 mg dose group, and three of six in the placebo group received concomitant medications.

Table 2. Number (%) of Subjects in each Group Receiving Concomitant Medications

	----------GAR-936 dose (mg)----------					
Medication category[a]	25	50	100	75	Placebo	Total
Antacids	0	2 (33.3)	0	0	2 (25.0)	4 (12.5)
Antidiarrheal microorganisms	1 (16.6)	2 (3.3)	1 (6.6)	1 (16.6)	0	5 (15.6)
Antiemetics and antinauseants	0	0	4 (66.6)	6 (100.0)	0	10 (31.2)
Antihistamines for systemic use	1 (16.6)	1 (16.6)	0	0	0	2 (6.2)
Antiinflammatory/antirheumatic products, non-steroidal	0	1 (16.60	0	0	1 (2.5)	2 (6.2)
Antipsychotics[b]	1 (16.6)	1 (16.6)	6 (100.0)	2 (33.3)	1 (12.5)	11 (34.3)
Corticosteroids, plain	1 (16.6)	0	0	0	0	1 (3.1)
IV solution additives	0	0	0	0	1 (12.5)	1 (3.1)
Other analgesics and antipyretics	1 (16.6)	0	2 (33.3)	1 (16.6)	2 (25.0)	6 (18.7)
Other beta-lactam antibacterials	0	1 (6.6)	0	0	0	1 (3.1)

a: Some subjects received more than one concomitant medication.
b: The antipsychotic medication was prochlorperazine edisylate (Compazine), given for nausea/vomiting.

The most commonly administered concomitant medications were given for nausea and vomiting. These were the antipsychotic medication prochlorperazine edisylate (11 subjects, 34.3%) and the antiemetic/antinauseant medication ondansetron hydrochloride (10 subjects, 31.2%).

Table 3 displays the listing if the PK parameters of GAR-936 (days 1 and 10).

Table 3. Pharmacokinetic Parameters of GAR-936 by Dose Group in Healthy Male Subjects Receiving Multiple Doses of GAR-936 as One Hour Intravenous Infusions

		***** DAY 1 *****		********** DAY 10 **********						
DOSE GROUP		CMAX (NG/ML)	AUC 0-TAU (NG*H/ML)	CMAX (NG/ML)	AUC,SS (NG*H/ML)	T 1/2 (H)	CL (L/H/KG)	VSS (L/KG)	MRT_IV (H)	R
25 MG Q/12H	MEAN	260.8	795.6	323.7	1482.1	49.30	0.204	8.58	43.88	1.28
	S.D.	37.2	62.6	54.4	259.4	35.49	0.035	1.97	15.79	0.23
	% CV	14.2	7.9	16.8	17.5	71.99	17.094	23.00	35.97	17.88
50 MG Q/12H	MEAN	487.4	1440.0	620.9	3069.1	36.86	0.204	7.18	35.49	1.31
	S.D.	80.7	195.2	93.4	381.5	11.64	0.018	0.51	4.62	0.13
	% CV	16.6	13.6	15.0	12.4	31.59	9.034	7.04	13.01	10.00
100 MG Q/12H	MEAN	816.3	2388.6	1173.1	4979.9	66.45	0.239	9.06	37.53	1.34
	S.D.	121.0	304.0	176.3	924.9	22.73	0.047	2.91	5.33	0.25
	% CV	14.8	12.7	15.0	18.6	34.21	19.818	32.13	14.22	18.27
75 MG Q/12H	MEAN	766.4	2149.0	.	.	.	.	.	.	.
	S.D.	119.7	215.9	.	.	.	.	.	.	.
	% CV	15.6	10.0	.	.	.	.	.	.	.
SOURCE		P-VALUES FROM A ONE-WAY ANALYSIS OF VARIANCE @								
DOSE GROUP		0.06	0.01	0.71	0.25	0.33	0.32	0.34	0.47	0.91
		TUKEY'S STUDENTIZED RANGE TEST (P=0.05)								
			25>100							

@ CMAX, AUC 0-TAU AND AUC,SS WERE DOSE NORMALIZED TO A 100 MG DOSE PRIOR TO ANALYSIS.
* DAY 10 VALUES FOR GAR-936 100 MG DOSE GROUP ARE DAY 9 VALUES. HOWEVER, COMPARISONS OF PHARMACOKINETIC PARAMETERS ARE VALID SINCE SERUM LEVELS OF GAR-936 ARE ASSUMED TO BE AT STEADY STATE BY DAY 7.
** DAY 10 VALUES FOR GAR-936 75 MG DOSE GROUP NOT REPORTED SINCE SUBJECTS DROPPED OUT OF THE STUDY BY DAY 7.

Results of the statistical analysis (using ANOVA) of each PK parameter across the dose groups are presented in Table 3. The values for C_{max}, $AUC_{0-\tau}$, and AUC_{ss} were normalized to those of the 100 mg dose group before statistical analysis. The mean dose-normalized serum C_{max} of GAR-936 in subjects on day 1 were not significantly different among dose groups and ranged from 261 ng/ml in the 25 mg dose group to 816 ng/ml in the 100 mg dose group. The mean $AUC_{0-\tau}$ values on day 1 ranged from 796 ng·h/ml in the GAR-936 25 mg dose group to 2389 ng·h/ml in the GAR-936 100 mg dose group.

None of the steady-state PK parameter values (obtained on day 10), including dose-normalized values for C_{max} and AUC, were significantly different among dose groups ($p>0.05$). Mean C_{max} obtained at steady state ranged from 324 ng/ml in the 25 mg dose group to 1173 ng/ml in the 100 mg dose group. Mean AUC_{ss} values increased proportionately from 1482 ng·h/ml to 4980 ng·h/ml with an increase in dose from 25 mg to 100 mg. Gar-936 was cleared with a mean value of 0.2 L/h/kg by all dose groups. The accumulation factor (R) was calculated to be 1.3 across all dose groups. The accumulation factor was calculated by dividing the C_{max} obtained at steady state by the C_{max} obtained on day 1. Mean elimination $t_{½}$ of GAR-936 obtained on day 10 ranged from 36.9 hours in the 50 mg dose group to 66.5 hours in the 100 mg dose group. High intersubject variability (CV > 30%) was seen in the $t_{½}$ of GAR-936 in each dose group examined.

Table 4 shows the urinary concentrations of GAR-936 obtained on day 10.

Table 4. Urinary amounts of GAR-936 on Day 10 in Subjects from the 25, 50, and 100 mg Q12

(UNIT = MG)

DOSE (MG)	SUBJECT	********** DAY 10 ********** 0-4 HR	4-8 HR	8-12 HR	12-24 HR	AE
25	2	(b) (4)				
	3					
	4					
	5					
	7					
	8					
	MEAN	2.49	1.42	0.93	.	3.44
	S.D.	0.96	0.23	.	.	2.06
	CV %	38.38	16.54	.	.	60.01
50	9	(b) (4)				
	10					
	11					
	14					
	16					
	MEAN	3.59	1.91	1.28	.	4.39
	S.D.	0.89	.	.	.	2.17
	CV %	24.89	.	.	.	49.36
100	17	(b) (4)				
	19					
	20					
	22					
	23					
	24					
	MEAN	5.90	2.68	1.67	3.79	12.22
	S.D.	1.53	1.01	0.22	2.58	4.56
	CV %	25.87	37.71	13.32	68.12	37.34

75 MG COHORT NOT SHOWN SINCE ALL SUBJECTS DROPPED OUT OF THE STUDY BY DAY 10
DAY 10 VALUES FOR THE 100 MG DOSE GROUP ARE DAY 9 VALUES

Table 4 shows moderately high levels of GAR-936 in urine. The mean concentrations of GAR-936 on day 10 (steady state) in the 0-4 hour interval after dose administration ranged from 5.7 µg/ml after a 25 mg q12h dose to 10.4 µg/ml after a 100 mg q12h dose. Concentrations in most subjects in the 4 -8 hour interval after dose administration (day 10) were below the limit of quantitation (b) (4) ml) in most subjects in the 25 mg and 50 mg q12h dose groups. Urinary concentrations in the 4-8 hour interval after dose administration in the 3 subjects who completed the study in the 100 mg q12h dose group ranged from (b) (4). The 25 and 50 mg dose administrations were well tolerated by the subjects in those groups. Gastrointestinal (GI) intolerance occurred in all six subjects in the tigecycline 100mg dose group (nausea in six, vomiting in four). Treatment of the 100mg dose group was discontinued after nine days (total of 17 doses). An intermediate tigecycline 75mg dose group was then initiated, and treatment was also discontinued early (day five) because of GI intolerance (nausea and vomiting in all six tigecycline treated subjects). The study was closed after the 75 mg dose group was discontinued.

CONCLUSION:

The pharmacokinetics of GAR-936 in healthy subjects after multiple doses were linear in the dose range between 25 and 100 mg given IV q12h. GAR-936 has a long $t_{½}$ and a high volume of distribution, indicating extensive tissue distribution. Assuming a 40 hour half-life, steady state would be achieved in 8 days. The sponsor determined the accumulation factor by dividing the C_{max} at day 10 in multiple dose state by the C_{max} at day 1 in single dose. The accumulation factor is 1.3, which means that with steady state conditions the levels are approximately 30% higher than single dose.

The safety and tolerability of tigecycline administered in various concentrations and infusion rates in healthy subjects(Protocol 3074A1-109-US)

Dates: May 15, 2001 to June 4, 2001
Clinical site: Clinical Pharmacology Unit, Wyeth Research, 1300 Wolf Street, Philadelphia, PA 19148
Analytical sites:

1. Serum samples for tigecycline determination were analyzed at (b) (4)
2. Urine samples for 5-HIAA determination were analyzed at (b) (4)
3. Laboratory determinations for subjects with clinically important changes in Laboratory test results were performed at (b) (4)

OBJECTIVES:
The primary objective of this study was to evaluate the safety and tolerability of various concentrations and infusion rates of tigecyline in comparison with those of the previously studied dosage regimen. A secondary objective was to investigate the potential role of serotonin in tigecycline-related nausea and vomiting, and to evaluate a limited PK profile of tigecycline administered at various infusion rates and concentrations.

FORMULATION:
Sterile tigecyline lyophilized powder was supplied in vials containing 100 mg of drug with batch #1997B0186. An assay of this sample batch showed that it contained (b) (4). The placebo for this study is 0.9% sterile saline injection, supplied by the investigator.

STUDY DESIGN:
This study was a multiple-dose, parallel, randomized, double blind, placebo-controlled, inpatient study conducted with healthy subjects at a single site. Twenty-seven subjects were planned, 28 enrolled, and 22 subjects completed the study. Six subjects were withdrawn from the study and 18 subjects were included in the PK analysis. Each subject was to participate for 22 days, with a 14 day screening period followed by a nine day/eight night inpatient period. The subjects were treated in three groups, each with a matched placebo group. All of the subjects in the tigecycline treatment groups received the same doses of tigecycline: a 100 mg loading dose and nine doses of 50 mg each, given every twelve hours. The three test groups differed only in the volume of tigecycline or placebo infused or in the rate of administration. Group one subjects received tigecycline or placebo in 100 ml of normal saline over 60 minutes. Seven subjects in group one received tigecycline and three received placebo. Group two subjects received tigecycline or placebo in 100 ml of normal saline over 30 minutes. Six subjects received tigecycline and three received placebo. Group three subjects received tigecycline or placebo in 250 ml of normal saline over 60 minutes. Six subjects received tigecycline and three received placebo.

Blood samples for PK evaluation were collected for determinations of tigecycline serum concentrations at time 0 (predose) and 15 and 30 minutes after the start of the test article infusion (either at 30 minutes for the 60 minute infusion, or at the end of the infusion for the 30 minute infusion). For the 60 minute infusion, additional samples were taken at 45 and 60 minutes (at the end of the 60 minute infusion) after the start of the test article infusion. Additionally, samples for serum trough level determinations were obtained on days 3, 4, and 5 before the morning dose of test article.

Urine for measurement of 5-hydroxyindolacetic acid (5-HIAA) and creatinine excretion was collected on days 1 through 5 over the following time periods: predose (within 2 hrs before the dose on day 1 only), 0 to 4hrs, 4 to 8 hrs, 8 to 12 hrs, and 12 to 24 hrs.

ASSAY METHODOLOGY:
Serum tigecycline concentrations were quantified by using a validated methodology that employed the (b) (4) system. The lower limit of quantitation was (b) (4) ml and the upper limit of quantitation was (b) (4) /ml. Quality control samples of tigecycline prepared in human serum at concentrations of (b) (4) (high), (b) (4) (medium) and (b) (4) /ml (low) were analyzed along with the subject's samples. The overall precision and accuracy for the standards and the QC samples were in the range (b) (4) respectively.

Urine 5-HIAA concentrations were quantified by using the (b) (4) S system. The lower limit of quantitation for HIAA was (b) (4) /ml, and the upper limit of quantitation was (b) (4) ml. Quality control samples of 5-HIAA at theoretical concentrations of (b) (4) ng/ml were analyzed along with the subjects samples. Two QC samples at the same concentration level were required to be within (b) (4) of the target value. At least four of six QC samples were required to have concentrations that differed from the target value by no more than (b) (4)

DATA ANALYSIS:
The PK measurements include the serum tigecycline C_{max} on day 1 and the trough serum tigecycline (C_{min}) observed on study days 3, 4, and 5. The statistical analysis performed for this study was primarily limited to summary statistics (eg, means, standard deviations, and coefficients of variation) for the PK measurements. The serum trough concentrations of tigecycline and 5-HIAA urine concentrations across dose groups were compared using a 1-factor analysis of variance.

RESULTS:
Twenty-eight subjects were enrolled and 22 completed the study. All six of the subjects withdrawn were due to adverse events. Table 1 shows the withdrawn subjects by group.

Table 1. Number (%) of Subjects Withdrawn from the Study by Primary Reason

	ADMINISTRATION RATE OF TIGECYCLINE OR PLACEBO				
	Group 1 — 100 mL/60 min	Group 2 — 100 mL/30 min	Group 3 — 250 mL/60 min		
Reason for Withdrawal	Tigecycline (n = 7)	Tigecycline (n = 6)	Tigecycline (n = 6)	Placebo (n = 3)	Total (n = 28)
Total	3 (43)	1 (17)	1 (17)	1 (33)	6 (21)
Adverse event	3 (43)	1 (17)	1 (17)	1 (33)	6 (21)

Three subjects in the tigecycline 100 ml/60 min group were withdrawn, including subject 1090010005, who was withdrawn because of preexisting gonorrhea and who was replaced by subject number 1090011005. One subject in the tigecycline 100 ml/30 min group was withdrawn, and one subject in the tigecycline 250 ml/ 60 min group and one in the matching placebo group were also withdrawn.

Table 2 shows the dempgraphic and baseline characteristics for all subjects by treatment group.

Table 2. Demographic and Baseline Characteristics of Subjects

	Administration Rate of Tigecycline or Placebo						
	Group 1 — 100 mL/60 min		Group 2 — 100 mL/30 min		Group 3 — 250 mL/60 min		
Attribute	Tigecycline (n = 7)	Placebo (n = 3)	Tigecycline (n = 6)	Placebo (n = 3)	Tigecycline (n = 6)	Placebo (n = 3)	Total (n = 28)
Age (years)							
Mean	35.1	36.0	40.3	39.3	34.3	36.3	36.8
SD	6.4	5.6	7.1	6.7	5.6	4.5	6.1
Minimum	28.0	30.0	32.0	32.0	27.0	32.0	27.0
Maximum	45.0	41.0	50.0	45.0	42.0	41.0	50.0
Ethnic origin, n (%)							
Black	4 (57)	1 (33)	5 (83)	1 (33)	5 (83)	2 (67)	18 (64)
Hispanic	1 (14)	1 (33)	0	0	0	0	2 (7)
White	2 (29)	1 (33)	1 (17)	2 (67)	1 (17)	1 (33)	8 (29)
Height, cm							
Mean	178.6	172.6	180.8	189.0	175.9	181.9	179.3
SD	6.1	2.7	7.8	5.1	4.6	4.2	6.8
Minimum	169.2	170.0	172.5	183.3	169.5	177.1	169.2
Maximum	187.4	175.3	195.0	193.2	183.3	185.1	195.0
Weight, kg							
Mean	79.8	78.6	88.0	95.2	78.5	79.6	82.8
SD	5.3	3.7	7.7	7.8	8.1	8.3	8.5
Minimum	71.6	74.3	79.0	86.9	66.2	70.9	66.2
Maximum	86.2	80.9	100.8	102.4	88.0	87.4	102.4

All subjects were male. There were no clinically important differences among the groups at baseline. All subjects were healthy and did not have any medical conditions that might have interfered with the metabolism or excretion of the study medication or the interpretation of the results. All subjects received some type of concomitant medication during the study. The most frequently used concomitant medications were

prochlorperazine edisylate and sucralfate. All concomitant medications were given as treatment for adverse events.

Ten subjects were excluded from the PK analysis. This includes all of the subjects who received placebo (and would have had no circulating levels of tigecycline) and subject 1090010005, who was replaced in the study by subject 1090011005.

The individual and mean tigecycline serum concentrations and C_{max} values obtained after the first tigecycline dose when given as a 60 minute unfusion with a flow rate of 100 ml/h, a 30 minute infusion with a flow rate of 200 ml/h, and a 60 minute infusion with a flow rate of 250 ml/h are shown in table 3, 4 and 5.

Table 3. Tigecycline Serum Concentrations and C_{max} on Day 1 in Healthy Subjects Receiving IV Doses of Tigecycline over 60 minutes at 100ml/h

(UNIT = ng/mL)

SUBJECT	TIME AFTER DOSE (HOURS) 0	0.25	0.5	0.75	1	Cmax (ng/mL)
9	(b) (4)					
12						
16						
23						
26						
1005						
MEAN	0.0	456.8	526.9	605.5	547.5	641.5
S.D.	0.0	83.8	138.6	149.8	136.9	153.3
% CV	.	18.3	26.3	24.7	25.0	23.9

NOTE: SUBJECTS RECEIVED 100 mg LOADING DOSE FOLLOWED BY 50 mg Q12H FOR A TOTAL OF 10 DOSES.
"." DENOTES DATA NOT AVAILABLE

Table 4. Tigecycline Serum Concentrations and C_{max} on Day 1 in Healthy Subjects Receiving IV Doses of Tigecycline over 30 minutes at 200ml/h

(UNIT = ng/mL)

SUBJECT	TIME AFTER DOSE (HOURS) 0	0.25	0.5	0.75	1	Cmax (ng/mL)
3	(b) (4)					
4						
10						
13						
19						
27						
MEAN	0.0	862.3	966.0	.	.	969.4
S.D.	0.0	135.1	146.1	.	.	141.7
% CV	.	15.7	15.1	.	.	14.6

NOTE: SUBJECTS RECEIVED 100 mg LOADING DOSE FOLLOWED BY 50 mg Q12H FOR A TOTAL OF 10 DOSES.
"." DENOTES DATA NOT AVAILABLE

Table 5. Tigeccycline serum concentrations and C_{max} on Day 1 in Healthy Subjects Receiving IV Doses of Tigecycline over 60 minutes at 250 ml/h

SUBJECT	(UNIT = ng/mL) ********** TIME AFTER DOSE (HOURS) ********** 0	0.25	0.5	0.75	1	Cmax (ng/mL)
2	(b) (4)					
7						
11						
18						
20						
24						
MEAN	0.0	453.5	529.8	595.9	668.1	668.1
S.D.	0.0	38.5	53.3	71.1	19.6	19.6
% CV	.	8.5	10.1	11.9	2.9	2.9

NOTE: SUBJECTS RECEIVED 100 mg LOADING DOSE FOLLOWED BY 50 mg Q12H FOR A TOTAL OF 10 DOSES.
"." DENOTES DATA NOT AVAILABLE

Tables 3 6, 7, and 8 shows the individual and mean trough concentrations of tigecycline obtained in three groups of subjects on days 3, 4, and 5.

Table 6. Tigecycline Trough Serum Concentrations in Healthy Subjects Receiving IV Doses of Tigecycline over 60 minutes at 100 ml/hr

SUBJECT	(UNIT = ng/mL) - TIME AFTER DOSE (HOURS) - Day 3 (48)	Day 4 (72)	Day 5 (96)
9	(b) (4)		
12			
16			
23			
26			
1005			
MEAN	71.7	90.8	83.8
S.D.	31.4	26.0	31.8
% CV	43.7	28.7	38.0

NOTE: SUBJECTS RECEIVED 100 mg LOADING DOSE FOLLOWED BY 50 mg Q12H FOR A TOTAL OF 10 DOSES.
"." DENOTES DATA NOT AVAILABLE

Table 7. Tigecycine Serum Trough Concentrations in Healthy Subjects Receiving IV Doses of Tigecycline over 30 minutes at 200 ml/hr

SUBJECT	(UNIT = ng/mL) - TIME AFTER DOSE (HOURS) - Day 3 (48)	Day 4 (72)	Day 5 (96)
3	(b) (4)		
4			
10			
13			
19			
27			
MEAN	69.2	92.3	99.1
S.D.	32.0	21.8	29.6
% CV	46.3	23.7	29.8

NOTE: SUBJECTS RECEIVED 100 mg LOADING DOSE FOLLOWED BY 50 mg Q12H FOR A TOTAL OF 10 DOSES.
"." DENOTES DATA NOT AVAILABLE

Table 8. Tigecycline Trough Serum Concentrations in Healthy Subjects Receiving IV Doses of Tigecycline over 60 minutes at 250 ml/hr

SUBJECT	(UNIT = ng/mL) - TIME AFTER DOSE (HOURS) - Day 3 (48)	Day 4 (72)	Day 5 (96)
2	(b) (4)		
7			
11			
18			
20			
24			
MEAN	97.7	104.3	106.6
S.D.	17.2	26.4	38.1
% CV	17.6	25.3	35.7

NOTE: SUBJECTS RECEIVED 100 mg LOADING DOSE FOLLOWED BY 50 mg Q12H FOR A TOTAL OF 10 DOSES.
"." DENOTES DATA NOT AVAILABLE

The tigecycline trough levels were not significantly different among days 3, 4, and 5, indicating that steady state is reached by day 3. As expected, the mean C_{max} was higher when tigecycline was given as a 30 minute infusion than when it was given as a 60 minute infusion.

The concentration of 5-HIAA in the urine of subjects showed no definite relationship between the occurrence of nausea and the excretion of 5-HIAA (the final metabolite of serotonin) in the urine of subjects. The sponsor suggests that the nausea and vomiting associated with the tigecycline administration are not associated with increased serotonin production.

CONCLUSIONS:
The administration of tigecycline in 100 ml of saline infused over 30 or 60 minutes is safe, and the adverse events related to IV infusion of tigecycline were comparable to those seen with the previously studied rate of administration of 250 ml/min.

Study of the Pharmacokinetic profile of Tigecycline in Serum and Blister Fluid after Multiple Intravenous Administrations in Healthy Adults(Protocol 3074A1-113-US)

Dates: October 29, 2003 to November 24, 2003
Clinical site: (b) (4)

Analytical Site: (b) (4)

OBJECTIVES:
The objective of the study was to determine the pharmacokinetic profile of tigecycline in blister fluid and serum in healthy subjects after multiple administrations of tigecycline.

FORMULATION:
Sterile tigecycline powder for injection was supplied by Wyeth Research in 5ml, flint glass vials, each containing lyophilized free base equivalent to 50mg of tigecycline. Batch number is 2001B0097. The vials were reconstituted with sterile normal saline (0.9% USP).

STUDY DESIGN:
This study was a multiple dose, open label, inpatient study conducted with 10 healthy men (age 18-45 years) at a single center. Each subject participated in the study for approximately 27 days, including a screening evaluation within 21 days before the initial tigecycline administration and a six day (five night) inpatient period. Each subject received IV tigecycline 100mg, followed by 50 mg every 12 hours, for a total of seven doses of tigecycline. On day one all of the subjects received 100mg IV as the loading dose, administered in 100 ml normal saline infused over 30 minutes at 200 ml/h. Twelve hours later, all of the subjects received tigecycline 50 mg IV as the maintenance dose, administered in 100 ml of normal saline infused over 30 minutes at 200 ml/hr. The subjects consumed a medium-fat meal approximately 30 minutes before each dose of tigecycline. On days two and three, at approximately 8:00 AM and 8:00 PM, and approximately 30 minutes after a medium-fat meal, tigecycline 50 mg was administered in 100 ml of normal saline solution infused over 30 minutes. On day three, blister induction was performed on the forearms of subjects approximately two hours before the second dose of tigecycline (approximately 14 hours before the first PK sample collection). After the skin was disinfected with alcohol, 0.2 ml of cantharidin ointment (compounded from 0.25% cantharidin powder in a standard ointment base) was measured with a syringe and applied to each forearm over approximately 1.5cm^2. Plastic cups 2.5 cm in diameter were placed around the ointment to protect it and to prevent its spread beyond the application area. In addition, a small amount of inert ointment base was applied to the interior base of the cup to prevent spread of the cantharidin ointment beyond the application area. The subject's forearms were then wrapped lightly with a bandage wrap to protect the blister area. The cantharidin ointment remained in contact with the skin for approximately 12 to 14 hours. Any excess ointment was carefully removed. The resulting blisters were sprayed lightly with a fast-drying plastic dressing to

maintain their integrity. Study days four and five the subjects received the tigecycline doses as described in day two and three.
Venous blood samples (7 ml) for tigecycline PK analysis were collected from each subject on day one and after the start of the tigecycline infusion on day four at 0 (predose), 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours. The samples were collected from an indwelling catheter or by direct venipuncture. If a catheter was used for blood collection, the approximately 0.5 ml of blood was to have been discarded before the sample was collected. Only saline was used to flush the catheter. The total amount of blood collected from each subject (including screening samples) was approximately 150 ml.

Skin blister fluid samples (50 to 100µL per sample) were collected from each subject on day four at hour 0 (predose) and after the start of the tigecycline infusion at hours 0.5, 1, 2, 3, 4, 6, 8, 12, and 24. These were the same time points used for the collection of the tigecycline venous blood samples, except for the day 1 (predose) blood sample.

ASSAY METHODOLOGY:
Concentrations of tigecycline in serum and skin blister fluid were determined by sensitive and specific liquid chromatography methods with [redacted] (b) (4) detection [redacted] (b) (4). The performance of the tigecycline assays during the analysis of the serum and blister fluid samples are summarized in Table 1, 2 and 3.

Table 1. Assay Range and Sensitivity

Standard Curve	Tigecycline/Serum (ng/mL)	Tigecycline/Blister Fluid (ng/mL)
Linear range	[redacted] (b) (4)	
Sensitivity		

Table 2. Summary of Assay Performance for Serum Assays

	---Low QC [redacted] (b) (4)			---Middle QC ([redacted] (b) (4)			--High QC [redacted] (b) (4)		
Analyte	Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
Tigecycline	[redacted] (b) (4)								

Abbreviations: CV = coefficient of variation; QC = quality control.

Table 3. Summary of Assay Performance for Skin Blister Assays

	---Low QC [redacted] (b) (4)			---Middle QC ([redacted] (b) (4)			--High QC ([redacted] (b) (4)		
Analyte	Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
Tigecycline	[redacted] (b) (4)								

Abbreviations: CV = coefficient of variation; QC = quality control.

DATA ANALYSIS:
The tigecycline serum and skin blister fluid concentration data for each subject were analyzed by using empirical, model-independent PK methods. The C_{max} and the time to peak concentration (t_{max}) were taken directly from the observed data. The apparent

terminal-phase disposition rate constant (λ_z) was estimated by a log-linear regression of the last two observed concentrations (12 and 24 hours after the last dose). The apparent terminal-phase disposition half-life ($t_{½}$) was calculated as $t_{½}=0.693/\lambda_z$. The area under the concentration-time curve over one steady state dosing interval (AUC or $AUC_{0\text{-}12h}$) was calculated by using the log-trapezoidal rule for decreasing concentrations and the linear-trapezoidal rule for increasing concentrations. Systemic clearance (Cl) was calculated as dose/AUC. Additionally, the degree of tigecycline penetration into skin blister fluid was calculated as $AUC_{blister\ fluid}/\ AUC_{serum}$. In this study, the estimate of $t_{½}$ may not be reliable because the serum and blister fluid were sampled only for 24 hours after the last dose of tigecycline, which is much shorter than the previously reported tigecycline $t_{½}$ after multiple-dose administration (35 to 55 hours). The tigecycline serum and skin blister fluid concentrations and PK parameters were analyzed by descriptive statistics (eg, mean, standard deviation [SD], and coefficient of variation).

RESULTS:

Ten subjects were enrolled and all completed the study after receiving a loading dose of 100 mg tigecycline followed by six doses of 50 mg IV tigecycline administered every 12 hours. All ten subjects had one or more adverse events, the most frequent of which was nausea and vomiting. Table 4 summarizes the mean with SD and geometric mean of estimates of tigecycline serum PK parameters for these subjects.

Table 4. Tigecycline Serum Pharmacokinetic Parameters (Mean ± SD and Geometric Mean)

Group	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC (ng·h/mL)	Cl (L/h)	V_{ss} (L)
All subjects (n=10)	819±113	0.5±0.0	34.3±20.3	2185±320	23.4±3.6	854±361
	812	0.5	42.9	2163	23.1	775

Abbreviations: AUC = total area under the concentration-time curve; Cl = systemic clearance; C_{max} = peak serum concentration; SD = standard deviation; t_{max} = time of peak concentration; $t_{1/2}$ = half-life; V_{ss} = volume of distribution at steady state.

The mean steady state tigecycline PK parameters are similar to the previously reported values. Table 5 summarizes the mean with SD and geometric mean of estimates of tigecycline skin blister fluid PK parameters for these subjects.

Table 5. Tigecycline Skin Blister Fluid Pharmacokinetic Parameters (Mean ± SD and Geometric Mean)

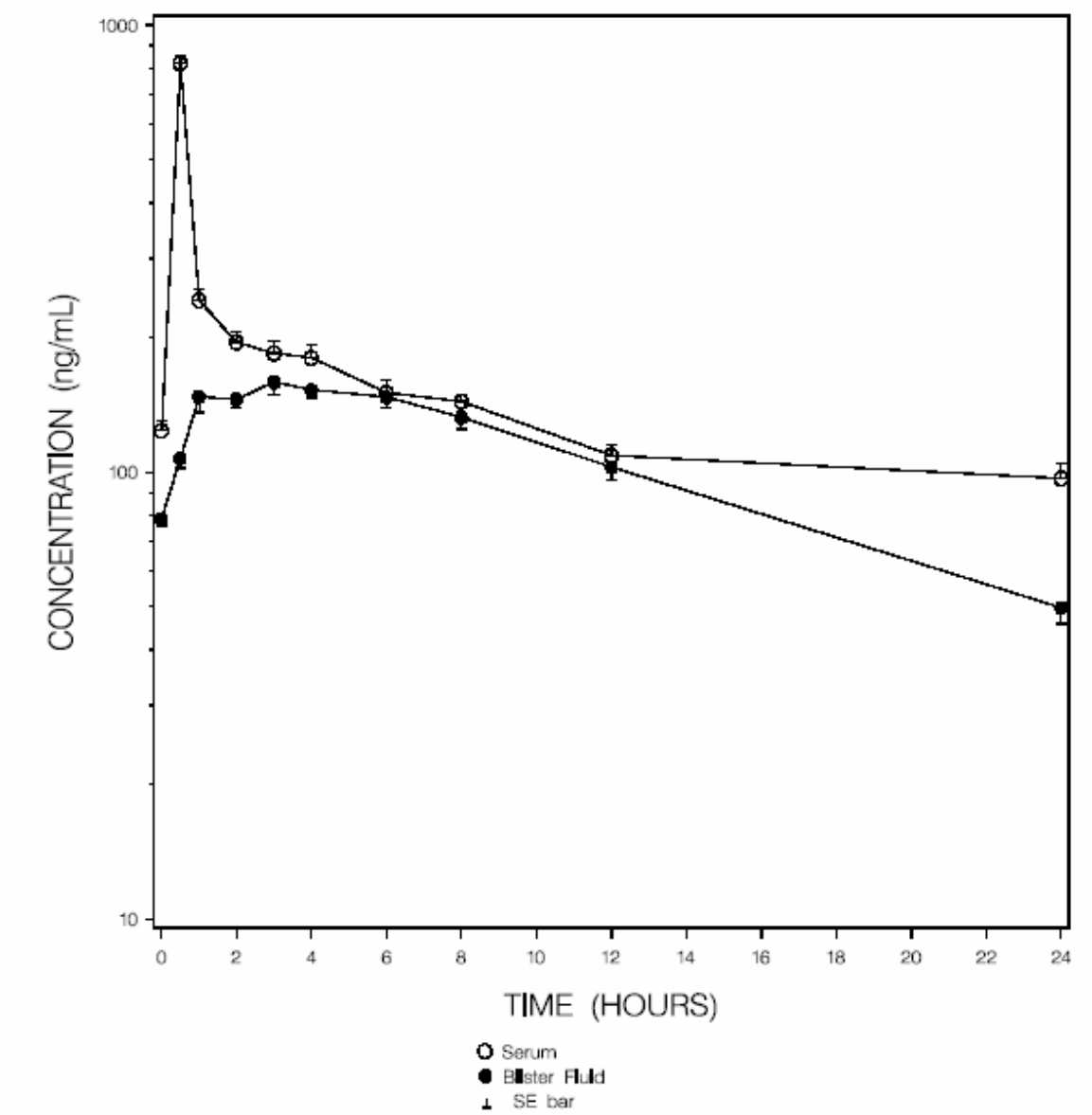
Group	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC (ng·h/mL)	$AUC_{Blister}/\ AUC_{Serum}$ (%)
All subjects (n=10)	173±28	2.8±2.0	11.8±2.5	1609±214	74.0±7.0
	171	2.0	11.5	1596	73.8

Abbreviations: AUC = total area under the concentration-time curve; C_{max} = peak serum concentration; SD = standard deviation; t_{max} = time of peak concentration; $t_{1/2}$ = half-life.

The peak tigecycline concentrations in blister fluid are attained 2.8 hours after the start of administration of a 30 minute IV infusion of tigecycline, indicating that there is a lag time

for distribution of tigecycline from the serum into the blister fluid. The degree of penetration into blister fluid as measured by the ratio of $AUC_{0\text{-}12h,Blister}/AUC_{0\text{-}12h,Serum}$ was 74%. Figure 1 presents the mean tigecycline serum concentrations from the ten subjects in this study.

Figure 1. Mean Tigecycline Concentrations in Serum and Blister Fluid



CONCLUSION:

This study showed that multiple-dose administration of tigecycline exhibited good penetration into cantharidin-induced skin blister fluid ($AUC_{0\text{-}12h,Blister}/AUC_{0\text{-}12h,Serum}$) and was generally safe and well tolerated in healthy adult men.

An Open-Label Evaluation of Tigecycline Concentrations in Selected Tissues: Interim Report(Protocol 3074A1-117-US)

Dates: Study began September 9, 2003 and is ongoing. This report contains data for subjects who completed the study as of March 2, 2004.
Clinical sites:

1. [redacted] (b) (4)

Analytical site: [redacted] (b) (4)

OBJECTIVES:
The objective of the study is to determine the tissue concentrations and corresponding serum concentrations of tigecycline at selected time points in lung, colon, gallbladder (and bile, if possible), bone (and synovial fluid, if possible), and cerebrospinal fluid following a single dose administration of tigecycline.

FORMULATION:
Tigecycline lyophilized powder was supplied by Wyeth in vials containing lyophilized free base equivalent to 50mg of tigecycline. The assay strength for batch # 2001B0022 was [redacted] (b) (4), for batch # 2000B0392 was [redacted] (b) (4) for batch # 046ETEC was [redacted] (b) (4).
The content of the vial was reconstituted with sterile normal saline (0.9% NaCl Injection, USP).

STUDY DESIGN:
This study was an open-label, single-dose study in 120 planned subjects. Fifty-five subjects completed the study before the cutoff date for this report and data was analyzed for 52 subjects. The subjects were scheduled for surgery or lumbar puncture at approximately nine facilities in the U.S. with one principal investigator. Prespecified

tissue sampling of lung, colon, gallbladder (and, if possible, bile), bone (and, if possible, synovial fluid), and CSF consistent with the planned procedure for each subject, was to be performed at approximately four hours (± two hours), eight hours (± two hours), twelve hours (± two hours), or 24 hours (± two hours) after the start of a 100 mg dose of IV tigecycline administered in 100 ml of normal saline, over 30 minutes.

Serum for tigecycline PK analysis was collected from all subjects at hour 0 (before the first dose), at approximately 30 minutes after the start of the infusion (end of infusion), and at the time corresponding to tissue/fluid sample collection (approximately four hours, eight hours, twelve hours, or 24 hours) after the start of the tigecycline infusion.

ASSAY METHODOLOGY:
Concentrations of tigecycline in serum and tissues/fluids were determined by sensitive and specific liquid chromatography methods with (b) (4) detection (b) (4). Tables 1 and 2 show the performance of the tigecycline assays during the analysis of the tissue and serum samples from the study.

Table 1. Tigecycline Assay Range and Sensitivity

Standard Curve	Serum (ng/mL)	Colon (ng/g)	Gallbladder (ng/g)	Lung (ng/g)	Bone (ng/g)	Synovial Fluid (ng/mL)
Linear range	(b) (4)					
Sensitivity						

Table 2. Summary of Tigecycline Assay Performance

	-------- Low QC --------			-------- Middle QC -------			-------- High QC --------		
Tissue/Fluid	Target QC Conc	CV%	Bias%	Target QC Conc	CV%	Bias%	Target QC Conc	CV%	Bias%
Serum	(b) (4)								
Colon									
Gallbladder									
Lung									
Bone									
Synovial fluid									

Abbreviations: CV=coefficient of variation; QC Conc=quality control concentration.

The low QC concentration for gallbladder and lung samples inidicated poorer assay performance at the lower concentrations (Bias%= (b) (4) respectively). However, the middle and high QC concentrations for gallbladder and lung samples exhibited acceptable accuracy and precision. The low, middle, and high QC concentrations for all other tissue/fluid samples exhibited acceptable accuracy and precision.

DATA ANALYSIS:
The limited sampling schedule used in this study did not permit the accurate calculation of PK parameters (eg, C_{max}, AUC, and $t_{½}$) for the various tissues/fluids. The data analyzed is of tissue/fluid concentrations over time. The tigecycline concentrations in

serum, colon, gallbladder, lung, bone, and synovial fluid were analyzed by descriptive statistics (eg, mean, standard deviation (SD), CV%, median, minimum, and maximum).

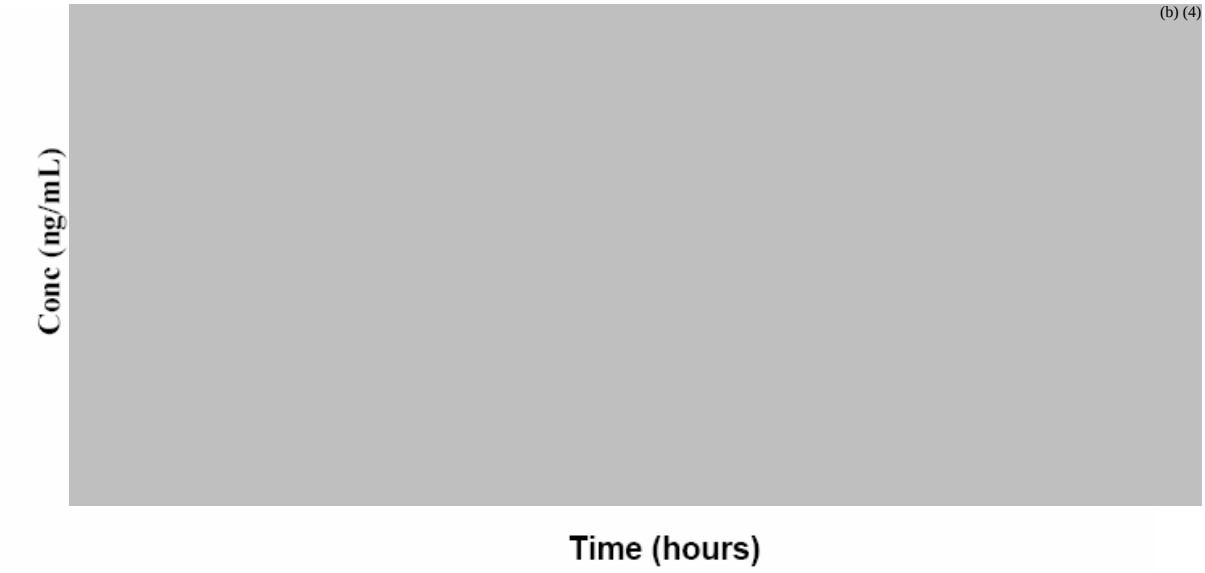
RESULTS:
Fifty-five subjects enrolled, and 52 completed this study to the cutoff date for this interim report. One subject withdrew consent before tigecycline administration. Additionally, two subjects received the tigecycline infusion but their surgical procedures were subsequently canceled and no tissue/fluid samples were obtained. Table 3 summarizes the subject's demographic baseline characteristics. None of the subjects were noted to have any medical condition that would have interfered with the distribution, metabolism, or excretion of tigecycline.

Table 3. Demographic and Baseline Characteristics for All Subjects

Characteristic	100 mg (Bone) N=21	100 mg (Colon) N=11	100 mg (Gallbladder) N=20	100 mg (Lung) N=2	Total N=54
Age (year)					
Mean	69.10	67.64	41.85	71.50	58.80
Standard deviation	8.06	14.85	11.65	4.95	16.97
Minimum	53.00	35.00	24.00	68.00	24.00
Maximum	83.00	83.00	62.00	75.00	83.00
Median	71.00	74.00	41.00	71.50	60.00
Sex					
Female (%)	8 (38)	8 (73)	19 (95)	1 (50)	36 (67)
Male (%)	13 (62)	3 (27)	1 (5)	1 (50)	18 (33)
Ethnic origin					
Black (%)	0	1 (9)	0	0	1 (2)
Other (%)	0	0	3 (15)	0	3 (6)
White (%)	21 (100)	10 (91)	17 (85)	2 (100)	50 (93)
Baseline height (cm)					
Mean	170.14	170.00	163.85	169.50	167.76
Standard deviation	8.19	7.13	7.10	10.61	8.02
Minimum	156.00	162.00	155.00	162.00	155.00
Maximum	185.00	188.00	180.00	177.00	188.00
Median	170.00	170.00	163.00	169.50	167.00
Baseline weight (kg)					
Mean	96.56	81.25	84.38	68.10	87.87
Standard deviation	20.23	14.18	16.81	17.96	18.93
Minimum	58.60	56.80	54.50	55.40	54.50
Maximum	125.00	106.80	115.40	80.80	125.00
Median	95.00	77.70	82.50	68.10	85.15
Body mass index (kg/m^2)					
Mean	33.27	28.15	31.47	23.45	31.20
Standard deviation	6.37	4.93	6.21	3.31	6.32
Minimum	21.01	19.65	22.15	21.11	19.65
Maximum	45.32	38.10	40.87	25.79	45.32
Median	32.72	27.86	30.27	23.45	30.86

The tigecycline serum concentrations in the predose serum samples were below the quantitative limit of detection in all subjects. Figure one shows the individual serum concentrations of the 52 study subjects following the 100 mg dose of tigecycline. The concentrations are evenly distributed around the schedules times of 0.5 (end of infusion), four, eight, 12, and 24 hours.

Figure 1. Tigecycline Serum Concentrations



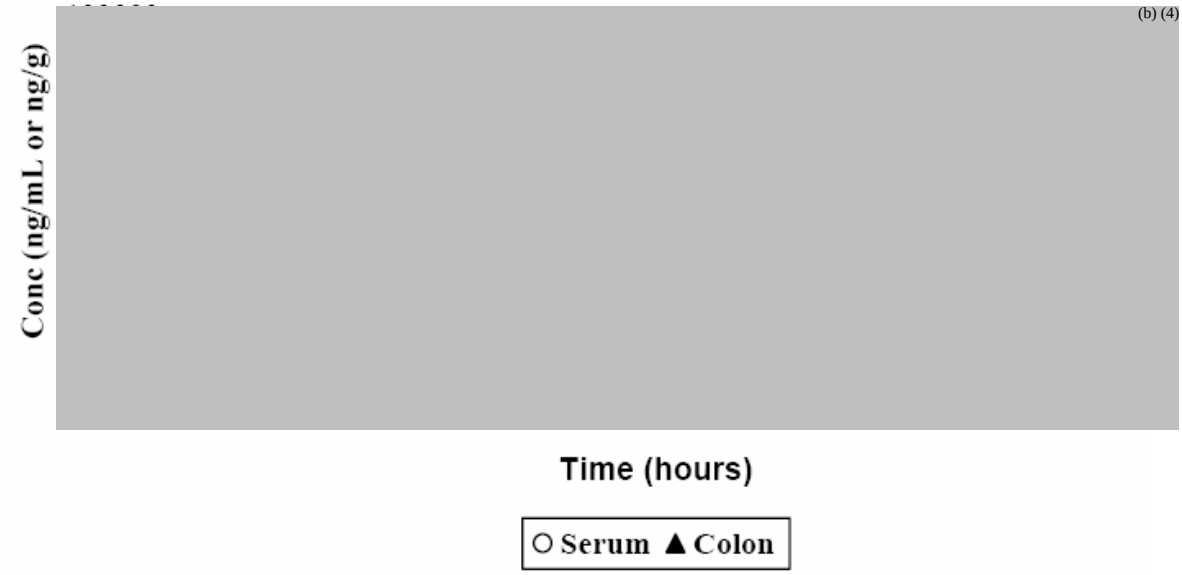
The tigecycline concentrations in the individual gallbladder tissue and serum concentrations are displayed in figure 2.

Figure 2. Tigecycline Gallbladder Concentrations

After the administration of the therapeutic loading dose of 100mg, the tigecycline quickly penetrated the gallbladder tissue. The concentration of tigecycline was 38 times higher in the gallbladder than in the serum as early as four hours after administration. The tigecycline concentrations in the gallbladder tissue remained considerably higher than the serum concentrations for at least 24 hours after single-dose administration.

Figure three shows the individual colon tissue and serum concentrations for the subjects in this study.

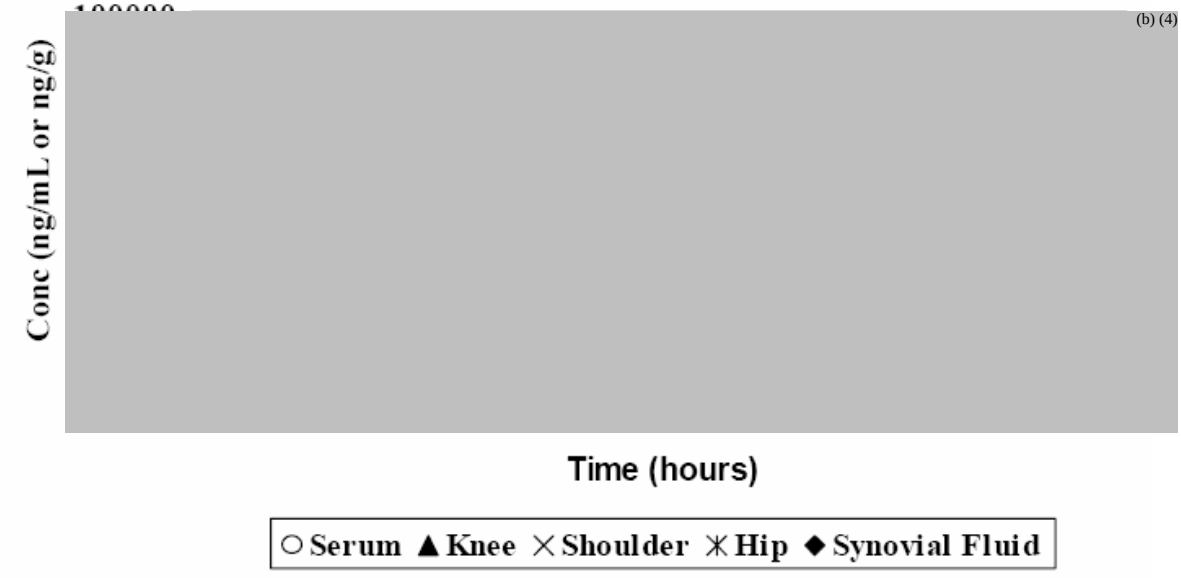
Figure 3. Tigecycline Colon Concentrations



After administration of the loading dose of 100mg, tigecycline quickly penetrated the colon tissue, attaining an approximately two-fold higher concentration in colon than in the serum as early as four hours after administration. The tigecycline concentrations in colon were similar to or higher than the serum concentrations for at least 24 hours after the single dose administration.

Figure four shows the individual bone, synovial fluid, and serum concentrations of tigecycline for the subjects in the study.

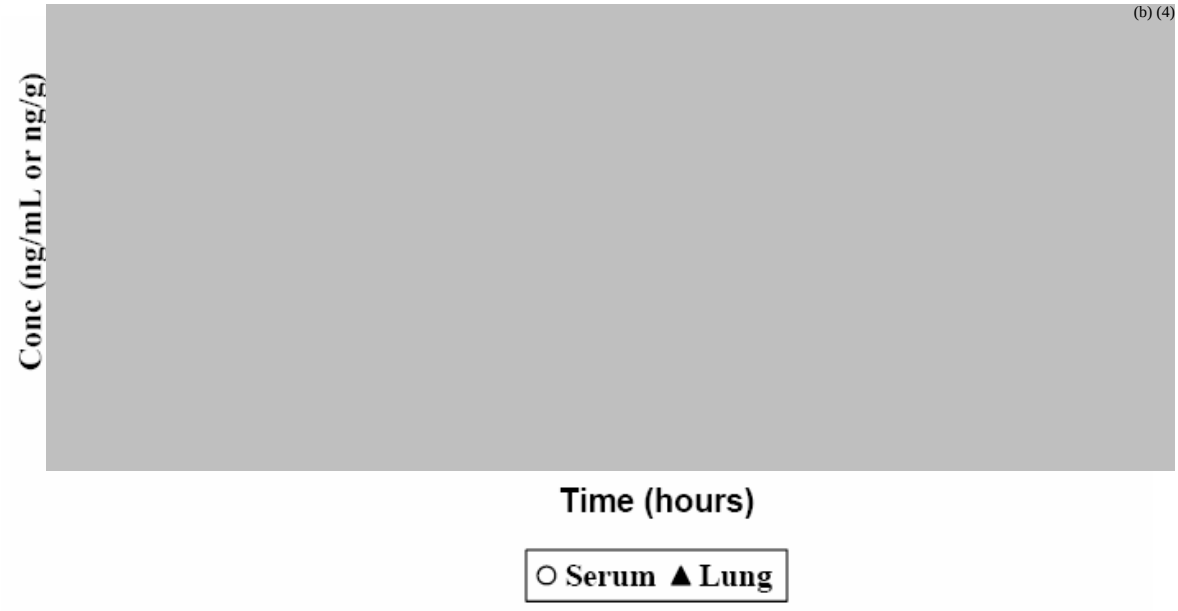
Figure 4. Tigecycline Bone and Synovial Fluid Concentrations



The tigecycline concentrations in bone that are below the quantifiable limit of the assay are shown with a value of (b) (4) at the bottom of the figure. After administration of the 100mg loading dose, the tigecycline quickly penetrated into the synovial fluid, attaining 60% of the concentration in the serum as early as four hours after administration. The tigecycline concentrations in synovial fluid consistently remained approximately 20 to 40% below the serum concentrations through at least 24 hours after the single-dose administration. Tigecycline did not appear to penetrate into the bone and showed lower concentrations than those seen in serum at four hours. The tigecycline concentrations measured in bone were below the quantifiable limit of the assay in one of six samples at four hours after administration, in zero of one sample at eight hours after administration, in three of six samples at 12 hours after administration, and in five of six samples at 24 hours after administration.

Figure five shows the individual lung tissue and serum concentrations in the two study subjects.

Figure 5. Tigecycline Lung Concentrations



After administration of the 100mg loading dose, tigecycline quickly penetrated the lung tissue. The concentration of tigecycline at four hours was approximately 8.6 times higher in the lungs than in the serum at four hours after administration. The tigecycline concentrations in the lung tissue remained higher than the serum concentrations at 24 hours after single-dose administration.

CONCLUSIONS:
The median serum concentration obtained at the end of the 30-minute administration of 100 mg of IV tigecycline was 1425 ng/ml. The serum concentration of tigecycline declined to median values of 198 ng/ml and 60 ng/ml at four hours and 24 hours, respectively, after the start of the infusion. The highest tigecycline concentrations were

seen in the gallbladder (range: [redacted]) and lung (range: [redacted] samples. The range of individual ratios of concentrations in gallbladder tissue to serum was [redacted], and the tissue to serum ratios for the two subjects with lung samples were [redacted] and the range of individual ratios of cocnetrations in the colon tissue was 0.1 to [redacted] The lowest concentrations in tissues and fluids were reported in synovial fluid (range: [redacted]) and bone (range:below the quantitative limit of detection [BQL] to [redacted] The range of individual ratios of concentrations in synovial fluid to concentrations in serum was [redacted]. The individual bone to serum ratios ranged from BQL to [redacted] The sponsor believes that the low concentration in human bone from this study may be related to tight binding of tigecycline to bone with poor extraction for assay. The data in this study was obtained following single dose administration of 100mg tigecycline.

The Effect of Tigecycline on the Oropharyngeal and Intestinal Microflora in Healthy Adults(Protocol 3074A1-116-EU) Interim Report

Dates: October 28, 2003 to December 19, 2003
Clinical site: (b) (4)

Analytical site:

1. Serum samples were assayed for tigecycline at (b) (4)
2. Serum, saliva, and fecal samples were analyzed for microbiologic activity at (b) (4)

OBJECTIVES:
The primary objective of this study was to assess the effect of antimicrobial treatment on the oropharyngeal and intestinal microflora before, during, and after administration of tigecycline to healthy subjects. Secondary objectives were to explore the potential for development of resistance by measuring the susceptibility (MIC) of isolated microbial strains before, during and after administration of tigecycline; and to assess the PK, safety, and tolerability of tigecycline under these study conditions.

FORMULATION:
Sterile tigecycline powder for injection was supplied by Wyeth Research in 5 ml, flint glass vials, each containing lyophilized free base equivalent to 50 mg of tigecycline without additives or preservatives to be reconstituted by the clinical pharmacist. The assay strength for batch# 2001B0097 was (b) (4)/vial (free-base). The formulation # was 0931179J.

STUDY DESIGN:
This was an open-label, non-randomized, multiple-dose, inpatient study conducted at a single investigational site. Thirteen healthy men and women aged 18 to 40 years enrolled in the study. Of the thirteen subjects enrolled in the study, 12 received 19 doses of tigecycline, an initial dose of 100 mg followed by 18 doses of 50 mg administered as an IV solution in 100 ml of normal saline infused over 30 minutes. One subject (116-001-0011) received an initial dose of 100 mg tigecycline followed by 3 doses of 50 mg, then discontinued the study on day two due to adverse event.

Serum samples for PK analysis were collected on days 1, 2, and 5 before the morning infusion, on day 9 before the morning and evening infusions, and on day 10 before the infusion; and then at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours after the start of the infusion. Saliva samples were to be collected at the same time points as for the PK blood samples in addition to days 2, 5, 10, 12, 15, 18, 24, and 31 for microbiological analyses.

Fecal samples were to be collected on day 1 before dose administration then on days 2, 5, 8, 10, 12, 15, 18, 24 and 31 for microbiological cultivation and for assay for tigecycline

levels (days 2, 5, 10, 12 and 15). The first specimen passed on a given day was to be analyzed if several were produced on the day. If none were passed on a given day, the first specimen after that day was to be kept for PK and microbiological assays.

ASSAY METHODOLOGY:
The concentrations of tigecycline in serum were determined by sensitive and specific liquid chromatography methods [redacted] (b) (4)
Table 1 shows the assay range and sensitivity. Table 2 shows the summary of assay performance.

Table 1. Assay Range and Sensitivity

Standard Curve	Tigecycline/Serum (ng/mL)	Tigecycline/Urine (µg/mL)
Linear range	[redacted] (b) (4)	
Sensitivity		

Table 2. Summary of Assay Performance for Serum Assays

	---Low QC ([redacted] (b) (4)--			---Middle QC ([redacted] (b) (4)--			--High QC ([redacted] (b) (4)		
Analyte	Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
Tigecycline	[redacted] (b) (4)								

Abbreviations: CV=coefficient of variation; QC=quality control.

The minimum inhibitory concentrations (MIC) for tigecycline were determined for isolated strains from the saliva and fecal samples by the agar dilution method on at least 3 occasions. The final inocula for aerobic and anaerobic bacteria were 10^4 CFU and 10^5 CFU per spot, respectively. Inoculated plates for aerobic and anaerobic bacteria were incubated for 24 hours and 48 hours respectively.
The reference strains were *Escherichia coli* American Type Culture Collection (ATCC) 25922, *Enterococcus faecalis* ATCC 29212, *Bacteroides fragilis* ATCC 25285, and *Bacteroides thetaiomicron* ATCC 29741. The MIC was defined as the lowest concentration of tigecycline that inhibited growth completely. The MIC_{50} and MIC_{90} were the concentration that inhibited growth in 50% and 90% of the strains, respectively.
The test organism used for the tigecycline microbiology bioassay was *Bacillus cereus* ATCC 11778. The inoculum was prepared by diluting a 0.9ml spore suspension of [redacted] (b) (4) *Bacillus cereus* [redacted] (b) (4)) in 9 ml of sterile saline. This diluted spore suspension yielded a bacterial density of approximately 10^7 CFU/ml. The agar medium was prepared by adding 8 g [redacted] (b) (4)) and 11 g agarose [redacted] (b) (4)) per liter of distilled water. After autoclaving at 121ºC for 15 minutes, the medium was allowed to equilibrate to a temperature range of 48ºC to 50ºC for approximately 1 hour in a water bath. The diluted *B. cereus* spore suspension was inoculated into the cooled agar to a final concentration of 0.9% (v/v, 0.9 ml/100 ml). A 100 ml aliquot of the suspension

was added to a [redacted] BioAssay Dish (245 x 245 x 20 mm) and the agar was allowed to solidify at room temperature on a level surface.
The stock solution of tigecycline (1000µg/ml) was prepared by accurately weighing and dissolving the standard tigecycline lyophilate in a sufficient quantity of 0.06 M phosphate-buffered saline (PBS), pH 7.8. This solution was then diluted with sterile human serum. Serial 2-fold dilutions were prepared for concentrations of 4, 2, 1, 0.5, 0.25, 0.12 and 0.06 µg/ml for the standard curve.

DATA ANALYSIS:
The tigecycline serum concentration data for each subject were analyzed by using empirical, model-independent pharmacokinetic methods. The peak concentration (C_{max}) and the time to peak concentration (t_{max}) were taken directly from the observed data. The area under the concentration-time curve over one steady-state dosing interval (AUC_{0-12h} or AUC) was calculated by using the log-trapezoidal rule for decreasing concentrations and at the linear-trapezoidal rule for increasing concentrations. Systemic clearance (Cl) was calculated as dose/AUC, and the mean residence time (MRT) was calculated as $AUC_{0-\tau}/(AUMC_{0-\tau} + \tau \cdot AUC_{\tau-\infty}) - T_{inf}/2$, where τ is the length of the dosing interval (12h), AUMC is the total area under the first moment curve and T_{inf} is the duration of infusion (0.5 hour). Then apparent steady-state volume of distribution (V_{ss}) was estimated as $Cl \cdot MRT$. The apparent terminal-phase disposition rate constant (λ_z) could not be estimated reliably because the duration of sampling (24 hours) was less than the reported terminal-phase disposition half-life ($t_{½}$) in other studies (24 to 48 hours). The statistical analysis is limited to providing descriptive statistics (eg, means and frequency tables).

RESULTS:
Thirteen subjects were enrolled, and 12 completed the study. Subject 116-001-0011 withdrew from the study because of an adverse reaction (urticaria). This subject withdrew from the study prior to providing on-therapy PK and PD data. Table three summarizes the demographic and baseline characteristics for all subjects in the study.

Table 3. Demographic and Baseline Characteristics of Subjects in Tigecycline Study 307A1-116-EU

Attribute	Tigecycline (n = 13)
Age, years	
Mean	25.54
SD	3.26
Minimum	20.00
Maximum	31.00
Median	26.00
Sex, n (%)	
Female	6 (46.15)
Male	7 (53.85)
Race, n (%)	
White	13 (100)
Ethnic origin, n (%)	
Hispanic or Latino	1 (7.69)
Non-Hispanic and Non-Latino	12 (92.31)
Height, cm	
Mean	174.23
SD	12.21
Minimum	153.00
Maximum	189.00
Median	174.00
Weight, kg	
Mean	68.08
SD	11.96
Minimum	50.00
Maximum	89.60
Median	65.20
Body Mass Index, kg/m^2	
Mean	22.28
SD	1.79
Minimum	19.98
Maximum	25.08
Median	21.42

Source: CDR DEMO4, 06 APR 2004

The serum, saliva, and fecal concentrations determined by microbiological assays are not available for this interim report. Table four summarizes the estimates of tigecycline steady-state PK parameters for the 12 subjects in the PK analyses.

Table 4. Tigecycline Steady-State Pharmacokinetic Parameters (Mean ± SD and Geometric Mean)

Group	C_{max} (ng/mL)	t_{max} (h)	AUC (ng·h/mL)	Cl (L/h)	V_{ss} (L)
All subjects (n=12)	1027±329	0.48±0.07	3350±1271	16.5±4.7	605±259
	985	0.47	3175	15.7	550

Abbreviations: AUC = total area under the plasma concentration-time curve, Cl = systemic clearance, C_{max} = peak serum concentration, V_{ss} = volume of distribution at steady state

The mean steady-state tigecycline PK parameters are similar to those seen in other studies in which the subjects received 50mg q12h as 30 minute IV infusions.
The pharmacodynamic results of tigecycline show that the number of enterococci was reduced by one log kill and *Escherichia coli* was reduced by more than 2 log kill, while the number of other enterobacteria and yeasts increased. Also, tigecycline reduced the number of lactobacilli and bifidobacteria, while tigecycline did not affect the colonization of bacteroides. No *Clostridium difficile* strains were found, which is ecologically favorable. Additionally, two *Klebsiella pneumonia*, two *Klebsiella oxytoca* strains, seven *Enterobacter cloacae* strains, one *Enterobacter amnisensus* strain, and one *Enterobacter asslomerans* strain resistant to tigecycline (≥8 µg/ml) were found.

CONCLUSION:
Based on the available data in this interim report, the observed effect of tigecycline on the intestinal microflora was expected due to the spectrum of antibacterial activity and the intestinal cocncetrations of tigecycline. No *Clostridium difficile* strains were found.

The Pharmacokinetics of GAR-936 in Adults Subjects with Various Degrees of Renal Function(Protocol 3074A1-103-US)

Dates:May 10, 2003 to July 27, 2001
Clinical sites:

1. Clinical Research Pharmacology Unit, Wyeth Research, 1300 Wolf Street, Philadelphia, PA 19148
2. (b) (4)
3. (b) (4)

Analytical site: (b) (4)

OBJECTIVES:
The primary objective was to evaluate the effect of renal impairment on the pharmacokinetics of tigecycline in adult subjects. The secondary objective was to assess the safety and tolerability of tigecycline in adult subjects with various degrees of renal function.

FORMULATION:
Wyeth supplied vials containing 100mg of lyophilized tigecycline powder to be reconstituted and diluted in sterile saline. The assay strength for batch # 1997B0186 is (b) (4)/vial.

STUDY DESIGN:
This study was an open-label, parallel-group, nonrandomized, single-dose, inpatient study conducted in 20 enrolled subjects who were 25 to 75 years of age. Six subjects had normal renal function. Six subjects had severe renal impairment with a creatinine clearance < 30ml/minute. Eight subjects had ESRD and were receiving HD (four received tigecycline before dialysis and four received it after HD). All twenty patients completed the study and were analyzed. Thirty minutes after a standard medium-fat meal, each subject was administered a single 100mg IV dose of tigecycline in 200 ml of sterile saline infused over 60 minutes.

For PK parameters, blood samples were collected at time 0 (predose), 0.5 hour (midinfusion), 1 hour (end of infusion), and at 1.5, 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, and 96 hours after the start of the tigecycline infusion for determination of tigecycline serum concentrations. Additional blood samples at 3, 4, and 6 (end of dialysis) hours were taken from the ESRD subjects receiving tigecycline before dialysis from each of the incoming and outgoing dialysis lines (6 additional samples).

Subjects with ESRD who received tigecycline before dialysis had two 20-ml aliquots of the dialysate collected before the initiation of dialysis. During dialysis, the total volume of dialysate fluid was recorded for the hour 0 to 1, 1 to 2, 2 to 3, and 3 to 4 intervals aftet the start of dialysis. Two 20 ml aliquots were collected at each interval.

Urine samples were collected at time 0 (predose) and at intervals of 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 36, and 36 to 48 hours for determination of tigecycline concentrations.

ASSAY METHODOLOGY:
Concentrations of tigecycline in serum and urine were determined by sensitive and specific liquid chromatography methods (b) (4) The performance of the tigecycline assays during the analysis of the serum and urine samples from this study is summarized in Tables 1, 2, and 3.

Table 1. Assay Range and Sensitivity

Standard Curve	Tigecycline/Serum (ng/mL)	Tigecycline/Urine (µg/mL)
Linear range	(b) (4)	
Sensitivity		

Table 2. Summary of Assay Performance for Serum Assays

	---Low QC (b) (4)--			---Middle QC (b) (4)--			--High QC (b) (4)-		
Analyte	Mean Conc	CV%	Bias%	Mean Conc	CV%	Bias%	Mean Conc	CV%	Bias%
Tigecycline	(b) (4)								

Abbreviations: Conc = concentration; CV = coefficient of variation; QC = quality control.

Table 3. Summary of Assay Performance for Urine Assays

	---Low QC (b) (4)--			---Middle QC (b) (4)-			--High QC (b) (4)-		
Analyte	Mean Conc	CV%	Bias%	Mean Conc	CV%	Bias%	Mean Conc	CV%	Bias%
Tigecycline	(b) (4)								

Abbreviations: Conc = concentration; CV = coefficient of variation; QC = quality control.

DATA ANALYSIS:
The tigecycline serum concentration data for each subject were analyzed by using empirical, model-independent pharmacokinetic methods. The peak concentration (C_{max}) and the time to peak concentration (t_{max}) were taken directly from the observed data. The apparent terminal-phase disposition rate constant (λ_z) was estimated by a log-linear regression of the last two to five observed serum concentrations that were determined to be in the log-linear elimination phase by visual inspection. The apparent terminal phase disposition half-life ($t_{½}$) was calculated as $t_{½} = 0.693/\lambda_z$. The area under the concentration-time curve (AUC_T) to the last observable concentration (C_T) at time T was calculated by using the log-trapezoidal rule for increasing concentrations. Total AUC was then estimated by $AUC = AUC_T + C_T/\lambda_z$. Systemic clearance (Cl) was calculated as dose/AUC, and the mean residence time (MRT) was calculated as $AUC/AUMC\text{-}T_{inf}/2$, where AUMC is the total area under the first moment curve and T_{inf} is the duration of infusion (1 hour). Finally, the apparent steady-state volume of distribution (V_{ss}) was

estimated as Cl·MRT. Statistical comparisons of the mean tigecycline serum concentrations at each sample collection time, tigecycline urinary recovery of each collection interval, and estimates of the tigecycline PK parameters were made among the four renal function groups by using 1-factor analysis of variance.

RESULTS:

Three of the twenty subjects enrolled in the ESRD group enrolled in both the predialysis and postdialysis treatment groups under different subject numbers (subject 6 = subject 4, subject 7 = subject 3, and subject 8 =subject 2). There were no protocol violations during the study, but there were two approved protocol deviations, subject 019 and 024 were aged 74 and 75 years respectively. Table 4 shows the demographic and baseline characteristics for all subjects.

Table 4. Demographic and Baseline Characteristics for all Subjects Sorted by Subject Category

			---------- ESRD ----------		
Characteristic	Healthy Matched Controls (n=6)	Severely Renal-Impaired (n=6)	(Tigecycline before dialysis) (n=4)	(Tigecycline after dialysis) (n=4)	Total (n=20)
Age (year)					
Mean	53.8	55.7	43.8	36.8	49.0
Standard deviation	11.9	10.4	12.7	12.2	13.2
Min, max[a]	44.0,75.0	46.0,74.0	26.0,55.0	25.0,50.0	25.0,75.0
Median	50.0	52.0	47.0	36.0	49.5
Sex, n (%)					
Female	1 (17)	1 (17)	2 (50)	1 (25)	5 (25)
Male	5 (83)	5 (83)	2 (50)	3 (75)	15 (75)
Ethnic origin, n (%)					
Black	1 (17)	2 (33)	4 (100)	4 (100)	11 (55)
White	5 (83)	4 (67)	0	0	9 (45)
Weight (kg)					
Mean	89.3	88.0	66.3	62.9	79.1
Standard deviation	14.4	18.3	9.4	9.9	17.9
Min, max	76.1,108.3	67.8,105.8	52.3,71.8	53.0,72.5	52.3,108.3
Median	87.1	89.7	70.6	63.1	74.1
Height (cm)					
Mean	173.7	173.7	172.6	174.9	173.7
Standard deviation	13.4	6.6	11.0	7.6	9.3
Min, max	158.0,189.9	165.1,181.1	158.8,185.4	168.0,185.4	158.0,189.9
Median	171.3	174.5	173.1	173.1	172.8
BMI					
Mean	29.5	28.8	22.1	19.9	25.9
Standard deviation	3.3	4.4	4.9	2.0	5.5
Min, max	24.7,34.5	23.0,33.1	17.3,28.7	17.3,22.1	17.3,34.5
Median	29.6	29.6	21.3	20.1	26.2

Abbreviations: BMI = body/mass index; ESRD = end-stage renal disease.

a: Two (2) subjects > 70 years of age were admitted to the study. Subject 002-024 was a healthy 75-year-old man, and subject 003-19 was a 74-year-old man with severe renal impairment.

Eighteen subjects received some type of concomitant therapy during the study. The medications were those that are most frequently used by subjects with renal impairment (eg., antihypertensives, vitamin and mineral supplements, and antianemic preparation).

Table 5 summarizes the mean ± SD and geometric mean of estimates of tigecycline PK parameter values for the four renal function groups, including statistical comparisons among the groups.

Table 5. Tigecycline Pharmacokinetic Parameters (Mean ± SD and Geometric Mean)

Group	C_{max} (ng/mL)	t_{max} (h)	$t_{½}$ (h)	AUC (ng·h/mL)	Cl (L/h)	Vss (L)	Cl_r (L/h)
Healthy subjects	604±243	0.7±0.3	27.3±5.2	3330±709	31.1±5.9	940±328	6.6±3.0
	572	0.6	26.9	3273	30.6	889	6.1
Severe impairment	605±166	1.0±0.0	26.8±7.0	4758±1811	23.4±7.6	761±260	1.4±0.6
	585	1.0	26.0	4501	22.2	723	1.3
ESRD before dialysis	982±161	0.9±0.3	17.8±3.6	4152±458	24.3±2.8	465±92	--
	971	0.8	17.5	4133	24.2	457	
ESRD after dialysis	940±342	1.0±0.0	31.8±19.2	3929±1023	26.9±7.8	628±274	--
	899	1.0	27.8	3822	26.2	579	
1-Factor Analysis of Variance of Log-Transformed Data							
Group	0.02	0.02	0.19	0.24	0.24	0.07	0.001
Tukey Pairwise Comparisons[a]							
	- -[b]	1<2	--	--	--	--	1>2
	- -	1<4	--	--	--	--	--

a: 1 = Healthy subjects, 2 = Severe renal impairment, 3 = ESRD before dialysis, 4 = ESRD after dialysis.
b: Tukey pairwise comparisons show no significant pairwise differences in C_{max} (overall p=0.02).

Following administration of tigecycline to healthy subjects, approximately 10% to 13% of the administered dose is recovered unchanged in the urine, and tigecycline renal clearance accounts for approximately 10% to 15% of the total systemic clearance of the drug. Moderate to severe renal impairment was expected to reduce tigecycline total systemic clearance by approximately 10% to 15%. In the healthy subjects and the renally impaired subjects, the renal clearance of tigecycline was similar to the creatinine (a surrogate for GFR), indicating that renal tubular secretion and tubular reabsorption are approximately equal. Tigecycline's protein binding (approximately 13% unbound at C_{max}) and its size (molecular weight 586 daltons) do not preclude the drug from being dialyzable. However, tigecycline has a large volume of distribution ($V_{ss} \approx 900$ L), indicating that tigecycline is extensively distributed into the tissue and not appreciably available to the dialysis membrane for removal. The tigecyclnie PK profile was not affected by HD, and tigecycline is not considered dialyzable. Tigecycline clearance in this study was reduced by approximately 20% (25 vs. 31 L/h) in subjects with severe or end stage renal impairment compared with healthy subjects, and the total exposure (AUC) was approximately 30% higher in the renally impaired groups than in the healthy subjects. Dosage adjustment based on renal function is not necessary. Since tigecycline is not dialyzable. Tigecycline can be administered before HD.

ECG's were performed during prestudy screening, on study day -1, on study day 1 before and after tigecycline administration, and then as part of the final study evaluation. Three subjects were noted to have QT_c interval values > 450 msec which met the criteria for potential clinical importance. Subject 002 (also participated as subject 008), a 44-year-old man with ESRD, had QT_c values > 450 msec on all ten study ECG's, but had no additional increase in the QT_c interval on the ECG taken one hour after the tigecycline dose. Subject 005, a 54 year old woman with severe renal impairment, had a predose QT_c interval of 486 msec, which decreased on the postdose ECG. Subject 001 had a predose QT_c interval of 445 msec, which increased to 473 msec on the ECG taken one hour after the tigecycline dose, and which decreased to 467 on the ECG taken at final evaluation.

CONCLUSION:
The study shows that administration of a single 100 mg dose of tigecycline is safe and well tolerated in healthy subjects, in subjects with severe renal impairment, and in subjects with end-stage renal disease. The tigecycline dose does not need to be adjusted in subjects with renal impairment, including subjects with end-stage renal disease. Also, treatment with tigecycline does not need to be delayed until after HD because tigecycline is not dialyzable.

The Pharmacokinetics and Safety of Tigecycline (GAR-936) in Patients with Compensated and Decompensated Cirrhosis and in Matched Adults(Protocol 3074A1-105-EU)

Dates: January 10, 2001 to December 6, 2003
Clinical sites:

1. (b) (4)

Analytical site: (b) (4)

OBJECTIVES:
The primary objective of this study was to assess the pharmacokinetics (PK) of tigecycline in subjects with compensated and decompensated cirrhosis and in matched healthy adults. A secondary objective was to assess the safety and tolerability of tigecycline in these subjects.

FORMULATION:
Sterile tigecycline powder for injection was supplied by Wyeth Research in 5 ml, flint glass vials, each containing lyophilized free base equivalent to 100mg or 50 mg of tigecycline without additives or preservatives. At the clinical site in (b) (4) they used 100 mg vials, and at the clinical site in (b) (4) they used two 50 mg vials to prepare the study dose. Table 1 shows the strengths and batch numbers.

Table 1. Study Medication Batch Data

Study Medication	Assay Strength (mg/vial)	Batch Number	Source
Tigecycline	(b) (4)	1997B0184	Wyeth Research Gosport, England
		1997B0186	
		1998B0350	
		2000B0253	
		2001B0022	

STUDY DESIGN:
This is a single-dose, open label, inpatient/outpatient, nonrandomized study conducted at two investigational sites in subjects with compensated and decompensated cirrhosis and in healthy adults matched by age, sex, weight, and smoking habit. The study enrolled healthy men and women from 18 to 75 years of age with weight 50 kg or greater who were willing to abstain from caffeine-containing products, grapefruit –containing products, and alcoholic beverages from 24 hours before the test article administration until the end of the study. The study also enrolled cirrhotic men and women with compensated and uncompensated cirrhosis from 18 to 65 years of age and with weight greater than 50 kg were eligible to participate. Hepatically impaired subjects had to have been on a stable therapeutic regimen for two weeks prior to administration of the test

item. All hepatically impaired subjects agreed to restrict ingestion of alcoholic beverages, caffeine-containing products, and grapefruit-containing products during the study. Forty-eight subjects at both sites were enrolled. Ten of the subjects had compensated cirrhosis (Child-Pugh-A, score of five or six in the Child-Pugh classification), ten subjects had mildly to moderately decompensated cirrhosis (Child-Pugh-B, score of seven to nine in the Child-Pugh classification), and five subjects had severly decompensated cirrhosis (Child-Pugh-C, score of ten to 13 in the Child-Pugh classification) without severe encephalopathy. Twentythree healthy subjects matching the cirrhotic subjects for age, sex, weight, and smoking habit were enrolled in the study. Subjects with large amounts of ascites were matched with subjects of the same "dry" weight rather than "wet" weight, that is, one healthy subject was matched with a body weight corresponding to the subject without ascites, calculated from the subject's medical history. In addition, three subjects with large amounts of ascites were double-matched with three healthy subjects according to the "dry" as well as the "wet" weight, to assess any significant influence of ascites. Each subject participated in the study for approximately three weeks including a screening phase of two weeks. Subjects with severly decompensated cirrhosis (Child-Pugh classification C) remained inpatients for six days. Healthy adults and subjects with compensated and mild to moderately decompensated cirrhosis (Child-Pugh classifications A and B) remained inpatients for three days followed by four outpatient visits over the following four days. On day one of the study, each subject received a single IV dose of tigecycline infused over 60 minutes, administered approximately 30 minutes after a standard breakfast. The end of the study evaluation was done on Day 6 (eg. 120 hours after dose administration).

Blood samples were collected at time 0(predose), 0.5, 1 (end of infusion), 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, 96, and 120 hours after the start of the tigecycline infusion for determination of the tigecycline serum concentrations.

Urine samples were collected for determination of tigecycline urinary recovery at predose, 0-4, 4-8, 8-12, 12-24, 24-36, 36-48 hours following the start of the tigecycline infusion.

ASSAY METHODOLOGY:
Concentrations of tigecycline in serum and urine were determined by sensitive and specific liquid chromatography methods (b) (4) Tables 2, 3, and 4 show the assays performed during the analysis of the serum and urine samples from the study.

Table 2. Assay Range and Sensitivity

Standard Curve	Tigecycline/Serum (ng/mL)	Tigecycline/Urine (µg/mL)
Linear range	(b) (4)	
Sensitivity		

Table 3. Summary of Assay Performance for Serum Assays

	---Low QC (b) (4)--			---Middle QC (b) (4)-			--High QC (b) (4)--		
Analyte	Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
Tigecycline	(b) (4)								

Abbreviations: CV=coefficient of variation; QC=quality control.

Table 4. Summary of Assay Performance for Urine Assays

	---Low QC (b) (4)--			---Middle QC (b) (4)-			--High QC (b) (4)-		
Analyte	Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
Tigecycline	(b) (4)								

Abbreviations: CV=coefficient of variation; QC=quality control.

DATA ANALYSIS:

The tigecycline serum concentration data for each subject were analyzed by using empirical, model-independent PK methods. The peak concentration (C_{max}) and the time to peak concentration (t_{max}) were taken directly from the observed data. The apparent terminal-phase disposition rate constant (λ_z) was estimated by a log-linear regression of the last two to five observed serum concentrations that were determined to be in the log-linear elimination phase by visual inspection. The apparent terminal phase disposition half-life ($t_{½}$) was calculated as $t_{½} = 0.693/\lambda_z$. The area under the concentration time curve (AUC_T) to the last observable concentration (C_T) at time T was calculated by using the log-trapezoidal rule for decreasing concentrations and the linear-trapezoidal rule for increasing concentrations. Total AUC was estimated by $AUC = AUC_T + C_T/\lambda_z$. Systemic clearance (Cl) was calculated as dose/AUC, and the mean residence time (MRT) was calculated as AUC/AUMC-T_{inf}/2, where AUMC is the total area under the first moment curve and T_{inf} is the duration of infusion (1 hour). The apparent steady-state volume of distribution (V_{ss}) was estimated as Cl·MRT. Statistical comparisons of the tigecycline serum concentrations, urinary recovery, and PK parameters among the groups of subjects with various degrees of hepatic impairment and the healthy subjects were performed using a 1-factor analysis of variance (ANOVA). The statistical analysis, other than PK, is descriptive (eg. means and frequency tables).

RESULTS:

None of the subjects were withdrawn from the study, and all subjects were included in the PK analysis. Table 5 summarizes the subjects demographics and baseline characteristics by the subjects Child-Pugh classification as well as for the healthy matched subjects.

Table 5. Demographic and Baseline Characteristics for all Subjects: Number (%) of Subjects

Characteristic	Healthy (n = 23)	Child-Pugh-A (n = 10)	Child-Pugh-B (n = 10)	Child-Pugh-C (n = 5)	Total (n = 48)
Age (year)					
Mean	45.8	53.4	52.5	51.2	49.4
Standard deviation	8.2	3.9	5.7	9.7	7.8
Min, max	31.0, 60.0	46.0, 58.0	43.0, 60.0	38.0, 64.0	31.0, 64.0
Median	45.0	54.0	53.0	50.0	50.0
Sex, n (%)					
Female	4 (17)	2 (20)	1 (10)	1 (20)	8 (17)
Male	19 (83)	8 (80)	9 (90)	4 (80)	40 (83)
Ethnic origin, n (%)					
Asian	2 (9)	1 (10)		1 (20)	4 (8)
Black	1 (4)				1 (2)
White	20 (87)	9 (90)	10 (100)	4 (80)	43 (90)
Baseline height (cm)					
Mean	175.8	175.2	172.3	170.8	174.4

Table 5 (cont) Demographic and Baseline Characteristics for all Subjects: Number (%) of Subjects

Characteristic	Healthy (n = 23)	Child-Pugh-A (n = 10)	Child-Pugh-B (n = 10)	Child-Pugh-C (n = 5)	Total (n = 48)
Standard deviation	8.6	10.3	6.5	4.1	8.2
Min, max	155.0, 186.0	153.0, 188.0	163.0, 185.0	164.0, 175.0	153.0, 188.0
Median	178.0	177.5	173.0	172.0	175.0
Baseline weight (kg)					
Mean	78.0	80.3	70.5	69.3	76.0
Standard deviation	10.9	14.0	8.8	14.2	11.9
Min, max	58.2, 98.5	64.1, 108.5	56.7, 80.1	54.8, 87.0	54.8, 108.5
Median	79.5	81.5	73.1	70.0	77.6
Body mass index (kg/m^2)					
Mean	25.2	26.1	23.8	23.7	24.9
Standard deviation	2.8	3.4	2.6	4.6	3.1
Min, max	20.9, 30.7	20.5, 30.7	18.9, 26.5	18.5, 29.8	18.5, 30.7
Median	24.7	26.3	24.0	22.9	24.8

Table 6 summarizes the mean plus or minus the standard deviation (± SD) and geometric mean of estimates of tigecycline PK Parameters for the four hepatic function groups, including statistical comparisons among the groups.

Table 6. Tigecycline Pharmacokinetic Parameters (Mean ± SD and Goemetric Mean)

Group	C_{max} (ng/mL)	t_{max} (h)	$t_{½}$ (h)	AUC (ng·h/mL)	Cl (L/h)	V_{ss} (L)	A_e (%)
Healthy (n=23)	981±536	0.9±0.2	18.7±7.2	3749±1315	29.8±11.3	524±157	14.5±4.2
	901	0.9	17.6	3550	28.2	502	14.1
Child-Pugh-A (n=10)	865±382	0.7±0.3	19.1±5.4	3835±1814	31.2±13.9	617±234	15.5±7.6
	796	0.7	18.4	3496	28.6	572	13.0
Child-Pugh-B (n=10)	914±551	1.0±0.0	23.0±5.0	5636±3419	22.1±9.3	542±246	22.2±7.4
	785	1.0	22.4	4980	20.1	479	20.9
Child-Pugh-C (n=5)	1207±414	1.0±0.0	26.8±6.1	7656±1534	13.5±2.7	378±107	28.6±7.9
	1141	1.0	26.2	7533	13.3	223	27.7
1-Factor Analysis of Variance of Log-Transformed Data							
Group	0.40	0.001	0.03	0.001	0.001	0.07	0.008
Tukey Pairwise Comparisons[a]							
	- -	A<H=B=C	- -	C>H C>A	C<H C<A	- -	H<C A<C

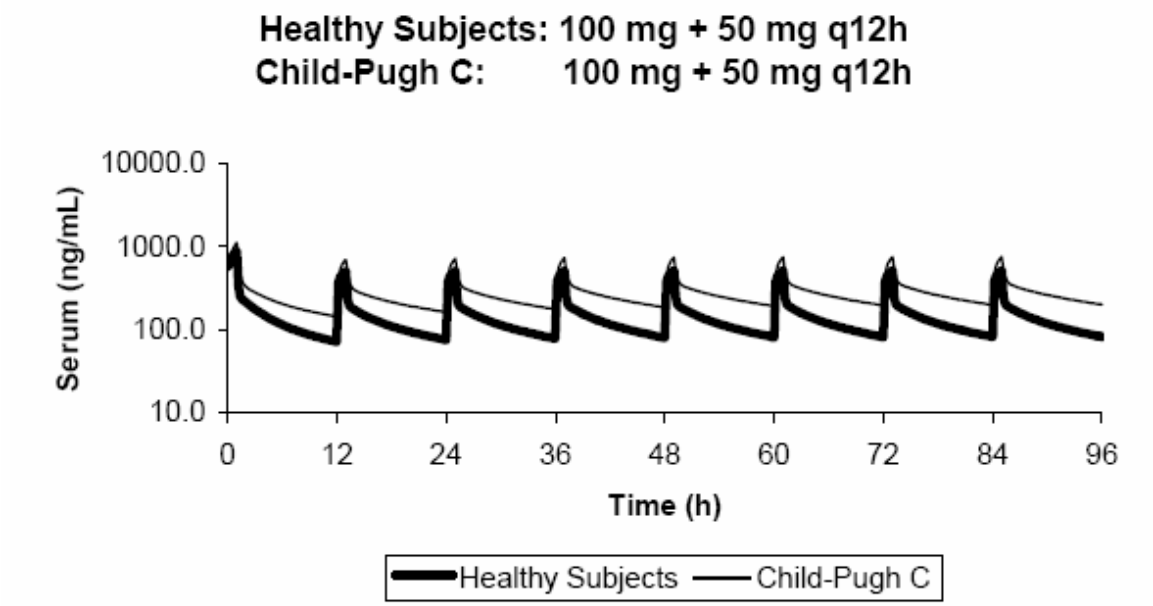
Abbreviations: A_e=amount excreted in urine as unchanged drug; AUC=area under the concentration vs. time curve; Cl=clearance; n=number of subjects; SD=standard deviation; $t_{1/2}$=terminal-phase elimination half-life; t_{max}=time at which peak concentration occurs; V_{ss}=apparent volume of distribution at steady-state.
a: H = Healthy subjects, A = Child-Pugh-A, B = Child-Pugh-B, C = Child-Pugh-C.

The mean tigecyline serum concentrations were statistically significantly higher in the Child-Pugh-C subjects than in the other subjects beginning at 1.5 hours after the start of the infusion (eg. 0.5 hour after the end of the infusion), and they remained significantly higher at the majority of the observation times. In three of the 23 healthy subjects and six of the ten Child-Pugh A subjects, the serum tigecycline concentration at 30 minutes ($C_{30\ minutes}$, midinfusion) was higher than the concentration at 60 minutes (end of infusion). A possible explanation is that in these subjects, the 60 minute blood sample may have been taken shortly after the infusion was completed, while the tigecycline serum concentrations were already rapidly declining. In these subjects, the tigecycline true C_{max} may have been underestimated by $C_{30\ minutes}$, which may explain why the mean tigecycline C_{max} in the Child-Pugh A subjects was slightly lower than in the mean C_{max} in the healthy subjects and the Child-Pugh B subjects. The mean tigecycline C_{max} was approximately 25% higher in the Child-Pugh C subjects than in the healthy subjects. In the healthy subjects, the mean tigecycline renal clearance (Cl_r) was 4.8 L/h (80 ml/min), which is slightly lower than the typical glomerular filtration rate (GFR) in subjects with healthy renal function (typically 100 to 120 ml/min), and the Cl_r represented approximately 16% of the total systemic clearance. The mean tigecycline Cl_r in the Child-Pugh A, Child-Pugh B, and Child-Pugh C subjects (5.3, 5.1, and 4.9 L/h, respectively) was similar to the mean Cl_r in the healthy subjects.
Tigecycline Cl and $t_{½}$ were similar in the Child-Pugh A subjects and in the healthy subjects. However, the mean tigecycline Cl was approximately 25% and 55% lower in the Child-Pugh B and Child-Pugh C subjects, respectively, than in the healthy subjects.

The mean tigecycline $t_{½}$ was approximately 25% and 45% longer in the Child-Pugh B and Child-Pugh C subjects, respectively, than in the healthy subjects. Consequently, the mean total exposure (AUC) in the Child-Pugh A subjects was similar to the mean AUC in the healthy subjects, but the AUC in the Child-Pugh B and Child-Pugh C subjects was approximately 50% and 105% higher respectively, than the mean AUC in the healthy subjects. The mean urinary recovery of tigecycline ($A_e = Cl_r \cdot AUC$), was higher in the Child-Pugh B and Child-Pugh C subjects than in the healthy subjects.

The dosage regimen currently being evaluated in clinical trials is a 100 mg loading dose of tigeycline infused over 30 to 60 minutes followed 12 hours later by a maintenance regimen of 50 mg q12h. In this study, the tigecycline single–dose C_{max} was approximately 25% higher in patients with severe hepatic impairment (Child-Pugh-C) than in healthy subjects. Consequently, patients with severe hepatic impairment receiving the standard therapeutic regimen initially would be exposed to only slightly higher serum concentrations of tigecycline over the first few hours compared to patients with normal hepatic function. However, upon multiple dose administration, patients with severe hepatic impairment (Child-Pugh-C) receiving the standard therapeutic regimen would be exposed to approximately twice the steady-state serum concentrations of tigecycline as the patients in the clinical efficacy trials (or the equivalent dose of 100 mg q12h). The amount of safety information in healthy subjects and infected patients at this exposure level is very limited. Simulated steady-state serum concentrations of tigecycline in healthy subjects receiving the therapeutic regimen of 100 mg followed by 50 mg q12h and in subjects with severe hepatic impairment (Child-Pugh-C) receiving either the standard therapeutic regimen or a 50% reduction in the maintenance dose (25 mg q12h) are shown in Tables 7 and 8. The simulations used one hour infusions for all doses and each simulation is shown in both log-linear scale and a linear scale for clarity.

Table 7. Simulated Tigecycline Serum Concentrations (Full Dose)



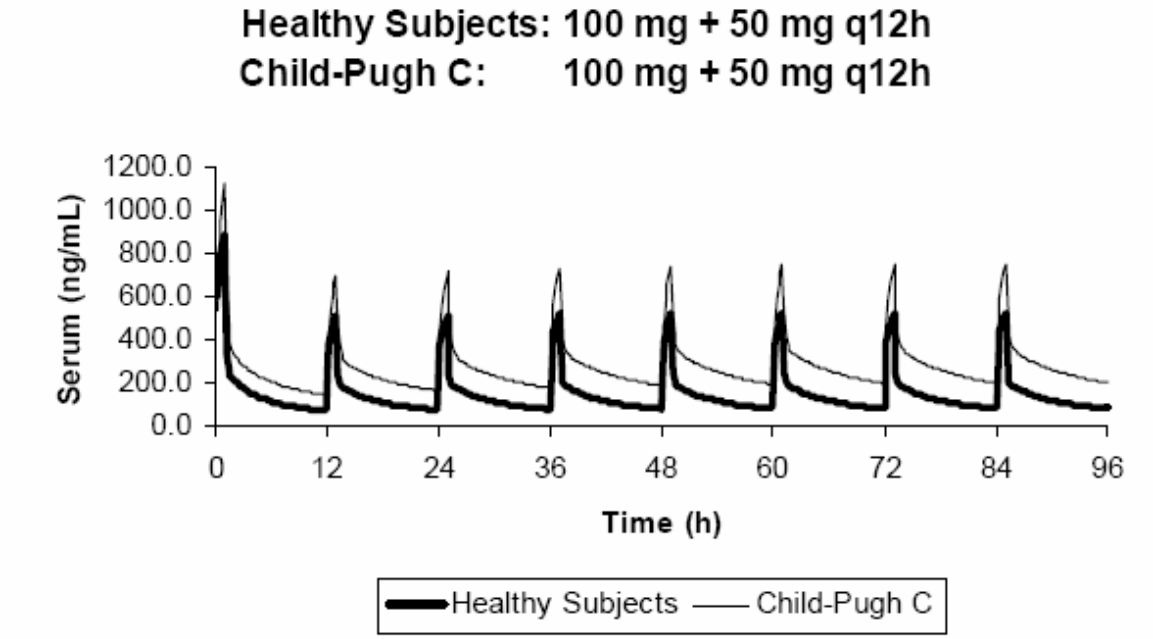
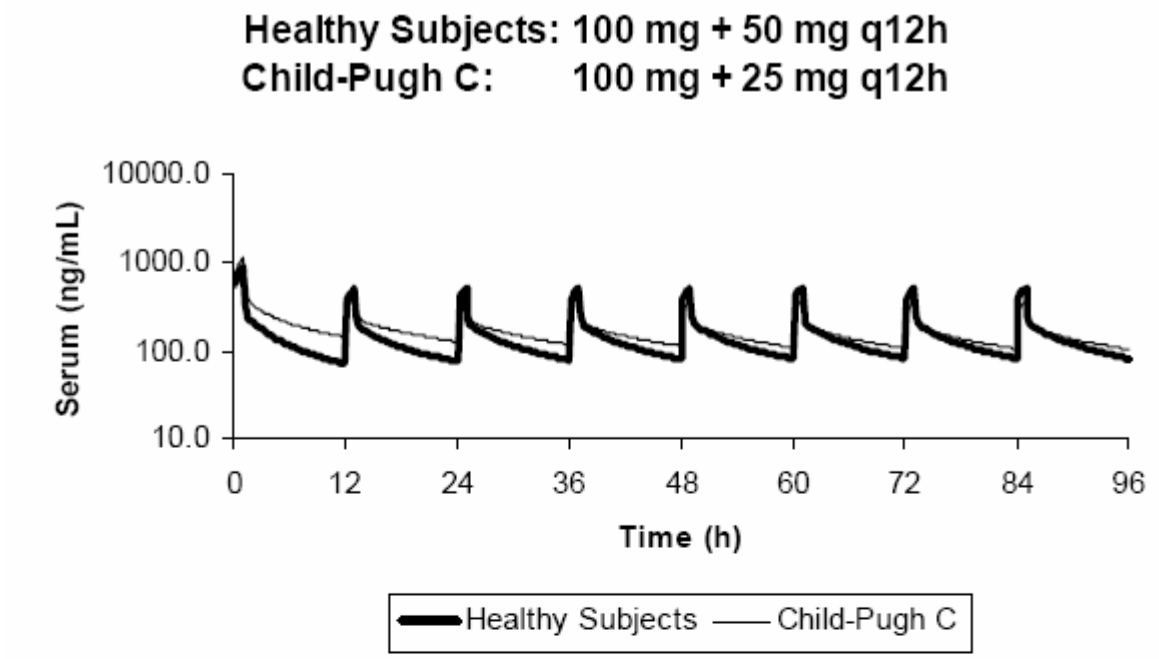


Table 8. Simulated Tigecycline Serum Concentrations (Reduced Dose)



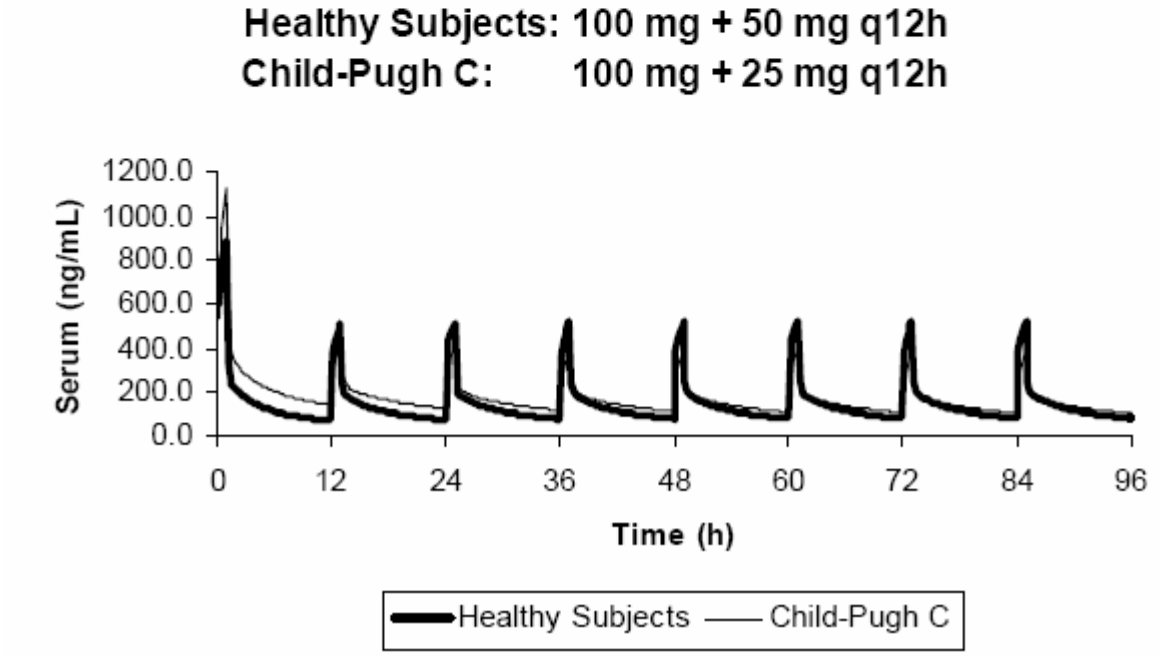


Table nine shows the simulated PK exposures.

Table 9. Simulated Tigecycline Serum Concentrations

Group	Dosage Regimen	Day 1 C_{max} (ng/mL)	Day 1 C_{12h} (ng/mL)	Day 1 C_{24h} (ng/mL)	Steady-State C_{max} (ng/mL)	Steady-State C_{min} (ng/mL)	Steady-State AUC_{0-24h} (ng·h/mL)
Healthy	100 mg + 50 mg q12h	891	72	75	523	82	3637
Child-Pugh-C	100 mg + 50 mg q12h	1125	144	163	760	204	7294
Child-Pugh-C	100 mg + 25 mg q12h	1125	144	127	380	102	3647

The simulated day-1 C_{max} and C_{12h} values for the 100 mg loading dose were very similar to the observed mean values in the healthy subjects and Child-Pugh-C subjects from this study. Also, the simulated daily AUC_{0-24h} values for the 50 mg q12h (100 mg/day) maintenance regimen were similar to the observed mean single-dose AUC for the 100 mg dose administered in this study. The PK parameters used to create the simulations were consistent with the results of this study. The AUC of tigecycline is approximately twice as high in Child-Pugh-C subjects than in the healthy subjects receiving the same dosage regimen of tigecycline. However, as expected, when the maintenance dose is reduced by 50% (to 25 mg q12h), the tigecycline AUC will be similar to that of the healthy subjects. Also, Child-Pugh-C subjects receiving the full maintenance dose of tigecycline will have approximately 45% higher C_{max} and 150% higher C_{min} values than healthy subjects, but Child-Pugh-C subjects receiving the reduced maintenance dose will have approximately 27% lower C_{max} and 24% higher C_{min} values than healthy subjects.

CONCLUSION:
The study shows that the clearance of tigecycline is reduced by approximately 25% in subjects with moderate hepatic impairment (Child-Pugh-B) and by approximately 55% in subjects with severe hepatic impairment (Child-Pugh-C). The tigecycline exposure (AUC) was increased by approximately 50% in subjects with moderate hepatic impairment and by approximately 105% in subjects with severe hepatic impairment. No dosage adjustment is warranted in subjects with mild to moderate hepatic impairment (Child-Pugh-A and Child-Pugh-B) who should receive the standard therapeutic dose of 100 mg followed twelve hours later by 50 mg q12h. A dosage adjustment in subjects with severe hepatic impairment (Child-Pugh-C) is warranted to 100 mg followed twelve hours later by 25 mg q12h.

A Pharmacokinetic Study of the Potential Drug Interaction between Tigecycline and Warfarin in Healthy Subjects(Protocol 3074A1-115-US)

Dates: July 17, 2003 to October 20, 2003
Clinical site: (b) (4)

Analytical sites:

1. Plasma samples for warfarin were analyzed at (b) (4)
2. Serum samples for tigecycline were analyzed at (b) (4)

OBJECTIVES:
The objectives of the study were to assess the potential PK interactions between tigecycline and warfarin and to evaluate the safety of tigecycline and warfarin administered concomitantly in healthy subjects.

FORMULATION:
Sterile tigecycyline powder for injection was supplied by Wyeth Research in 5 ml flint glass vials, each containing lyophilized free base equivalent to 50 mg of tigecycline without additives or preservatives. The batch numbers were 2001B0022 and 2000B0392. The contents of the vials were reconstituted with sterile normal saline (0.9% NaCl Injection, USP). Warfarin sodium was supplied by Wyeth Research as (b) (4) 10 mg and 5 mg tablets. The (b) (4) batch numbers were EQF261A and ERA058A, respectively.

STUDY DESIGN:
This was an open-label, nonrandomized, inpatient/outpatient, 2-period, 2-treatment study performed with healthy men and women age 18 to 45 years. Sixteen subjects were planned; 19 were enrolled and eight completed the study. All subjects received a 25 mg oral dose of warfarin on study day 1 of period 1. They were discharged from the study site on study day 8 to begin a 5-day washout interval, during which no test article was given. On study days 1 through 8 of period 2, subjects received a 100 mg loading dose of tigecycline, followed by 15 doses of 50 mg tigecycline, 1 dose every 12 hours. All tigecycline infusions were administered in a volume of 100 ml of normal saline over 30 minutes at 200 ml/h. On study day 5 of period 2, subjects received a 25 mg oral dose of warfarin. All subjects consumed a medium fat meal approximately 1 hour before administration of the test item.

Blood samples were collected on day 1 of period 2 before tigecycline infusion and on days 4 and 5 at time 0 (predose), 0.5 (end of infusion), 1, 1.5, 2, 3, 4, 6, 8, and 12 hours after the start of tigecycline infusion for determination of tigecycline serum concentrations. Additionally, blood samples were collected at time 0 (predose), 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours after the administration of warfarin on day 1 of period 1 and day 5 of period 2 for determination of R-warfarin and

S-warfarin plasma concentrations and for determination of prothrombin time (PT) and international normalized ratio (INR).

ASSAY METHODOLOGY:
Concentrations of tigecycline in serum were determined by sensitive and specific liquid chromatographic methods (b) (4) and concentrations of R(+)-warfarin and (S)-warfarin in plasma were determined by a separate (b) (4) procedure. The performance of the tigecycline and warfarin assays during the analysis of the serum and plasma samples from the study is shown in Tables 1, 2, and 3.

Table 1. Assay Range and Sensitivity

Standard Curve	Tigecycline/Serum (ng/mL)	R-warfarin/Plasma (ng/mL)	S-warfarin/Plasma (ng/mL)
Linear range	(b) (4)		
Sensitivity			

Table 2. Summary of Assay Performance for Tigecycline Serum Assays

	---Low QC (b) (4)			---Middle QC (b) (4)			--High QC ((b) (4)		
Analyte	Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
Tigecycline	(b) (4)								

Table 3. Summary of Assay Performance for Warfarin Plasma Assays

	---Low QC (b) (4)			---Middle QC (b) (4)			--High QC ((b) (4)		
Analyte	Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
R-warfarin	(b) (4)								
S-warfarin									

DATA ANALYSIS:
The steady state tigecycline serum concentration data for each subject were analyzed by using empirical model-independent PK methods. The peak concentration (C_{max}) and the time to peak concentration (t_{max}) were taken directly from the observed data. The area under the concentration time curve over 1 dosage interval (AUC_{0-12h}) was calculated by using the log-trapezoidal rule for decreasing concentrations and the linear-trapezoidal rule for increasing concentrations. Systemic clearance (Cl) was calculated as dose/AUC, and it is presented both corrected and uncorrected for body weight. The tigecycline serum concentrations were measured over 1 dosage interval (12 hours), which is much shorter than the observed steady-state $t_{½}$ (>36 hours), and thus the tigecycline $t_{½}$ could not be estimated reliably. The single-dose R-warfarin and S-warfarin plasma concentration dta for each subject also were analyzed by using empirical, model-independent PK methods. The apparent terminal-phase disposition rate constant (λ_z) was estimated by a log-linear

regression of the last two to five observed serum concentrations that were determined to be in the log-linear elimination phase by visual inspection. The apparent terminal-phase disposition half-life ($t_{½}$) was calculated as $t_{½} = 0.693/\lambda_z$. the area under the concentration-time curve (AUC_T) to the last observable concentration (C_T) at time T was calculated using the log-trapezoidal rule for decreasing concentrations and the linear-trapezoidal rule for increasing concentrations, and total AUC was estimated by $AUC = AUC_T + C_T/\lambda_z$. The oral dose clearance (Cl/F) was calculated as dose/AUC, and the apparent terminal-phase volume of distribution (V_z/F) was estimated as $V_z/F = [Cl/F]/\lambda_z$. To evaluate the clinical relevance of any potential effect of tigecycline on the plasma concentrations of R-warfarin and S-warfarin, the prothrombin time (expressed in seconds and INR) was measured at predose and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours after each single dose of warfarin. The peak INR (INR_{max}) and the time to peak INR were taken directly from the observed data. The area under the INR vs time curve over the 168-hour observation interval was calculated using the linear trapezoidal rule. Statistical comparisons of the tigecycline serum concentrations, R-warfarin and S-warfarin plasma concentrations and PK parameters between monotherapy and combination therapy was made using a 2-factor analysis of variance (ANOVA).

RESULTS:

Eight subjects completed the study out of 19 enrolled. Eleven subjects withdrew from the study, five because of adverse events and six for other reasons (eg., protocol violations). Thirteen subjects were analyzed for PK including the eight who completed the study. Table 4 summarizes the subjects demographic and baseline characteristics.

Table 4. Demographic and Baseline Characteristics for all Subjects

Characteristic	n = 19
Age (year)	
Mean	26.89
Standard deviation	5.95
Minimum	19.00
Maximum	41.00
Median	28.00
Sex , n (%)	
Male	19 (100%)
Ethnic origin , n (%)	
Asian	3 (16%)
Black	5 (26%)
Other	1 (5%)
White	10 (53%)
Baseline height (cm)	
Mean	177.95
Standard deviation	6.78
Minimum	165.00
Maximum	188.00
Median	179.00
Baseline weight (kg)	
Mean	81.46
Standard deviation	10.92
Minimum	58.80
Maximum	96.80
Median	82.20
Body mass index (kg/m^2)	
Mean	25.69
Standard deviation	2.84
Minimum	19.63
Maximum	29.60
Median	25.89

Table 5 summarizes the PK profile of tigecycline.

Table 5. Tigecycline Steady-State Pharmacokinetic Parameters (Mean ±SD)

Treatment	C_{max} (ng/mL)	t_{max} (h)	C_{12h} (ng/mL)	AUC_{0-12h} (ng·h/mL)	Cl (L/h/kg)
Tigecycline	886±202	0.50±0.00	109±28	2097±514	0.310±0.072
Tigecycline + warfarin	898±192	0.50±0.00	114±32	2192±494	0.296±0.057
p-value[a]	0.646	- -	0.255	0.004	0.004
Geo. Mean Ratio[b]	102%	- -	108%	107%	93%
90% CL[b]	94–112%	- -	96-123%	104-111%	90-96%

Abbreviations: AUC_{0-12h} = area under the concentration time curve during the dose interval 0 to 12 hours; C_{12h} = concentration at hour 12; C_{max} = peak concentration; CL = confidence limits; Cl = intravenous clearance; t_{max} = time to peak concentration
a: Treatment p-value from a 2-factor ANOVA of log-transformed data.
b: Geometric mean ratio and 90% confidence limits.

Following coadministration of a single dose of warfarin 25 mg, the mean steady-state tigecycline systemic clearance (Cl) and exposure (C_{max}, C_{12}, and AUC) were within 10% of the mean values for tigecycline monotherapy. The 90% confidence limits of the geometric mean parameter ration were all within the strict bioequivalence criteria of 80% to 125%. Based upon the data, coadministration of warfarin 25 mg does not alter the PK profile of steady-state tigecycline 50 mg q12h.

Table 6 summarizes the mean ±SD and geometric mean estimates of R-warfarin PK parameters for the two treatments, including statistical comparisons between treatments.

Table 6. R-Warfarin Pharmacokinetic Parameters (Mean ±SD and Geometric Mean)

Treatment	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng·h/mL)	Cl/F (mL/h/kg)
Warfarin monotherapy	1158±259	5.00±1.51	42.4±5.9	73289±18454	4.27±0.77
	1136	4.83	42.0	71440	4.20
Tigecycline + warfarin	1584±259	3.50±0.76	68.7±13.0	122249±27508	2.54±0.45
	1564	3.41	67.8	119680	2.51
2-Factor Analysis of Variance of Log-Transformed Data					
Subject	0.012	0.418	0.131	0.011	0.046
Treatment	0.001	0.029	0.001	0.001	0.001
Geometric Mean Relative Bioavailability and 90% Confidence Limits[a]					
Geo. Mean Ratio	138%	71%	161%	168%	60%
90% CL	126 – 151%	56 – 90%	144 – 180%	150 - 187%	54 – 67%

Abbreviations: $AUC_{0-\infty}$ = area under the concentration time curve during the dose interval hour 0 to infinity; C_{12h} = concentration at hour 12; C_{max} = peak concentration; CL= confidence limits; Cl = intravenous clearance; Cl/F = oral dose clearance; $t_{1/2}$ = half-life; t_{max} = time to peak concentration.
a: Warfarin monotherapy is the reference treatment.

The washout interval between single-dose administration of warfarin ranged from 16 to

18 days. Prior to administration of the second single dose of warfarin (combination therapy), there were low but measurable R-warfarin plasma concentrations in all subjects (11.3 ± 3.6 ng/ml, range (b) (4)), indicating that the R-warfarin plasma concentrations were not completely washed out prior to the administration of the second single dose administration. In all subjects, the predose plasma concentrations were less than 1% of the observed C_{max}. Following the coadministration of a multiple-dose regimen of tigecycline 50 mg q12h, the absorption of R-warfarin occurred slightly more rapidly, with an earlier t_{max} (mean, 3.5 vs 5 hr) and a 38% higher C_{max} (mean, 1584 vs 1158 ng/ml). The coadministration of tigecycline 50mg q12h reduced the mean R-warfarin oral dose clearance (Cl/F) by 40%, increased the mean R-warfarin AUC by approximately 68%, and increased the mean R-warfarin $t_{½}$ by approximately 61%. Since the R-warfarin $t_{½}$ was prolonged in addition to the reduced R-warfarin Cl/F, the reduction in R-warfarin Cl/F was probably caused by a decreased R-warfarin systemic clearance (Cl) rather than an increase in the R-warfarin systemic bioavailability (F).

Table 7 summarizes the mean ±SD and geometric mean of estimates of S-warfarin PK parameters for the two treatments, including statistical comparisons between the treatments.

Table 7. S-Warfarin Pharmacokinetic Parameters (Mean ± SD and Geometric Mean)

Treatment	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng·h/mL)	Cl/F (mL/h/kg)
Warfarin monotherapy	1104±282	5.00±1.51	32.0±4.0	46672±17729	7.04±1.85
	1076	4.83	31.8	44162	6.80
Tigecycline + warfarin	1555±236	3.25±0.71	37.0±5.3	59052±16866	5.38±1.17
	1539	3.18	36.7	57069	5.26
2-Factor Analysis of Variance of Log-Transformed Data					
Subject	0.014	0.199	0.006	0.001	0.001
Treatment	0.001	0.005	0.002	0.001	0.001
Geometric Mean Relative Bioavailability and 90% Confidence Limits[a]					
Geo. Mean Ratio	143%	66%	115%	129%	77%
90% CI	129 – 158%	54 – 80%	109 – 122%	119 - 140%	71 – 84%

Abbreviations: $AUC_{0-\infty}$ = area under the concentration time curve during the dose interval hour 0 to infinity ; C_{12h} = concentration at hour 12; C_{max} = peak concentration; CL = confidence limits; Cl = intravenous clearance; Cl/F = oral dose clearance; $t_{1/2}$ = half-life; t_{max} = time to peak concentration.
a: Warfarin monotherapy is the reference treatment.

The washout interval between single dose administration of warfarin ranged from 16 to 18 days. Prior to administration of the second single dose of warfarin (combination therapy), there were low but measurable S-warfarin plasma concentrations in all subjects (8.8 ± 0.9 ng/ml, range (b) (4) indicating that the S-warfarin plasma concentrations were not completely washed out prior to the administration of the second single dose administration. In all subjects, the predose plasma concentrations were less than 1% of the observed C_{max}. Following coadministration of a multiple dose regimen of

tigecyline 50mg q12h, the absorption of S-warfarin occurred slightly more rapidly, with an earlier t_{max} (mean, 3.25 vs 5 hr) and a higher C_{max} (mean, 15555 vs 1104 ng/ml). Coadministration of tigecycline 50 mg q12h reduced the mean S-warfarin oral dose clearance (Cl/F) by 23%, increased the mean S-warfarin AUC by approximately 29%, and increased the mean S-warfarin $t_{½}$ by approximately 15%. Since the R-warfarin $t_{½}$ was prolonged in addition to the reduced S-warfarin Cl/F, the reduction in S-warfarin was probably caused by a decreased S-warfarin systemic clearance (Cl) rather than an increase in the S-warfarin systemic bioavailability. Table 8 summarizes the mean ± SD and geometric mean of estimates of INR pharmacodynamic parameters for the two treatments, including the statistical comparisons between the treatments.

Table 8. INR Pharmacodynamic Parameters (Mean ± SD and Geometric Mean)

Treatment	INR_{max}	t_{max} (h)	$AUC_{0\text{-}168h}$ (h)
Warfarin monotherapy	1.8±0.3	30±9	216±14
	1.8	28	216
Tigecycline + warfarin	1.6±0.2	39±6	220±13
	1.6	39	220
2-Factor Analysis of Variance of Log-Transformed Data			
Subject	0.018	0.465	0.206
Treatment	0.036	0.068	0.564
Geometric Mean Relative Bioavailability and 90% Confidence Limits[a]			
Geo. Mean Ratio	90%	136%	102%
90% CL	84 – 107%	104 – 179%	97 - 107%

a: Warfarin monotherapy is the reference treatment.

After single dose administration of warfarin 25 mg, the INR exhibited little or no change from baseline for the first eight hours after administration, increased to peak values of 1.6 to 1.8 within approximately 30 to 40 hours after administration, and decreased back to baseline values of 1.1 to 1.2 within 144 hours (6 days) after administration. The INR t_{max} (30 to 40 hours) occurred much later than the R-warfarin and S-warfarin plasma concentration t_{max} values (3.25 to 5 hours). Although the warfarin PK t_{max} occurred slightly earlier with coadministration of tigecycline 50 mg q12h, the INR pharmacodynamic t_{max} occurred later with tigecycline coadministration.

Figure 1 shows stick plots of the INR_{max} and the INR AUC0-168 ratios for the subjects in this study

Figure 1. Stick plots demonstrating the INRmax(ratio) and INR AUC 0-168 (ratio*h) for subjects receiving warfarin alone (25 mg) and warfarin (25mg) with concurrent multiple IV doses of tigecycline 50mg q12h

There were 19 subjects who participated in the warfarin study. The sponsors obtained INRmax and INR AUC_{0-168} information on eight of them. There were six subjects who had a decrease, one subject who had an increase, and one subject who remained the same in the INRmax group (Range of individual ratios: (b) (4) There were three subjects who had a decrease and five subjects who had an increase in INR AUC_{0-168} (Range of individual ratios: (b) (4) The plots in figure one represent the graphing of individual subject data for the INR max (ratio) and INR AUC_{0-168}. The clinical significance of this is not known. It is recommended that patients who are receiving concomitant warfarin and tigecycline therapy monitor their prothrombin time or other coagulation test.

The R-warfarin and S-warfarin plasma concentrations were higher following tigecycline coadministration, but the mean INR_{max} values were 10% lower following administration of the combination treatment and the mean INR AUC_{0-168h} values were unchanged.

CONCLUSION:
Warfarin coadministration did not alter the PK profile of tigecycline. However, administration of the therapeutic regimen of tigecyclnie increased the exposure (AUC) of R-warfarin and S-warfarin by 68% and 29%, respectively, but this increase in AUCs did not significantly alter warfarins anticoagulant profile (measured by INR). No dosage adjustment is warranted with coadmnistration of tigecyclnie and warfarin.

A Pharmacokinetic Study of the potential drug interaction between Tigecycline and Digoxin in Healthy Subjects(Protocol 3074A1-111-US)

Dates: January 10, 2003 to March 28, 2003
Clinical site: Clinical Pharmacology Unit, Wyeth Research, 1300 Wolf Street, Philadelphia, PA 19148
Analytical sites:

1. Serum samples were assayed for tigecycline at (b) (4)
2. Plasma, serum, and urine samples were assayed for digoxin at (b) (4)
3. Plasma samples for trough measurements were assayed at (b) (4)

OBJECTIVES:
The primary objective was to assess the potential PK interaction between tigecycline and digoxin in healthy subjects. The secondary objective was to assess the safety of digoxin when administered concomitantly with tigecycline in healthy subjects.

FORMULATION:
Sterile tigecycline powder for injection was supplied by Wyeth Research in 5ml., flint glass vials, each containing lyophilized free base equivalent to 50 mg of tigecycline without additives or preservatives (batch # 2001B0023). The lyophilized tigecycline powder was reconstituted with sterile normal saline (0.9% NaCl for Injection, USP) and administered intravenously over 30 minutes. The digoxin was supplied as an oral tablet 0.25 mg (Formulation # (b) (4)

STUDY DESIGN:
This study is an open-label, single-sequence, 3-period, multiple-dose drug interaction study conducted with healthy subjects at a single investigational site. Since the study involved multiple doses of digoxin and tigecycline, a single-sequence crossover design was chosen. Compared with a multisequence crossover design, the study eliminated the need for multiple washout periods, which was an important consideration because both digoxin and tigecycline have long half-lives. The data from 20 subjects who completed the study were analyzed for PK. Each subject participated in the study for approximately six weeks, including a prescreening evaluation within three weeks before study drug administration, followed by three sequential, nonrandomized study periods. On day 1 of period 1 (days 1 to 5), each subject received a single infusion of 100 mg tigecycline (0.5-hour intravenous infusion). During period 2 (days 6 to 14), each subject received 0.5 mg of digoxin orally on day 7 followed by 0.l25 mg of digoxin daily on subsequent days. During period 3 (days 15 to 23), each subject continued receiving 0.25mg of digoxin daily through day 19; each subject also received 100mg tigecycline (0.5 hour infusion) as the first dose on day15 followed by 50 mg of tigecycline every 12 hours beginning with the second dose on day 15 and ending with the first dose on day 19.

For tigecycline concentration determination, serum blood samples were collected on day 1 (collected within 2 hours before tigecycline administration), and a t 0.5(end of infusion), 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, and 96 hours after tigecycline administration; and on day 19, predose, and at 0.5 (end of infusion), 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, and 96 hours after tigecycline administration. Blood samples were also collected for determination of digoxin concentrations in plasma on day 7, predose, on day 14 at 0.5, 1, 2, 4, 6, 8, 10, 12, 16, and 24 hours after digoxin administration, and on day 19 at 0.5, 1, 2, 4, 6, 8, 10, 12, 16, and 24 hours after digoxin administration. A serum digoxin level was collected at hour 0 of day 15. Also digoxin trough samples for determination of digoxin levels were collected within two hours before digoxin administration on days 10 through 19.

Urine samples for determination of digoxin concentrations were obtained on day 7 within two hours before digoxin administration, and on days 14 and 19 before test article administration, at 0 to 4 hours, 4 to 8 hours, 8 to 12 hours, and 12 to 24 hours after the morning digoxin administration.

ASSAY METHODOLOGY:

Serum tigecycline samples were analyzed by a validated liquid (b) (4) method. Tables 1 and 2 show the assay range, sensitivity and validations for serum samples of tigecycline

Table 1. Assay Range and Sensitivity

Standard Curve	Serum Tigecycline
Linear Range (ng/mL)	(b) (4)
Sensitivity (ng/mL)	

Table 2. Summary of Assay Validation and In-Process Performance

Analyte	Assay Standards/QCs	Inter-Day	Intra-Day
Serum Tigecycline	Validation standards (b) (4) mL)	(b) (4)	
	Bias (%)		
	Imprecision (%)		
	Validation QCs (b) (4) nL)		
	Bias (%)		
	Imprecision (%)		
	In-Process Standards		
	Bias (%)		
	Imprecision (%)		
	In-Process QCs		
	Bias (%)		
	Imprecision (%)		

Validated radioimmune assay (RIA) methods were used for the analysis of digoxin in plasma and urine samples. Table 3 and 4 show the range, sensitivity, and validation for the assays of the plasma and urine digoxin concentrations.

Table 3. Assay Range and Sensitivity

Standard Curve	Plasma Digoxin	Urine Digoxin
Linear Range (ng/mL)	[redacted] (b) (4)	
Sensitivity (ng/mL)		

Table 4. Summary of Assay Validation and In-Process Performance

Analyte	Assay Standards/QCs	Inter-Day	Intra-Day
Plasma Digoxin	Validation Standards (b) (4) Bias (%) Imprecision (%)	(b) (4)	
	Validation QCs (b) (4) Bias (%) Imprecision (%)		
	In-Process Standards Bias (%) Imprecision (%)		
	In-Process QCs (b) (4) Bias (%) Imprecision (%)		
Urine Digoxin	Validation Standards (b) (4) Bias (%) Imprecision (%)	(b) (4)	
	Validation QCs (b) (4) Bias (%) Imprecision (%)		
	In-Process Standards (b) (4) Bias (%) Imprecision (%)		
	In-Process QCs (b) (4) Bias (%) Imprecision (%)		

a: QC sample at (b) (4) mL.

DATA ANALYSIS:

Serum tigecycline and plasma digoxin concentration-versus-time data for each subject were analyzed by using noncompartmental methods. Tigecycline peak serum concentration (C_{max}) and time to peak concentration (t_{max}) were taken directly from the observed data. Concentrations that were judged to be in the terminal phase were used to obtain the terminal-phase disposition rate constant (λ_z) by log-linear regression. The $t_{½}$ was calculated as $0.693/\lambda_z$. The tigecycline concentrations over the time periods from 24

to 96 were used to estimate the $t_{½}$. After a single dose (period 1, days 1 to 5), the area under the concentration-time curve (AUC_t) and area under the first moment concentration-time curve ($AUMC_t$) truncated at the last observable concentration (C_t) at time t, were calculated by applying the linear trapezoidal rule to C_{max} and the log-linear trapezoidal rule thereafter. Total AUC and AUMC were estimated as follows: AUC= $(AUC_t) + C_t/\lambda_z$, and AUMC = $(AUMC_t) + t_{last} \cdot C_t/\lambda_z + C_t/\lambda_z^2$. The systemic mean residence time (MRT) was calculated as follows: MRT = (AUMC/AUC) - $T_{inf}/2$, where T_{inf} is the infusion time (0.5 hours). The IV clearance (Cl) was calculated and normalized by body weight (WT) as follows: Cl= Dose/(AUC·WT). The apparent V_{ss} was estimated by V_{ss} = Cl·MRT. After multiple doses (period 2, day 19), the steady-state AUC ($AUC_{0-\tau}$) and AUMC ($AUMC_{0-\tau}$) over the dose interval (τ=12 hours) were also calculated by applying the linear trapezoidal rule to C_{max} and the log-linear trapezoidal rule thereafter. However, the MRT was calculated as follows: MRT = $(AUMC_{0-\tau} + (\tau \cdot C_\tau/\lambda_z))/AUC_{0-\tau}$. The Cl and V_{ss} were calculated as previously described for a single dose. Since the dose regimens were different during period one and two, the sponsor defined the parameters that would be valid for determining the effect of digoxin on serum tigecycline PK. Tigecycline concentrations in individual patients without coadministration of digoxin during period one were based upon a single 100 mg tigecycline dose, while concentrations with coadministration of digoxin during period two were based upon a 50 mg every 12h multiple dose regimen. According to linear PK theory, the total AUC after a single dose ($AUC_{0-\infty}$) is equal to AUC over the dose interval τ at steady state ($AUC_{0-\tau}$). Therefore, it is possible to determine the effect of digoxin on serum tigecycline exposure by comparing the tigecycline $AUC_{0-\infty}$ after a single tigecycline dose alone (dose-normalized to 50 mg) with tigecycline $AUC_{0-\tau}$ after the concomitant multiple dose administration of tigecycline and digoxin. Since the ratio of the tigecycline doses during periods one (dose=100 mg) and two (dose=50 mg/12h) was nearly equal to the mean accumulation factor (R [eg, dose ratio] = 2.0; R [mean ± SD] = 2.1 ± 0.3), the effect of digoxin on serum tigecycline exposure could also be determined from a direct comparison of the area under the concentration-time curve during a 12 hour dose interval (AUC_{0-12h}) for the two periods.

Other valid parameters for determining the effect of digoxin on tigecycline PK include $t_{½}$, Cl, MRT, and V_{ss}. However, the use of the single point exposure parameters Cmax (peak) and C_{12h} (trough) would not be useful because of the PK characteristics of tigecycline. Tigecycline exhibits a 3-compartment disposition function after the cessation of a 0.5 hour IV infusion, in addition to showing a prolonged terminal $t_{½}$. Single dose (period one) and multiple dose (period two) profiles would not be expected to provide directly comparable C_{max} and C_{12h} values under these conditions. The value of t_{max} would not be useful because the infusion time remained constant during the two treatment periods. Plasma digoxin steady-state profiles were obtained on study days 14 (period two, digoxin alone) and 19 (period 3, digoxin with tigecycline). The C_{max} and t_{max} values were taken directly from the observed data. The λ_z and $t_{½}$ values were not estimable because blood samples were not collected during the terminal disposition phase. Estimates of the plasma steady-state AUC ($AUC_{0-\tau}$) on days 14 (period two) and 19 (period three) were obtained over 24 hour (AUC_{0-24}) intervals. The oral dose clearance (Cl/F) was calculated and normalized by body weight (WT) as follows: Cl/F = Dose/(AUC·WT). V_{ss}/F and MRT were not estimable because λ_z could not be estimated. The amount of digoxin excreted in

urine over the intervals of 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours on study days 14 (period one) and 19(period two) were determined in order to estimate the total amount of digoxin excreted in urine ($A_{e,0\text{-}24h}$). The percentage of the dose of digoxin excreted unchanged in urine ($A_{e,\%}$) was calculated using the formula: $A_{e,\%} = (A_{e,0\text{-}24h}/\text{Dose}) \cdot 100$. the renal clearance of digoxin (Cl_r) normalized by body weight (WT) was calculated from the formula: $Cl_r = A_{e,0\text{-}24h}/AUC_{0\text{-}24h}/WT$.
Descriptive statistics (mean, SD, CV, n, median, minimum, and maximum) were obtained for all demographic characteristics, drug concentrations, and PK parameters.

RESULTS:
Of the 30 subjects who were enrolled, 20 completed the study. All of the subjects were men. Nine of the subjects were withdrawn because of adverse reactions and one because of a problem with disruptive behavior. The ten subjects 001, 004, 005, 012, 013, 014, 015, 016, 024, and 028 were excluded from the pharmacokinetic and pharmacodynamic analysis. Table 5 summarizes the subjects demographic and baseline characteristics. All of the subjects were healthy men and did not have any medical conditions that might have interfered with the metabolism or excretion of study medication or the interpretation of the results.

Table 5. Demographic Characteristics of Subjects Included in the Pharmacokinetic Analysis

SUBJECT	AGE (y)	HEIGHT (cm)	WEIGHT (kg)	BMI (kg/m2)	SEX	ETHNIC ORIGIN
1*	40	178.0	80.0	25.2	MALE	WHITE
2	31	174.2	82.5	27.2	MALE	BLACK
3	34	182.0	76.7	23.2	MALE	BLACK
4*	38	187.5	84.6	24.1	MALE	BLACK
5*	34	169.5	78.8	27.4	MALE	BLACK
6	34	171.9	68.4	23.1	MALE	BLACK
7	33	188.5	77.3	21.8	MALE	WHITE
8	32	193.1	81.6	21.9	MALE	BLACK
9	42	189.7	85.2	23.7	MALE	WHITE
10	33	182.5	89.3	26.8	MALE	BLACK
11	44	180.6	73.1	22.4	MALE	WHITE
12*	24	179.5	78.9	24.5	MALE	BLACK
13*	27	175.4	76.7	24.9	MALE	OTHER
14*	42	184.2	93.4	27.5	MALE	BLACK
15*	40	179.2	76.4	23.8	MALE	BLACK
16*	35	186.2	87.0	25.1	MALE	BLACK
17	35	187.9	86.1	24.4	MALE	BLACK
18	42	188.3	89.6	25.3	MALE	WHITE
19	45	179.5	89.4	27.7	MALE	WHITE
20	37	182.7	100.5	30.1	MALE	BLACK
21	27	175.7	85.0	27.5	MALE	BLACK
22	44	181.0	92.3	28.2	MALE	BLACK
23	34	194.4	94.4	25.0	MALE	WHITE
24*	30	181.0	95.0	29.0	MALE	BLACK
25	29	194.2	84.7	22.5	MALE	BLACK
26	43	175.5	70.6	22.9	MALE	BLACK
27	37	174.8	80.9	26.5	MALE	WHITE
28*	38	181.1	77.7	23.7	MALE	WHITE
29	41	174.5	57.7	18.9	MALE	WHITE
30	34	180.5	82.5	25.3	MALE	BLACK
MEAN	36.6	182.6	82.4	24.7		
S.D.	5.4	7.1	9.9	2.7		
% CV	14.8	3.9	12.0	11.1		
N	20.0	20.0	20.0	20.0		
MIN	27.0	171.9	57.7	18.9		
MAX	45.0	194.4	100.5	30.1		

* SUBJECT DISCONTINUED PREMATURELY FROM THE STUDY AND WAS EXCLUDED FROM ALL STATISTICAL ANALYSES

Table 6 shows the statistical comparison of serum tigecycline PK parameters.

Table 6. Statistical Comparison of SerumTigecycline Pharmacokinetic Parameters (n=20)

Parameter (units)	Tigecycline Alone[a] (mean ± SD)	Tigecycline With Digoxin[b] (mean ± SD)	GLS Mean Ratio (%)[c] (90% CI)
$t_{1/2}$ (h)	27.7 ± 7.5	40.4 ± 11.9	146 (131-162)
AUC (ng•h/mL)[d]	2837± 732	2625 ± 524	94 (88-99)
CL (mL/h/kg)	229 ± 56	243 ± 54	107 (101-113)
V_{ss} (L/kg)	6.53 ± 1.30	8.13 ± 2.68	121 (109-134)
MRT (h)	30.0 ± 8.3	34.4 ± 10.8	113 (104-123)
AUC_{0-12h} (ng•h/mL)	2480 ± 379	2625 ± 524	105 (100-111)

Abbreviations: GLS = geometric least squares; SD = standard deviation; CI = confidence interval; $t_{1/2}$ = terminal phase elimination half-life; AUC = area under the concentration time curve; CL = intravenous clearance; V_{ss} = apparent steady-state volume of distribution, MRT = mean residence time.

a: Tigecycline single intravenous dose (100 mg). Estimates of AUC, V_{ss}, and MRT are based on tigecycline concentrations normalized to a 50-mg dose.

b: Tigecycline multiple intravenous doses (50 mg/12 hours).

c: Ratio of (tigecycline + digoxin)/(tigecycline alone).

d: AUC = dose-normalized $AUC_{0-\infty}$for tigecycline alone, and AUC = AUC_{0-12h} for tigecycline with digoxin.

The results of the bioequivalence analysis showed that the 90% CIs for the parameters AUC_{0-12h}, AUC, Cl, and MRT were all within the 80% to 125% equivalence window, but the 90% CIs for terminal phase elimination half-life ($t_{½}$, CI =131%-162%) and V_{ss} (CI = 109% -134%) were not. Thus, digoxin did not affect the AUC, Cl, or MRT of tigecycline. Although digoxin increased both tigecycline $t_{½}$ and V_{ss}, these increases did not affect the total exposure or intravenous clearance of tigecycline.

Table 7 shows the summary of the PK parameters for plasma and urine digoxin.

Table 7. Statistical Comparison of Plasma and Urine Digoxin Pharmacokinetic Parameters (n=20)

Parameter (units)	Digoxin Alone[a] (mean ± SD)	Digoxin With Tigecycline[a] (mean ± SD)	GLS Mean Ratio (%)[b] (90% CI)
Plasma			
C_{max} (ng/mL)	1.19 ± 0.20	1.09 ± 0.46	87 (77-98)
t_{max}	1.35 ± 0.56	1.48 ± 0.55	111 (91-135)
AUC_{0-24h} (ng•h/mL)	11.7 ± 2.3	11.2 ± 2.7	95 (88-103)
CL/F (mL/h/kg)	4.54 ± 1.08	4.79 ± 1.21	105 (97-113)
Urine			
$A_{e,\%}$	41.3 ± 9.0	37.8 ± 9.4	91 (80-102)
CL_r	1.82 ± 0.37	1.75 ± 0.40	95 (87-104)

Abbreviations: GLS = geometric least squares; SD = standard deviation; CI = confidence interval; C_{max} = peak concentration; t_{max} = time to peak concentration; AUC_{0-24h} = area under the concentration time curve during a dose interval; CL/F = oral-dose clearance; $A_{e,\%}$ = percentage of digoxin excreted in urine; CL_r = renal clearance.

a: Digoxin pharmacokinetic parameters for both treatments are based on daily 0.25-mg doses of digoxin.

b: Ratio of (digoxin + tigecycline)/(digoxin alone).

Based on the bioequivalence analysis, 90% CIs for the plasma digoxin AUC_{0-24h} and Cl/F were both within the 80% to 125% equivalence window, but the 90% CIs for C_{max} (CI = 77%-98%) and t_{max} (CI = 91% - 135%) were not. Therefore, tigecycline did not affect the digoxin total exposure (AUC) or oral dose clearance (Cl/F); but the digoxin absorption rate was slightly decreased. The sponsors do not believe that the small concurrent decrease in C_{max} (13%) and increase in t_{max} (11%) would be expected to alter the pharmacodynamic effect of digoxin. The 90% CI for plasma digoxin concentrations at 12, 16, and 24 hours were all within the equivalence window. These results are significant because the sponsor mentions that it is known that digoxin concentrations at < 8 hours after dose administration are a misleading indicator of inotropy. It is recommended that trough samples drawn just before the next scheduled dose of the drug be used for digoxin concentration monitoring. A bioequivalence analysis of urinary digoxin PK parameters showed that the 90% CIs for $A_{e,\%}$ and Cl_r were both within the 80% to 125% equivalence window. Therefore, tigecycline did not affect the digoxin urinary PK.
The protocol was designed to compare changes from baseline in ECG parameters (PR, QRS, QT, and QTc interval) at 24 hours after drug administration. At this time point, serum digoxin concentrations would be expected to be in equilibrium with tissue concentrations, and the ratio of inotropic response to serum concentrations would be relatively constant. Based on ANOVA, there was no significant differences in ECG parameters due to treatment effects at 24 hours after drug administration, except for the QT interval ($p = 0.0007$, period 1> 2=3). The QT interval decreased after digoxin (period 2) compared to tigecycline alone (period 1) but was not changed further when tigecycline was added to digoxin (period 3). However, the corrected QT interval (QTc) was not different between the three periods. The correction method was not mentioned in the study report. These results indicate that coadministration of tigecycline did not produce significant changes in steady state digoxin pharmacodynamics as measured from baseline in ECG parameters.

CONCLUSIONS:
The administration of a single 100 mg IV dose of tigecycline alone, multiple 0.25 mg daily oral doses of digoxin alone, and the combined multiple dose administration of tigecycline (50 mg/12 hours) and digoxin (0.25 mg daily) were generally safe in healthy subjects. The study showed that digoxin did not affect the steady state AUC, Cl, or MRT of tigecycline, although the GLS means ratios for serum tigecycline $t_{½}$ and V_{ss} fell outside the 80% to 125% equivalence window. Tigecycline did not affect the steady state plasma digoxin AUC_{0-24h}, oral dose Cl/F, or digoxin concentrations during the 12 to 24 hour period after the dose administration (therapeutic drug monitoring times), although the 90% CIs for C_{max} and t_{max} fell outside of the equivalence window. Tigecycline did not affect the steady state digoxin urinary PK as shown by measurement of digoxin $A_{e,\%}$ and digoxin Cl_r. Tigecycline did not affect the steady state digoxin pharmacodynamics as measured by changes from baseline in ECG parameters.

The effects of Age and Gender on the Safety, Tolerability, and Pharmacokinetics of tigecycline in Healthy Subjects(Protocol 3074A1-102-US) Final report

Dates: February 10, 1999 to May 24, 1999
Clinical site: Clinical Pharmacology Unit, Wyeth Research, 1300 Wolf Street, Philadelphia, PA 19148
Analytical site:

1. Serum samples were assayed for tigecycline at Drug Metabolism Division, Wyeth Research, 401 N. Middletown Road, Pearl River, NY 10965
2. Urine samples were analyzed by (b) (4)

OBJECTIVES:
The primary objective was to compare the PK profile of tigecycline (GAR-936) in groups of healthy men and women of different ages following a single intravenous infusion of 100mg tigecycline. The second objective was to compare the safety and tolerability of 100mg tigecycline in young, young-elderly, and elderly men and women.

FORMULATION:
Tigecycline lyophilized powder was supplied by Wyeth in vials containing 100mg of drug to be reconstituted by the study pharmacist. The contents of the vial were reconstituted with sterile normal saline (0.9% NaCl injection, USP). The assay strength for tigecycline batch number 1997B0186 was (b) (4) vial.

STUDY DESIGN:
This was an open-label, parallel, nonrandomized, single-dose, inpatient study was conducted at a single study site. The study included 46 healthy men and women (postmenopausal or surgically sterile) in 3 age categories, young (age 18 to 50 years, inclusive), young-elderly (age 65 to 75 years, inclusive), and elderly (age > 75 years). Each subject received a single 100mg IV dose of tigecycline in 100 ml of normal saline solution, infused over a 60 minute period. The study medication was administered 30 minutes after a standard medium-fat breakfast.

Serum samples for PK analysis of tigecycline concentrations were collected at 0 hour (predose), and at 0.5, 1, 1.5, 2, 3, 4, 5, 8, 12, 16, 24, 36, 48, 60, 72, 96, and 120 hours after the start of tigecycline infusion.

Urine samples were also collected for determination of tigecycline concentrations at the following intervals, 0 (predose), 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 36, and 36 to 48 hours following the start of tigecycline infusion.

ASSAY METHODOLOGY:
Serum tigecycline concentrations were quantified using a validated high performance liquid chromatography (HPLC) method with minor modification. The modification involved (b) (4) The method consists of (b) (4)

[redacted] The lower limit of quantitation was [redacted]l. A stock solution of tigecycline was prepared by dissolving tigecycline free base in methanol and further diluting with methanol. Working standard solutions were prepared by appropriate dilutions of the stock solution with methanol. Calibration standards were prepared fresh daily by spiking serum with appropriately diluted volumes of the stock solution to yield calibration standards in the range [redacted]. Serum was spiked with appropriately diluted stock solutions to yield quality control (QC) samples with the concentrations of [redacted]. The precision, expressed as coefficients of variation (CV), of tigecycline calibration standards was between [redacted] The accuracy, expressed as bias, values of the calibration standards were between [redacted] The CV of the tigecycline QC samples was between [redacted] and the bias values ranged from [redacted]

Tigecycyline in human urine samples was quantified by using a validated liquid chromatography/[redacted] method with the [redacted] vortexing. The lower limit of quantitation was [redacted] and the upper limit of quantitation was [redacted]. QC samples at [redacted] prepared in human urine and analyzed with each assay validation run to ensure acceptable precision and accuracy. During the aanalysis of the samples from this study, the interday precision of the QC samples was [redacted] or better and the aaccuracy was greater than [redacted]. Similarly, the interday precision of the standards was [redacted] or better and accuracy within the range [redacted]

DATA ANALYSIS:

The PK parameters based on serum data for tigecycline were estimated using noncompartmental methods. The values for C_{max} were obtained directly from the observed data. Indiviidual concentration-time profiles were plotted and the elimination rate constant (λ_z) was determined by the log-linear regression of at least three terminal points that, upon visual inspection, were considered to be in the terminal phase. The $t_{½}$ was estimated by $0.693/\lambda_z$. The area under the plasma concentration-time curve (AUC_T) was determined by log-linear trapezoidal rule from 0 to the time of last observed concentration (C_T). Total AUC was determined by the equation, $AUC = AUC_T + C_T/\lambda_z$. The systemic clearance (CL) for tigecycline was calculated using the formula, CL= (Dose/AUC). The CL was also normalized to body weight for each subject by dividing CL obtained for each subject by his/her body weight. The systemic mean residence time (MRT) was determined by the equation, $MRT = AUMC/AUC - T_{inf}/2$. The AUMC is the area under the first moment curve extrapolated to infinity, which was obtained using the following equation, $AUMC = AUMC_T + T \cdot C_T/\lambda_z + C_T/\lambda_z^2$. The tigecycline V_{ss} was calculated by multiplying the clearance by the MRT, $V_{ss} = CL \cdot MRT$.

The volume of urine excreted in each interval was determined. The values obtained for each subject were multiplied by the respective concentration obtained for each collection interval to determine the amount of tigecycline excreted in urine during each collection interval. Summation of the amount of tigecycline exctreted in urine during the various collection intervals gave the amount of tigecycline excreted in urine over 48 hours (Ae_{0-48}). The percentage of dose of tigecycline excreted in urine (fe) as unchanged tigecycline was calculated using the formula, fe(%) = (Ae_{0-48}/Dose) · 100. The renal clearance of tigecycline (CL_R) was calculated by the formula, $CL_R = Ae_{0-48}/AUC_{0-48}$. The creatinine clearance (CL_{cr}) in milliliters per minute was calculated by the formula, CL_{cr} = Urinary recovery of creatinine/ Serum concentration of creatinine. The creatinine clearance was obtained by dividing the product of urine creatinine concentration (µmol/L) and urine volume (mL) by the product of the serum creatinine concentration (mmol/L) obtained at the midpoint of the urine collection interval and the length of the collection interval (minutes). The calculation was performed for the 0-12 hour excretion interval and the 0-24 hour excretion interval.

A two factor analysis of variance was used to detect PK differences among groups with respect to the effects of age, gender and age by gender interaction. For purposes other than PK, the statistical analyses were descriptive (eg, means and frequency tables).

RESULTS:
Forty-six subjects enrolled, and 45 completed the study. These 45 patients were analyzed for PK. Subject 1020010025, a 46 year old woman withdrew from the study because a rash developed during then infusion of study drug. The subject was treated with diphenhydramine and the rash resolved in 12 hours. Table one summarizes the subjects demographic and baseline characteristics by age and gender.

Table 1. Demographic and Baseline Characteristics for All Subjects

Characteristic	Age range (y), Gender: 18-50 F (n = 9)	18-50 M (n = 9)	65-75 F (n = 7)	65-75 M (n = 8)	> 75 F (n = 5)	> 75 M (n = 8)	Total (n = 46)
Age, y							
Mean	39.9	32.2	67.9	68.4	78	77.5	58.3
Standard deviation	6.6	7.1	2	3.3	3.7	1.3	19.1
Minimum	27	25	64	65	75	76	25
Maximum	48	45	70	73	84	79	84
Median	42	32	68	67.5	76	77	67
Gender, n (%)							
Female	9 (100)	0	7 (100)	0	5 (100)	0	21 (46)
Male	0	9 (100)	0	8 (100)	0	8 (100)	25 (54)
Ethnic origin, n (%)							
Black	5 (56)	6 (67)	1 (14)	0	2 (40)	0	14 (30)
Hispanic	0	2 (22)	0	0	0	0	2 (4)
White	4 (44)	1 (11)	6 (86)	8 (100)	3 (60)	8 (100)	30 (65)
Height, cm							
Mean	165.3	180.1	160.6	178.3	162.4	172.7	170.7
Standard deviation	5.6	7.5	7.9	7	7.9	10.2	10.6
Minimum	158.0	169.5	149.7	171.0	152.0	156.2	149.7
Maximum	176.5	188.8	171.6	191.5	170.9	187.4	191.5
Median	163.9	178.5	160.9	177.6	166	170.5	170.7
Weight, kg							
Mean	65	81	61.5	85.8	68.9	74.4	73.3
Standard deviation	9.7	10.5	9.1	13.5	9.5	11.6	13.5
Minimum	54.7	70.8	49.6	68.8	53.6	57.5	49.6
Maximum	82.8	102.2	79.3	112.1	78.4	93.5	112.1
Median	64.7	77.7	61.7	83.4	70.3	73	71.9

All subjects were reported by the sponsor to be healthy and not have any medical conditions that might interfere with the metabolism or excretion of study medication or the interpretation of the results. None of the subjects who completed the study were excluded from the PK analysis.

Table two shows the mean PK parameters of tigecycline in the young, young-elderly and elderly male and female subjects.

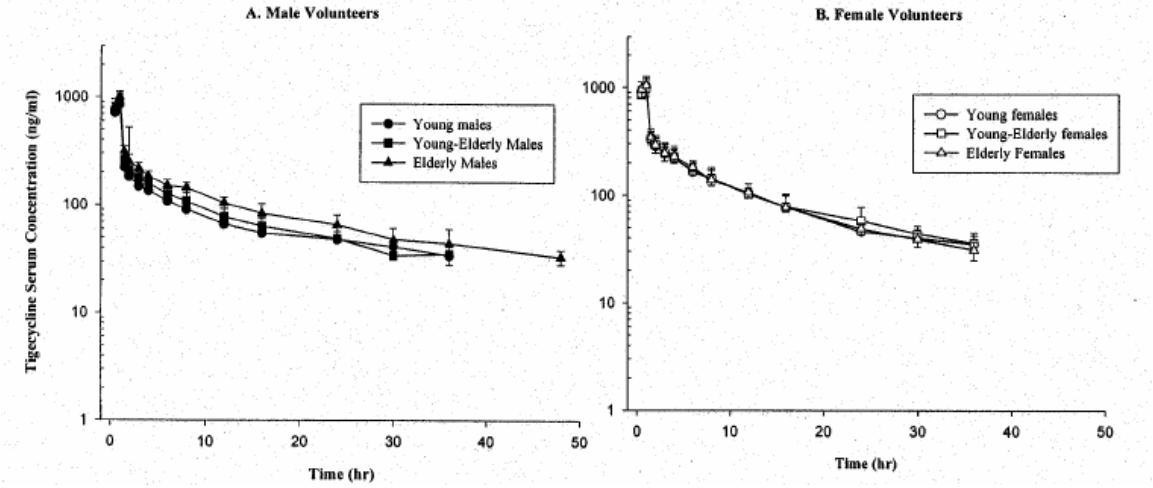
Table 2. Mean (CV%) Pharmacokinetic Parameters of Tigecycline in Young (18-50 Years), Young-Elderly (65-74 Years) and Elderly (>75 Years) Male and Female Subjects

Pharmacokinetic Parameters	Young Men	Young Women	Young-Elderly Men	Young-Elderly Women	Elderly Men	Elderly Women
C_{max} (ng/mL)	861 (18)	1033 (15)	900 (19)	993 (27)	1017 (11)	1088 (14)
AUC (ng•h/mL)	4218 (48)	5112 (26)	4317 (16)	5120 (23)	5472 (17)	5273 (21)
$t_{1/2}$ (h)	22.3 (69)	17.1 (49)	19.5 (16)	16.5 (25)	19.0 (26)	21.2 (59)
CL (L/h)	28.5 (41)	20.6 (23)	23.8 (18)	20.4 (23)	18.7 (16)	19.6 (19)
CL (L/h/kg)	0.34 (33)	0.31 (18)	0.28 (10)	0.34 (31)	0.26 (22)	0.29 (17)
CL_R (L/h)	2.9 (40)	2.6 (37)	2.3 (68)	2.2 (33)	2.6 (22)	2.2 (13)
V_{ss} (L)*	554 (29)	355 (27)	499 (16)	367 (26)	401 (15)	377 (33)
V_{ss} (L/kg)	7.1 (37)	5.6 (38)	5.9 (20)	6.1 (34)	5.5 (22)	5.6 (35)
Fe (%)	8.3 (20)	9.5 (39)	7.4 (52)	9.2 (39)	11.3 (27)	9.6 (8)

Abbreviation: CV = coefficient of variation.
*Significantly different (p=0.05) between men and women.

The mean C_{max} values for tigecycline for both genders and across all age groups were in the range of 861 to 1088 ng/ml. The values for C_{max} were slightly higher in the female subjects than in the male subjects (p=0.05), but there was no significant effect of age on the mean C_{max} values (p=0.15). Mean tigecycline AUCs ranged from 4218 ng·h/mL in young male subjects to 5472 ng·h/mL in the elderly male subjects with the effects of age, gender, or age by gender interaction being statistically insignificant (p>0.05). The AUC, clearance, and $t_{½}$ values for the young men (18 to 50 years) showed a high degree of intersubject variability as reflected in the high CV (approximately 40 to 70 %) for these parameters. The mean AUC values in each of the three female age groups were approximately 5µg·h/mL compared to 4 to 5 µg·h/mL in men. Values for V_{ss} were large and approximated 350L in female subjects and 500 L in male subjects. The mean $t_{½}$ of tigecycline obtained in this study was approximately 17 to 22 hours. The clearance and renal clearance were not significantly different between the genders and age groups (p>0.05). The mean fractions of tigecycline excreted in urine (f_e) were relatively constant, ranging from 8% to 11% among the various groups of subjects. A comparison of estimates of creatinine clearance for the young and elderly subjects showed the expected decline in estimated creatinine clearance with advanced age. Tigecycline C_{max} was lowest in young men and highest in elderly women, but the difference in C_{max} values between these groups was only 26%. Additionally, tigecycline AUC values were approximately 21% higher in young women compared to young men, and there was only a 4% difference in tigecycline AUC values between elderly men and women. These differences are small and would probably not warrant a dosage adjustment from a PK viewpoint based upon age or sex. Figure one shows the plots of mean serum concentrations of tigecycline in male and female subjects for each of the three age groups represented.

Figure 1. Mean Tigecycline Concentrations in Male and Female Subjects Belonging to Different Age Groups After a 100 mg Single Dose of Tigecycline given as a 1 hour Intravenous Infusion.



These plots clearly show the multicompartmental nature of tigecycline PK and its disposition.

CONCLUSION:

The differences in tigecycline PK among age and sex groups in this study were not markedly different and it appears that dosage adjustment based upon age and sex would not be necessary.

A Study of the Steady-State Intrapulmonary Pharmacokinetics of Tigecycline in Healthy Adults(Protocol 3074A1-112-US)

Dates: November 4, 2002 to December 16, 2003
Clinical site: (b) (4)

Analytical site: (b) (4)

OBJECTIVES:
The objective of this study was to determine the PK profile of tigecycline in serum, lung epithelial lining fluid (ELF), and alveolar cells (AC) in healthy subjects after administration of tigecycline to steady state.

FORMULATION:
Wyeth research supplied the 50 mg dosage form of sterile tigecycline powder for injection in 5 ml flint glass vials. The 50 mg dosage form formulation number 0931179J and batch number 2001B0023 contained 52.2 mg of tigecycline free base. The 50 mg dosage form formulation number 0931179J and batch number 2001B0097 contained (b) (4) of tigecycline free base. Each of the 5 ml vials of the 50 mg dosage form were reconstituted before use with 5 ml of normal saline (0.9% NaCl Injection, USP).

STUDY DESIGN:
This was a single center, multiple dose, open label, nonrandomized study which enrolled 34 subjects. The subjects received a loading dose of 100 mg of tigecycline as a 30 minute IV infusion on the morning of day 1. Following this dose every 12 hours through day 4, the subjects received a 50 mg maintenance dose of tigecycline as a 30 minute infusion for a total of seven doses. All subjects except those with a bronchoalveolar lavage (BAL) at hour 12, received 100 mg of tigecycline on day 1 in the morning, and then a 50 mg dose every 12 hours. The subjects with a BAL at hour 12 received the first dose on day 1 in the evening, so that the BAL could be done the morning of day 5. The subjects were fed a standard medium-fat meal approximately 30 minutes before receiving study medication, except for subjects whose BAL was scheduled for day 4 at the 2, 3, or 4 hour time points, who were required to fast in accordance with the standard operating procedures used for bronchoscopy the study unit.

Predose blood samples for PK analyses were collected on day 1. Thereafter, blood samples for determination of tigecycline concentrations in serum were collected at time 0 (predose), at the end of infusion (0.5 hours), and at 1, 2, 3, 4, 6, 8, 12, and 24 hours after the start of the last tigecycline infusion. A single BAL for PK analyses of lung ELF and AC was scheduled for subjects at 2, 3, 4, 6, 12, or 24 hours after last tigecycline infusion.

ASSAY METHODOLOGY:
Concentrations of tigecyline in serum, BAL fluid, AC were detemined by sensitive and specific liquid chromatography methods (b) (4) (b) (4). The performance of the tigecycline assays during the analysis of the

serum, AC, and BAL fluid samples from this study are summarized in tables 1, 2, 3 and 4.

Table 1. Assay Range and Sensitivity

	----------Tigecycline----------		
Standard Curve	Serum (ng/mL)	Alveolar Cells (ng/mL)	BAL Fluid (ng/mL)
Linear range	[redacted] (b) (4)		
Sensitivity			

Abbreviations: BAL = bronchoalveolar.

Table 2. Summary of Assay Performance for Serum Assays

	---Low QC [redacted] (b) (4)			---Middle QC [redacted] (b) (4)			--High QC [redacted] (b) (4)		
Analyte	Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
Tigecycline	[redacted] (b) (4)								

Abbreviations: QC = quality control; CV = coefficient of variation.

Table 3. Summary of Assay Performance for Alveolar Cell Assays

	---Low QC [redacted] (b) (4)			---Middle QC [redacted] (b) (4)			--High QC [redacted] (b) (4)		
Analyte	Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
Tigecycline	[redacted] (b) (4)								

Abbreviations: QC = quality control; CV = coefficient of variation.

Table 4. Summary of Assay Performance for BAL Fluid Assays

	---Low QC [redacted] (b) (4)			---Middle QC [redacted] (b) (4)			--High QC [redacted] (b) (4)		
Analyte	Mean	CV%	Bias%	Mean	CV%	Bias%	Mean	CV%	Bias%
Tigecycline	[redacted] (b) (4)								

Abbreviations: QC = quality control; CV = coefficient of variation.

Then concentration of tigecycline in the lung epithelial lining fluid (C_{ELF}) was calculated as $C_{BAL} \cdot (V_{BAL}/V_{ELF})$, where C_{BAL} is the concentration in the BAL fluid, V_{BAL} is the volume of the aspirated BAL fluid, and V_{ELF} is the volume of lung ELF. The volume of the lung ELF within the BAL fluid was estimated by $V_{BAL} \cdot (Urea_{BAL}/Urea_{serum})$, where $Urea_{BAL}$ and $Urea_{serum}$ represent the concentration of urea in the BAL fluid and serum, respectively.

DATA ANALYSIS:

The tigecycline serum concentration data for each subject was analyzed by using empirical, model-independent PK methods. The C_{max} and the time to peak concentration (t_{max}) were taken directly from the observed data. The area under the concentration-time curve over one steady-state dosing interval (AUC or $AUC_{0\text{-}12h}$) was calculated by using the log-trapezoidal rule. Systemic clearance (CL) was calculated as dose/AUC. The tigecycline concentrations in AC and lung ELF were measured at only one observation time for each subject, and the data from all subjects were pooled for a PK analysis of the mean concentrations. Additionally, the degree of tigecycline penetration into AC or lung ELF was calculated as AUC_{AC}/AUC_{serum} or AUC_{ELF}/AUC_{serum}, respectively.

STATISTICAL ANALYSIS:
Descriptive statistics (eg, mean,standard deviation [SD], and coefficient of variation) were used to analyze tigecycline concentrations in serum, AC, and lung ELF and tigecycline PK parameters. No formal statistical comparisons were made in this study.

RESULTS:
Of the 34 subjects who enrolled in this study, 30 completed the study and were used for data analysis. Table 5 summarizes the demographic and baseline characteristics of the subjects in this study.

Table 5. Demographic and Baseline Characteristics of All Subjects

Characteristic	n=34
Age, years	
Mean	33.41
Standard deviation	8.83
Minimum	18.00
Maximum	53.00
Median	32.50
Sex	
Female, n (%)	9 (26.47)
Male, n (%)	25 (73.53)
Ethnic origin	
Black, n (%)	4 (11.76)
Oriental (Asian), n (%)	3 (8.82)
White, n (%)	27 (79.41)
Baseline height, cm	
Mean	175.14
Standard deviation	10.93
Minimum	149.70
Maximum	191.40
Median	176.75
Baseline weight, kg	
Mean	76.19
Standard deviation	14.19
Minimum	50.70
Maximum	102.50
Median	76.35
Body mass index, kg/m^2	
Mean	24.69
Standard deviation	3.10
Minimum	18.22
Maximum	30.49
Median	24.08

Source: Clinical Data Report DEMO4

The sponsor reported all of the patients to be healthy and did not have any medical conditions that might have interfered with the metabolism or excretion of study medication or with the interpretation of the results.

Table 6 summarizes the mean ± standard deviation (SD) and geometric mean of estimates of tigecycline serum PK parameters for these subjects.

Table 6. Tigecycline Serum Pharmacokinetic Parameters of All Subjects (n=30)

Parameter	C_{max} (μg/mL)	t_{max} (h)	C_{0h} (μg/mL)	AUC (μg·h/mL)	CL (L/h)	CL (L/h/kg)
Mean±SD	0.75±0.23	0.5±0.0	0.12±0.12	1.73±0.64	31.4±7.7	0.41±0.07
Geometric mean	0.72	0.5	0.10	1.65	30.2	0.40

Abbreviations: C_{max} = maximum plasma concentration; t_{max} = time peak plasma concentration occurs; C_{0h} = AUC = total area under the serum concentration-time curve; CL = clearance

The mean steady state tigecycline exposure (C_{max} and AUC) in this study is slightly lower than that in other studies for healthy subjects receiving tigecycline 50 mg q12h, where the mean AUC ranged from 2.1 to 3.0 μg·h/ml.

Table 7 summarizes the tigecycline lung ELF PK parameters from the mean ELF concentrations.

Table 7. Tigecycline Lung ELF Pharmacokinetic Parameters

Parameter	C_{max} (μg/mL)	t_{max} (h)	C_{min} (μg/mL)	$t_{1/2}$ (h)	AUC (μg·h/mL)	AUC_{ELF}/AUC_{serum}
All subjects (n=30)	0.37	6.0	0.0	39.1	2.28	1.32

Abbreviations: C_{max} = maximum plasma concentration; t_{max} = time peak plasma concentration occurs; C_{min} = minimum plasma concentration; $t_{1/2}$ = terminal-phase elimination half-life; AUC = total area under the plasma concentration-time curve; AUC_{ELF}=AUC in epithelial lining fluid; AUC_{serum}=AUC in serum

Peak tigecycline concentrations in lung ELF were observed at 6 hours after administration of tigecycline, indicating that tigecycline has delayed penentration into the lung ELF. The degree of penetration into the lung ELF cells as measured by the ratio of $AUC_{0\text{-}12h,ELF}/AUC_{0\text{-}12h,serum}$ was 1.32, indicating that tigecycline exposure in lung ELF is approximately 30% higher in lung ELF than in serum.

Table 8 summarizes the tigecycline AC PK parameters from the mean AC concentrations.

Table 8. Tigecycline Alveolar Cell Pharmacokinetic Parameters

Parameter	C_{max} (μg/mL)	t_{max} (h)	C_{min} (μg/mL)	$t_{1/2}$ (h)	AUC (μg·h/mL)	AUC_{AC}/AUC_{serum}
All subjects (n=30)	15.2	2.0	6.4	23.7	134	77.5

Abbreviations: C_{max} = maximum plasma concentration; t_{max} = time peak plasma concentration occurs; C_{min} = minimum plasma concentration; $t_{1/2}$ = terminal-phase elimination half-life; AUC = total area under the plasma concentration-time curve; AUC_{AC}=AUC in alveolar cells; AUC_{serum}=AUC in serum

The degree of penetration into AC as measured by the ratio of $AUC_{0\text{-}12h,AC}/AUC_{0\text{-}12h,serum}$ was 77.5 indicating that tigecycline exhibited significant penetration into AC.

CONCLUSION:
Tigecycline exhibited good penetration into serum, lung ELF, and AC of healthy subjects. After treatment, the exposure (AUC) of tigecycline in lung ELF was approximately 30% higher than the exposure in serum, and the exposure of tigecycline in AC was approximately 77 fold higher than the exposure in serum.

Office of Clinical Pharmacology and Biopharmaceutics

Pharmacometrics Consult Request Form

NDA:	21821	**Sponsor:**	Wyeth Pharmaceuticals
IND:	56518		
Brand Name:	Tygacil	**Priority Classification:**	1P
Generic Name:	Tigecycline	**Indication(s):**	cSSSI, cIAI
Dosage Form:	I.V.	**Date of Submission:**	12/15/04
Dosing Regimen:	100mg load, followed by 50mg q12	**Due Date of PM Review:**	
Division:	DPE-III	**Medical Division:**	Anti-Infectives
Reviewer:	Jeffrey J. Tworzyanski	**Team Leader:**	Venkat Jarugula

Tabular Listing of All Human Studies That Contain PK/PD information (This can be requested at the pre-NDA stage as indicated on the PM roadmap)
(may attach tabular summary of all studies from NDA to this document)

Phase 1/2/3 studies included in Population PK analysis:
Drug interactions (3074A1-111-US, 3074A1-115-US)
Renal impairment (3074A1-103-US)
Hepatic impairment (3074A1-105-EU)
Geriatric (3074A1-102-US)
Multiple dose (3074A1-116-EU)

Phase 2 studies:
3074A1-200-US
3074A1-202-US

Phase 3 studies:
3074A1-300-US/CA
3074A1-305-WW
3074A1-301-WW
3074A1-306-WW

List the following for this compound (if known. The list will be confirmed by PM Scientist during the review):

Clinical endpoint(s):
Surrogate endpoint(s):
Biomarker(s):
Any reported optimal dose based on PK/PD ?:
Any reported dose/concentrations associated with efficacy/ toxicity ?:

Principal adverse event(s):

Pharmacometrics Request: (Jointly filled out with PM Scientist)

(Briefly state the objective(s) of the consult. The request should be as explicit as possible, and should state whether a review or additional analysis is needed. An assessment of the impact that the data will have on labeling should be included (Questions to be answered in QBR). The proposed labeling and the HPK Summary along with the relevant volumes should be available to the PM Scientist.)

1. Is there any effect of covariates (eg. age, body weight, sex, race) on the pharmacokinetics of tigecycline?
2. Is there a need for dose adjustment of tigecycline based upon these pharmacokinetic changes?
3. Is there a PK/PD relationship for tigecycline in terms of both efficacy and adverse reactions based upon population PK/PD analysis?

Due Date to the Reviewer _____

The __X__ PM Scientist or the ____ Primary Reviewer (select one) will perform the PM Review

PM Briefing ____ or PM Peer Review ____ requested (for criteria see the PM Road Map of QA/QC process)

Primary Reviewer _____ Signature _____ Date _____

PM Scientist _____ Signature _____ Date _____

CC: HFD-880 (John Lazor, Yaning Wang, Venkat Jarugula, Jeff Tworzyanski) HFD-850 (Electronic Entry or Lee)

Office of Clinical Pharmacology and Biopharmaceutics

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	**21821**	**Brand Name**	**Tygacil**
OCPB Division (I, II, III)	**III**	**Generic Name**	**Tigecycline**
Medical Division	**520-ANTIINFECTIVE**	**Drug Class**	**Glycylcycline**
OCPB Reviewer	**Jeffrey J. Tworzyanski**	**Indication(s)**	**cIAI, cSSSI**
OCPB Team Leader	**Venkat Jarugula**	**Dosage Form**	**IV**
		Dosing Regimen	**100mg load, 50mg q12**
Date of Submission	**12/15/04**	**Route of Administration**	**IV**
Estimated Due Date of OCPB Review		**Sponsor**	**Wyeth Pharmaceuticals**
PDUFA Due Date	**6/15/04**	**Priority Classification**	
Division Due Date			

Clin. Pharm. and Biopharm. 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				
I. Clinical Pharmacology	**x**			
Mass balance:	**x**	**1**		
Isozyme characterization:	**x**	**2**		
Blood/plasma ratio:				
Plasma protein binding:	**x**	**1**		
Pharmacokinetics (e.g., Phase I) -	**x**	**17**		
Healthy Volunteers-				
single dose:	x	7		
multiple dose:	x	10		
Patients-				
single dose:	x	1		
multiple dose:	x			
Dose proportionality -				
fasting / non-fasting single dose:	x	1		
fasting / non-fasting multiple dose:	x			
Drug-drug interaction studies -				
In-vivo effects on primary drug:	x	2		
In-vivo effects of primary drug:	x	2		
In-vitro:	x			
Subpopulation studies -				
ethnicity:	x	4		
gender:	x	1		
pediatrics:				
geriatrics:	x			
renal impairment:	x	1		
hepatic impairment:	x	1		
PD:				
Phase 2:	**x**	**2**		
Phase 3:	**x**	**4**		
PK/PD:				

Phase 1 and/or 2, proof of concept:	x	2		
Phase 3 clinical trial:	x	4		
Population Analyses -				
Data rich:				
Data sparse:	x	6		
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		**23**	**19**	

Filability and QBR comments

	"X" if yes	**Comments**
Application filable ?	**x**	Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?
Comments sent to firm ?		Comments have been sent to firm (or attachment included). FDA letter date if applicable.
QBR questions (key issues to be considered)		
Other comments or information not included above		
Primary reviewer Signature and Date		
Secondary reviewer Signature and Date		

CC: NDA XX-XXX, HFD-850(Electronic Entry or Lee), HFD-XXX(CSO), HFD-8XX(TL, DD, DDD), CDR (B. Murphy)

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/s/

Jeffrey Tworzyanski
6/15/05 11:34:27 AM
BIOPHARMACEUTICS

Venkateswar Jarugula
6/15/05 12:21:34 PM
BIOPHARMACEUTICS

Yaning Wang
6/15/05 12:28:02 PM
BIOPHARMACEUTICS

Jogarao Gobburu
6/15/05 12:37:20 PM
BIOPHARMACEUTICS